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Das Elektrovenogramm (Evg) der isolierten Flughautvene (*Chiroptera*)

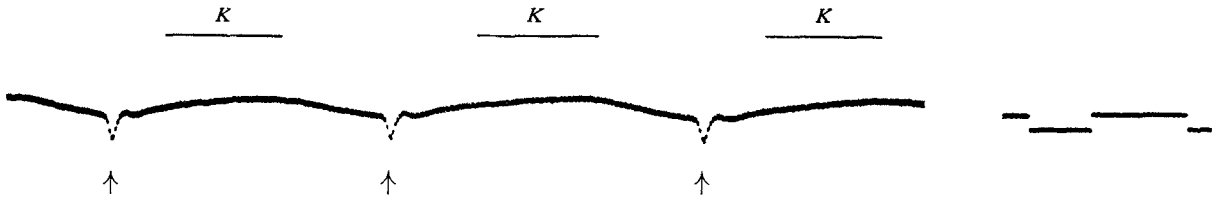
Bis heute ist es unseres Wissens nicht gelungen, von selbständig tätigen Gefäßen Aktionsströme abzuleiten. Die früher publizierten Elektroangiogramme sollen sich bei der Nachprüfung als Artefakte erwiesen haben[1]. Elektroarterio- und Elektrovenogramme, die 1940 veröffentlicht worden sind[1], wurden aus herznahen Gebieten abgegriffen und sind darum nicht beweisend. Wir haben deshalb versucht, von einem völlig isolierten und autonom arbeitenden Gefäß bioelektrische Ströme abzuleiten.

Mit Hilfe des Mikromanipulators kann man die Flughautvene präparieren und zum Venensäckchen abbinden[2]. Das Präparat gestattet die Bestimmung der Temperatur- und Druckabhängigkeit der isolierten, autonom tätigen Flughautvene an der Mikrokanüle. Es wurde weiterhin nachgewiesen, daß neben der autochthonen Automatie der intravaskuläre Dehnungsreiz zusammen mit dem Temperaturreiz das pulsauslösende Moment ist[3]. Die histologische Analyse der Venenwand ergibt ein elementares Geflecht aus spiralig angeordneten, glatten und offenbar nicht innervierten Muskelfasern[4].

Der Vorschlag liegt etwa 0,3 sec vor dem Beginn der Gefäßsystole. Er beginnt mit einer Potentialsenke, die von einem ungefähr gleich steil verlaufenden, vollständigen oder leicht überhöhten Anstieg gefolgt ist. Die nachfolgende schwache Senke ist etwas in die Länge gezogen. Die Verzögerung dürfte wohl auf das verspätete Eintreffen der eigentlichen Kontraktionswelle am Ort der Registrierungselektrode beruhen. Der nun einsetzende Potentialanstieg läuft synchron mit dem visuell überwachten und signalisierten Kontraktionsvorgang und geht meist glatt in einen der Gefäßdilatation folgenden allmählichen Potentialabfall über. Gelegentlich scheint der Erschlaffungsphase ebenfalls eine deutliche Potentialschwankung kurz vorauszugehen. Die Amplitude der Vorschlagskomponente wird bei erhöhter Pulsfrequenz vergrößert. Zwei Beispiele: wir messen bei einer Frequenz von 10 pro min 80 μV, bei einer Frequenz von 16 pro min 120 μV.

H. MISLIN

Zoologische Anstalt der Universität Basel, den 5. Dezember 1947.



Elektrovenogramm *Eichkurve*

K = Kontraktionsdauer (Pulsfrequenz 16 pro Min.).
↑ = Vorschlag (Initialwelle).

Die Ausschläge der Eichkurve entsprechen je 80 μV.

An diesem Präparat lassen sich Aktionsströme ableiten, die sehr übersichtliche und elementare Verhältnisse zeigen. Der Aktionsstrom wird vom 2 mm langen Venensäckchen mit unpolarisierbaren Silberelektroden innen und außen abgeleitet. Die Innenelektrode befindet sich in der Mikrokanüle, während die Außenelektrode mit einer feinen Schwammbrücke in unmittelbarer Nachbarschaft der pulsierenden Stelle angelegt wird. Zur Registrierung des Aktionsstromes wurde ein dreistufiger, gegengekoppelter Gleichstromverstärker mit einer Braunschen Röhre am Ausgang verwendet[5]. Die Eichimpulse an der Eichkurve entsprechen 80 μV. Die Außenelektrode bildete dabei den positiven Pol.

In der vorliegenden Notiz wird nur über den photokymographisch registrierten Hauptbefund berichtet. Auf die nähere Analyse der zeitlichen Beziehungen, die zwischen Erregungsvorgang und Ablauf der Kontraktionswelle bestehen, wird verzichtet. Ebenfalls können erst in der ausführlichen Arbeit die abfallenden bzw. ansteigenden Potentialänderungen den bekannten Komponenten des Wirbeltier-Ekg zugeordnet werden.

Am Evg lassen sich bei diphasischem Aktionsstrom in der Hauptsache zwei Stromschwankungen unterscheiden: eine schneller ablaufende Initialwelle (Vorschlag) und eine stark in die Länge gezogene Nachwelle.

Herrn Prof. M. MONNIER in Zürich danke ich für die freundlicherweise zur Verfügung gestellte Registrierapparatur, Herrn Dr. F. BOEHM, Zürich, Herrn Dr. G. LABHARDT, Basel, und Fräulein M. KAUFFMANN, Basel, für ihre wertvolle Mitarbeit und Hilfe bei der Registrierung des Evg.

Der Stiftung für biologisch-medizinische Stipendien danke ich für ihre Unterstützung.

Summary

The electrovenogram of the isolated vein of the wing membrane (*Chiroptera*) exhibits two main irregularities in its diphasic action current—a fast initial wave before the beginning of the systole and a very protracted afterwave. Following this the rise and fall of potential run parallel with the contraction and dilatation of the vessels.

[1] L. UNGHVÁRY und F. OBÁL, Z. Kreislaufforsch. *32*, 667 (1940).
[2] H. MISLIN, Helv. physiol. Acta *5*, C 3 (1947).
[3] H. MISLIN, Helv. physiol. Acta *5*, C 18 (1947).
[4] H. MISLIN und M. KAUFFMANN, Rev. suisse Zool. *54*, 240 (1947).
[5] F. BOEHM, B. SIGG und M. MONNIER, Helv. physiol. Acta *2*, 481 (1944).

Notizie preliminari sull'azione fitodinamica dell'acido difenililacetico sulla pianta del tabacco

Durante un gruppo di recerche sul dosaggio della attività dei discerbanti chimici selettivi abbiamo ottenuto dei risultati particolarmente interessanti usando l'acido difenililacetico

Questo acido, che dallo spoglio della letteratura[1] non ci risulta essere mai stato considerato come auxinosimile,

[1] Per la bibliografia fondamentale sull'argomento vedi: FR. A. GILBERT, Chem. Rev. *36*, 199 (1946), e vedi pure: R. CIFERRI, Riv. Soc. Tosc. Ortic. *31*, 11 (1947).

tein and fluid also occurs in the lymph nodes, but some controversy exists about the net direction of both of these.

Blood-lymph interrelationships

In regions with only continuous capillaries, apart from protein which is normally lysed in the tissues and which may be up to about a third of that which leaves the blood vessels (vide infra), the remaining protein which leaves them is returned via the lymphatic system. If this does not function properly considerable oedema results. A relatively small amount of fluid is also returned, but this amount is still very important in avoiding tissue oedema[2,3,23].

In regions where there are many fenestrated capillaries, it is becoming increasingly probable that there is a considerable circulation of plasma proteins, through the tissues, which re-enter the blood via the fenestrae on the venous side of the circulation[2,3,4,12]. It appears, however, that the lymphatics in this situation still are vital for the avoidance of oedema[24]. The amount of protein they remove may only amount to about 10–30%, but because this is in a concentrated form the mean concentration in the tissues is considerably reduced, and with it the colloidal osmotic pressures and tissue hydrostatic pressures. It appears that this reduction is essential for the avoidance of a mean positive tissue pressure and oedema.

Evolution

Primitive chordates have continuously lined great vessels, but the endothelial cells gradually become further and further apart in the smaller vessels, until even the basement membranes of the vessels may partly disappear and the tissue spaces are directly continuous with the blood vessels[2,3]. The higher animals have more continuous endothelial vascular linings, until in the elasmobranch the venous intercellular junctions in the body wall can still be opened by muscular movements, but in the viscera fenestrated capillaries appear, allowing macromolecular uptake there. In the teleosts the increased blood hydrostatic pressure no longer allows any blood vascular intercellular junctions to be openable, but also necessitates a raised plasma protein concentration. A separate lymphatic system now develops, which is filled by variations in the solid tissue pressure, but which propels the lymph and discharges it into the blood by means of the contractions of muscle in its walls. Thus, aside from part of the raised protein circulation through the tissues in fenestrated regions, protein is now returned to the blood via a separate system of pumps – the initial lymphatics, the segments in the collecting lymphatics and the lymph hearts, when these exist.

[24] J. R. CASLEY-SMITH, Microvasc. Res., submitted for publication (1976).

Active Contractility of the Lymphangion and Coordination of Lymphangion Chains

by H. MISLIN

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A 'lymph drainage capable of compensation' (FÖLDI[1]) is always based on the functional interplay between differentiated lymph drainage mechanisms, and specially effective importance is shown to be attached to the vasomotoric lymph drainage[2]. The initial lymphatics do not show any active contractions because of lack of muscle in their walls. The lack of that autorhythm of course does not mean the lacking of contractility. First information about open junctions in the lymphatic capillaries is given by CASLEY-SMITH and FLOREY[3]. My collaborator SCHIPP[4] observed in the peripheric lymph vessels intraendothelial filaments, which could be interpreted in the sense of a contractility of the endothelium. SCHIPP and SCHÄFER[5] found no open junctions inspite of experimental lymphostasis. LEAK[6] postulated in his studies on the permeability of lymphatic capillaries, that these small vessels are able to open and close the interendothelial cell joints by active contraction an relaxation of the endothelium. The larger musculous lymphatic vessels have a pronounced autorhythm. From the morphological point of view, the lymphatic vessel is characterized by its segmentation; it consists of valve segments with central muscle collars and directed multiple innervation of the smooth muscles. Physiologically the valve segment, we called it lymphangion[7], is an autochthonous efficiency element with its typical action potential[8]. The in vivo experiments of SMITH[9] and our own in vitro experiments demonstrated that the single

[1] M. FÖLDI, in *Handbuch der Allgemeinen Pathologie* (Springer Verlag, Berlin, Heidelberg, New York 1971), vol. 3/6, p. 329.
[2] H. MISLIN, in *Handbuch der Allgemeinen Pathologie* (Springer Verlag, Berlin, Heidelberg, New York 1971), vol. 3/6, p. 219.
[3] J. R. CASLEY-SMITH and H. W. FLOREY, Q. Jl. exp. Physiol. *46*, 101 (1961).
[4] R. SCHIPP, Acta anat. *71*, 341 (1968).
[5] R. SCHIPP and A. SCHÄFER, Zool. Anz. Suppl. *33*, 407 (1969).
[6] L. V. LEAK, J. Cell Biol. *50*, 300 (1971).
[7] H. MISLIN, Experientia *17*, 19 (1961).
[8] H. MISLIN, *Lymphographie und Pharmakolymphographie* (Gustav Fischer Verlag, Stuttgart 1975), p. 10.
[9] R. O. SMITH, J. exp. Med. *90*, 498 (1949).

lymphangion is able to contract independently, but at the same time it plays a key role in the activation and coordination of lymphangion chains because of its extensive distention. In order to measure the dependence of the pulse rate on the pressure, flow-through canulae were put into the isolated lymphangion chains[2]. Pulsating segments of vessels normally show typical inherent frequencies with a characteristic amplitude of the vessel and in relation to the present angiotonus (f 8–55 min). With increasing internal pressure of 2–25 cm H_2O, the frequency of the lymphangions increases proportionally and reaches a temperature-dependent frequency maximum. In pressure experiments at 12 cm H_2O the maximal frequency of the active pulse was 55/min (short term). Long persisting and continually regular pulse is normally found at the mesenterial lymphangion at an internal pressure of 4 cm H_2O. In connection with the specific condition of the single angions, the active pulse is often released by an internal pressure between 4 and 8 cm H_2O or between 10 and 12 cm H_2O (f 15–25/min). In flowthrough experiments, and by employing the discharge drop counting method, the volume per min is doubled if the pressure increases from 8 to 10 cm H_2O and increases 3-fold at an increase from 10 to 12 cm H_2O. These investigations were carried out on the intestinal lymphangions of the guinea-pig, which proved the importance of the active contractions for the transport of lymph; they were completed by pressure measurements at lymphatic vessels of the intestine of rats after occlusion of the vessels carried out by WALDECK[10a]. Inside an in situ occluded lymphatic vessel, an increase of pressure from 15 to 40 cm H_2O has been registered within 10–20 min. The mean value was about 27 cm H_2O. The increase of frequency taking place continuously with the increase of pressure could reach up to 65/min in the in situ experiments.

In relation to the mean pressure, the pressure amplitudes varied as well. Even as the in case of the isolated single angion, they are small, if the pressure is low; they increase with increasing pressure to about 15 cm H_2O ($^1/_2$–$^2/_3$ of the diameter of the angion), then they decrease rapidly with still increasing pressure. With further increase in pressure, the volume per min as well as the volume per pulse decreases. Because of the small mass of muscle of the lymphangions, the isotonic as well as the isometric maximum curves run near the repose-distention-curve, as is known from the heart. WALDECK[10b] determined the work of the pressure volume of a segment of a lymphatic vessel in direct dependence on the pressure by multiplying the volume of the lymphpulse with the respective pressure. Starting at zero, he found the curve reaches its maximum at about 10–15 cm H_2O and then decreases rapidly with further increase in pressure. According to our experiments with isolated lymphangions, it is obvious that the high pressures found by WALDECK in occluded lymphatic vessels were caused by addition of the pressure in the single lymphangions. At isovolumetric contractions, the isolated chains of the lymphagions create pressures up to 2 cm H_2O. The final pressure is defined by the number of contractile unities and their capability to produce pressure. Physiologically the lymphangion chains can be compared with the multiple lymphatic hearts of the gymnophions, which, as is well known, produce an intermittent rhythm. With lymphatic fistulas in non-anesthetized, freely moving sheep HALL, MORRIS and WOOLEY[11] carried out long-term experiment with lymph canula, where lateral as well as final pressures could be measured. The decisive result of those fistula experiments was the proof of an autonomic intermittent rhythm of the lymph flow. The measured pulse frequencies were between 1 and 30/min, the pressures so caused were between 1 and 25 mm Hg. These experiments also show a good or relation between the volumes of the lymph flow and the contractions of the lymphangion chains. In theoretical investigations, REDDY[12] and REDDY et al.[13] have confirmed the theory of coordination of lymphangion chains. They expressed intralymphatic pressure of the terminal lymphatics (P_{tl}) in terms of the tissue pressure and wall hoop stress

$$P_{tl} = T + h/a\,(\sigma_{hoop}) \qquad [1]$$

where T is the tissue pressure, h is the wall thickness, a is the radius of the vessel and σ_{hoop} is the hoop stress which is a function of the modulus of elasticity, the instantaneous radius, and the unstressed radius of the vessel. Similarly the authors expressed the intralymphangion pressure (P_{ly}) in terms of the external pressure, the hoop stress and the stress due to active muscular contractions:

$$P_{ly} = P_{ext} + h/a\,(\sigma_{hoop} + \sigma_{act}) \qquad [2]$$

where σ_{act} is the stress developed due to active contractility in the walls of the lymphangion, and P_{ext} is the external pressure on the lymphangion. The stress developed due to active contractility σ_{act} depends on the time as well as distention of the lymphangion. We showed with our experiments on the isolated lymphangion chains that each active contraction lasts for a given period of time which is followed by a period of refraction. During the refractory period, a lymphangion does not contract even if the wall of the angion is dilated beyond the threshold. Together with HALL et al.[11] we concluded that the force amplitude developed due to an active contraction is dependent on the di-

[10] F. WALDECK, a) Pflügers Arch. ges. Physiol. *283* (1965); b) *284*, 294 (1965).
[11] J. G. HALL, B. MORRIS and G. WOOLEY, J. Physiol., Lond. *180*, 336 (1965).
[12] N. P. REDDY, Ph. D. Dissertation, Texas A & M University (1974).
[13] N. P. REDDY, TH. A. KROUSKOP and TH. A. NEWELL JR., Microvasc. Res. *10*, 214 (1975).

stention of the lymphangion wall just before the onset of the contraction, findings which are consistent with Starling's law of the heart.

With [1] REDDY observed that a rise in the interstitial fluid pressure causes the intraluminal pressure of the terminal lymphatics to increase. Therefore, if the pressure in the terminal lymphatics is larger than the pressure in the lymphangion adjacent to the terminal lymphatics, then fluid flows from the terminal lymphatics into the lymphangion adjacent to the terminal lymphatics. From [2] it can be seen that the lymphangion pressure increases with the inflow due to the contribution of the hoop stress in this terms. If, now, the dilation of the lymphangion wall reaches its threshold, an active contraction in the lymphangion is initiated so as to propel the lymph into the lymphangion in front of it. It is clear that the outflow from the lymphangion causes the lumen size to decrease. During the recovery phase of the active contraction, the contribution of active contractility stress terms is zero. In their note on the mechanisms of lymph flow through the terminal lymphatics, REDDY, KROUSKOP and NELL JR.[13] offer an explicit explanation of these mechanisms: 'The negative pressure is responsible for the lymph absorption by the terminal lymphatics. If the threshold strain required to initiate an active contraction in the lymphangion adjacent to the initial lymphatics is zero or just above zero, then this lymphangion acts as a pump regulated by the tissue pressure'. This suction effect of the terminal lymphatics is very unlikely, as is shown by actual measurements of intralymphatic pressure given by ZWEIFACH and explained by CASLEY-SMITH[14], who proposes an alternative explanation for terminal lymphatic filling. However, even with this, variations in tissue pressure will affect the filling of the terminal lymphatics. Thus, the rest of the explanation of REDDY et al. will hold.

The duration of a contraction and the duration of the refractory period can be altered by several pharmacological agents: MISLIN[15], TIRONE et al.[16].

This theory suggests that the lymph absorption is dependent on the pressure gradient between the interstitial pressure and the pressure in the lymphangion adjacent to the initial lymphatics and can be in agreement with the conception of CASLEY-SMITH.

[14] J. R. CASLEY-SMITH, Experientia *32*, 1 (1976).
[15] H. MISLIN, Arzneimittel-Forsch. *21*, 852 (1971).
[16] P. TIRONE, P. SCHIANTARELLI and G. ROSATTI, Lymphology *6*, 65 (1973).

Diseases of Lymphostasis

by M. FÖLDI

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Definition

Lymphoedema of the extremities is the only sequela of chronic stagnation of lymph on which interest is focused. It is, however, a well established fact that lymphostasis will induce not only extracellular oedema, but an intracellular too, i.e. it causes parenchymal cell lesions. A whole series of lymphostatic syndromes; 'diseases of lymphostasis' have been identified.

'Diseases of lymphostasis' may appear in any organ drained by lymphatics, independently of whether the lymph capillaries lie inside the parenchyma organs with short prelymphatics (e.g. dermis, gut) or outside (organs with long or very long prelymphatics, e.g. liver). They are characterized by typical functional and pathological alterations. 'Diseases of lymphostasis' form a new chapter in medicine. Although most of them are described up to now as experimental syndromes and still await clinical application, it is possible to outline our knowledge of this new field.

Experimental lymphostatic syndromes

Experimental 'diseases of lymphostasis' are induced surgically. The usual methods are: a) By ligating or resecting all lymph vessels and lymph nodes found, or by an intralymphatic injection of phlogogenic substances, thus provoking lymphangitis and lymph-vessel thrombosis, an acute or subacute lymphatic disease may be induced which will be terminated by lymphatico-lymphatic and/or lymphatico-venous anastomoses. b) To induce chronic forms of lymphostatic diseases, not only lymphatics must be resected but also a strip of connective tissue, even the adventitia of blood vessels, too. Such techniques have been devized, by CLODIUS (extremities) and LIE (liver), in order to delay regenerative processes. Repeated intralymphatic injections of sclerosing substances may be helpful too.

Pathomechanism

As a result of lymphostasis, the plasma proteins accumulate in the interstitium; this leads to an increase in interstitial colloid-osmotic pressure and to lymphoedema. Intracellular oedema and cell damage will soon follow due to the increase of interstitial pressure, disturbed cell nutrition and transport of metabolites. Stagnation impairs the 'vehicle function' of proteins; hormones and vitamins bound to plasma proteins participate in their extravascular circulation as well as

Etant donné le rôle éminent que jouent les peptones dans le métabolisme de notre microorganisme, il était indiqué d'étudier leur action en relation avec celle des sulfamidés. Onze peptones différentes ont été employées: toutes, à des concentrations variables, ont un pouvoir antisulfamide net (tableau 2).

Nous retrouvons le fait essentiel mis en évidence par LOCKWOOD et LYNCH[1] en 1940 ainsi que par NITTI, TABONE et MOUSSET[2] relatif à l'action antisulfamide de la peptone.

Le filtrat d'une solution de peptone à 6%, dont la teneur en matière sèche a passé de 6 à 0,515% et le taux en azote de 0,378% à 0,0022%, manifeste encore une action antisulfamide nette, quoique plus faible que celle de la peptone pure, non traitée par la norite.

Comme, selon toute apparence, la peptone est privée d'acide p-aminobenzoïque et que d'autre part ce dernier est retenu par la norite, il faut envisager, ici aussi, la possibilité d'une action antisulfamide indépendante de l'acide p-aminobenzoïque.

W. H. SCHOPFER et Mlle M. GUILLOUD

Institut de Botanique de l'Université de Berne, le 5 novembre 1945.

Nous remercions les Etablissements F. Hoffmann-La Roche & Co. (Bâle) pour les produits qu'ils nous ont fait aimablement parvenir, ainsi que les Etablissements CIBA (Bâle), Geigy (Bâle) et CILAG (Schaffhouse) pour les sulfamidés qu'ils ont obligeamment mis à notre disposition.

Summary

Different sulphanilamides show an inhibiting action on *Eremothecium Ashbyii*. This effect is marked by a diminution in the formation of flavine, visible already before the decrease of the weight of the culture. Peptones possess a very high anti-sulphanilamide power.

[1] LOCKWOOD and LYNCH, J. Amer. med. Ass. *114*, 935 (1940).
[2] NITTI, TABONE et MOUSSET, Ann. Inst. Pasteur *68*, 474 (1942). TABONE, Bull. Soc. Chim. biol., Paris *26*, 137 (1944).

L'acide thymonucléique polymérisé, principe paraissant susceptible de déterminer la spécificité sérologique et l'équipement enzymatique des bactéries. Signification pour la biochimie de l'hérédité

Dans des publications antérieures[1], nous avons montré que les colibacilles existent sous de très nombreux types distincts, dont chacun est défini par la possession d'un polysaccharide spécifique particulier, se caractérisant par sa constitution chimique et par son comportement sérologique. En outre, des différences dans le détail des propriétés biochimiques se rencontrent parmi tous ces colibacilles.

Lorsqu'on vient à cultiver un de ces types sur bouillon, puis à filtrer la culture à travers une bougie et enfin à ensemencer le filtrat avec un autre type, on peut obtenir des résultats très différents selon les germes utilisés:

1. Le second type ne se multiplie pas ou se multiplie très mal dans le milieu où s'est préalablement développé le premier type; tantôt cela tient à l'intervention d'un phage porté par le premier en mode inapparent et auquel le second se trouve être fort sensible; tantôt aucun phage n'entre en ligne et on est en face d'un phénomène d'antagonisme bactérien vrai, un principe (ou des principes?) bactériostatique pour le second type étant élaboré par le premier.

2. Le second type se multiplie bien, sans subir aucune modification (c'est le cas le plus fréquent).

3. Le second type se multiplie abondamment, mais en subissant des transformations dans ses propriétés biochimiques ou antigéniques. Dans le premier cas, le comportement du germe à l'égard de certains «substrats» change, pour s'aligner sur celui que manifeste le premier type à l'égard des mêmes substrats[1]. Dans le second cas, le deuxième type perd son antigène «somatique» (passage de la forme «smooth» normale, pourvue de son polysaccharide spécifique, à la forme dégradée «rough», dépourvue du même polysaccharide) ou *quelquefois* il subit un changement de spécificité, en acquérant l'exacte spécificité propre au premier type (remaniement du polysaccharide). La transformation de type semble bien se faire par les étapes suivantes: forme smooth N° 2 → forme rough correspondant au N° 2 → forme smooth N° 1.

De pareilles constatations montrent bien que l'antagonisme microbien – dont il est tant parlé et à juste raison depuis la découverte de la pénicilline – n'est que l'un des aspects possibles des interactions entre deux bactéries différentes, interactions qui doivent jouer un grand rôle dans l'établissement des flores microbiennes dans le sol, dans les eaux, etc., et aussi, dans l'organisme, à la surface des diverses muqueuses, dans le gros intestin. etc.

Nous avons étudié, en détail, un cas d'induction d'une spécificité sérologique nouvelle et d'un équipement enzymatique nouveau par action d'un colibacille sur un autre colibacille et nous avons pu préciser la nature du principe inducteur élaboré par le premier germe.

Il s'agit de deux colibacilles retirés des matières fécales humaines normales, que nous désignerons par C_1 et C_2. C_1 renferme un polysaccharide donnant la réaction des acides uroniques; il ne fait pas fermenter le saccharose, même après passages répétés sur des milieux contenant ce disaccharide. C_2 renferme un polysaccharide de tout autre spécificité sérologique, ne donnant pas la réaction des acides uroniques; il fait fermenter le saccharose avec production d'acides (intervention d'enzymes «constitutifs» au sens de KARSTRÖM). Or, si l'on vient à cultiver pendant quelques jours C_2 smooth dans un filtrat de culture de C_1 smooth, on obtient côte-à-côte des germes répondant à C_2 smooth, à C_2 rough et à C_1 smooth (on les sépare par l'artifice habituel des colonies isolées sur gélose); si l'on cultive C_2 rough dans les mêmes conditions, on passe à un mélange de C_2 rough et de C_1 smooth; si enfin on cultive C_1 rough, on aboutit à un mélange de C_1 rough et de C_1 smooth. C_1 issu de C_2 présente les mêmes caractères antigéniques et enzymatiques que C_1 naturel. Des résultats identiques peuvent être atteints en substituant au filtrat de culture de C_1 smooth du bouillon auquel on a ajouté un autolysat de C_1 smooth (tuer les germes par le toluène, les laisser s'autolyser à 37°C, centrifuger pour éliminer les cadavres microbiens). Le principe actif se retrouve dans la fraction nucléoprotéidique qu'on peut isoler de l'autolysat par précipitation à

[1] A. BOIVIN, L. CORRE et Y. LEHOULT, C. R. Soc. Biol. *136*, 98, 257 et 432 (1942); *137*, 42, 138, 410 et 714 (1943). — Bull. Acad. Méd. *127*, 95, 125 et 162 (1943). — Rev. Immunol. *7*, 97 (1942).

[1] Des phénomènes du même ordre ont été rencontrés par LISBONNE, NÈGRE, ROMAN et SEIGNEURIN (Ann. Inst. Pasteur *61*, 822 [1938]) au cours de leurs ingénieuses expériences de «parabiose» (culture de deux bactéries dans deux portions d'un même milieu séparées par une membrane de collodion infranchissable pour les germes, mais perméable à leurs produits métaboliques).

pH 3,5. Il se retrouve dans l'acide nucléique qu'on libère de ce nucléoprotéide par digestion pepsique, suivie de précipitations fractionnées par l'alcool additionné d'acide chlorhydrique. En réalité, on aboutit ainsi à un mélange d'acide ribonucléique et d'acide thymonucléique. L'activité persiste après action de la ribopolynucléotidase, mais disparaît après celle de la thymopolynucléotidase (enzymes pancréatiques). Le principe actif apparaît donc comme étant un acide thymonucléique à l'état polymérisé. Cette conclusion s'accorde pleinement avec la découverte, faite tout récemment par Avery, MacLeod et MacCarty[1], de la nature thymonucléique du principe susceptible de provoquer la transformation de type chez les pneumocoques; mais les Américains ne paraissent pas avoir rencontré de transformations dans l'équipement enzymatique de leurs germes.

Il semble bien établi, maintenant, que la cellule bactérienne possède un petit noyau à acide thymonucléique, noyé dans un cytoplasme à acide ribonucléique. Le principe issu de C_1 et qui se montre capable d'imposer à C_2 une constitution moléculaire nouvelle pour son polysaccharide et un équipement enzymatique nouveau, ne résulte-t-il pas d'une simple «solubilisation» de l'appareil chromosomien rudimentaire de C_1? L'hypothèse offre quelque vraisemblance. Si elle répond à la réalité, elle ouvre des horizons tout à fait nouveaux et combien prometteurs en ce qui concerne la biochimie de l'hérédité. En particulier, c'est du côté de l'acide nucléique et non plus de la protéine de la macromolécule nucléoprotéidique constituant un gène qu'il faudrait chercher la raison des propriétés inductrices propres à ce gène. Cela amènerait à envisager la possibilité d'une structure («primaire» ou plus vraisemblablement «secondaire») susceptible de différencier entre eux les divers acides nucléiques à désoxyribose, sous leur état naturel de polymérisation.

André Boivin, Albert Delaunay,
Roger Vendrely et Yvonne Lehoult

Institut Pasteur (Paris – Garches), le 10 novembre 1945.

Summary

Interactions between two types of bacteria can produce either inhibition of the growth of one of these bacteria or transformation of their biochemical or antigenic properties. Authors have shown and studied one case of induction of a new serological specificity and a new enzymic equipment in a *Bacterium coli*. This induction was produced through a substance liberated, during autolysis, by an other *Bacterium coli*. Active principle is a thymonucleic acid which, maybe, results from a solubilisation of chromosomes of the inductor bacteria. Hypothesis appears likely.

[1] J. exp. Med. *79*, 137 (1944).

Di alcuni fenomeni corticali che accompagnano la fecondazione e le prime divisioni dell'uovo di riccio di mare

Dalle osservazioni in luce polarizzata mie e di A. Monroy Oddo[1] è risultato che il cortex dell'uovo vergine di riccio di mare ha struttura submicroscopica radiale con asse ottico normale e birifrangenza *positiva* (rispetto al raggio). Le nostre osservazioni ci hanno inoltre portato ad ammettere che il cortex è costituito esclusivamente da lipidi (probabilmente fosfolipidi + colesterina) e che per le sue caratteristiche ottiche e proprietà fisiche, si può considerare come un cristallo fluido di tipo smettico[1].

Quando l'uovo viene fecondato, non appena lo spermio tocca il cortex dell'uovo, scompare la birifrangenza corticale. Questa reazione sembra precedere, se pur di poco, l'elevazione della membrana di fecondazione. La quale, come è noto, presenta una birifrangenza *negativa* (rispetto al raggio) assai evidente, dovuta alla sua struttura foliare ed alla sua natura proteica.

All'inizio dell'anafase della prima mitosi però, la birifrangenza corticale riappare; dapprima è assai debole, ma aumenta rapidamente di intensità, pur senza raggiungere quella che ha nell'uovo vergine, *e sempre con carattere ottico positivo*. Tra i ricci da me studiati solo nelle uova di *Arbacia pust.* non mi è riuscito finora di mettere in evidenza il riapparire della birifrangenza corticale all'inizio dell'anafase della prima mitosi, né nelle uova normali né in quelle centrifugate (e cioè dopo avere dislocato il pigmento). Anche il valore della birifrangenza — calcolato col metodo di Schmitt e Bear[2] (v. A. Monroy e A. Monroy Oddo[3]) — ha fornito valori assai vicini a quelli dell'uovo vergine; il che fa pensare che la costituzione chimica della formazione sia sostanzialmente la stessa.

Con l'inizio della formazione del solco equatoriale la birifrangenza scompare ai poli dell'uovo mentre rimane ancora evidente nelle zone equatoriale e subequatoriale. Ciò è probabilmente in relazione con l'espansione che in questo stadio subisce lo strato corticale delle regioni polari (Dan, Yanagita e Sugiyama[4]), espansione che, data la natura liquocristallina del cortex, si traduce in uno scompigliamento della sua tessitura con conseguente scomparsa della birifrangenza. Anche nelle zone equatoriali e subequatoriali la scomparsa della birifrangenza si ha durante la fase espansionale, e cioè durante la telofase, quando il solco si è molto approfondito.

Formatisi i due blastomeri, nessuna birifrangenza corticale è in essi dimostrabile fino all'inizio della seconda anafase, momento in cui essa riappare, ma molto debole, su ognuno dei due blastomeri, per scomparire ancora alla metà della telofase. Nulla di simile ho potuto osservare durante la terza divisione e nelle divisioni successive; ciò però non esclude — anzi è molto probabile — che il fenomeno si verifichi, ma è forse così debole che non è rivelabile nelle condizioni delle mie osservazioni.

Il fatto che un tale ordinamento strutturistico submicroscopico dello strato corticale — del quale sono espressione i descritti fenomeni osservabili in luce polarizzata — si manifesta nel periodo ana-telofasico, fa

[1] A. Monroy e A. Monroy Oddo, Boll. Soc. it. Biol. sper. XIX, p. 70 (1945); Pubbl. Staz. Zool. di Napoli (in corso di pubblicazione).

[1] Da una lettera privata del Prof. Runnström, Stockholm, apprendo che anche egli ha visto la birifrangenza corticale positiva dell'uovo vergine di riccio di mare e che su questo argomento ha un lavoro in pubblicazione, mentre una nota preventiva sarebbe già apparsa; ma le attuali difficoltà degli scambi mi hanno finora impedito di prenderne visione.

[2] F. O. Schmitt e R. S. Bear, J. cell. comp. Physiol. IX, p. 261 (1937).

[3] A. Monroy e A. Monroy Oddo, Boll. Soc. it. Biol. sper. XIX, p. 70 (1945); Pubbl. Staz. Zool. di Napoli (in corso di pubblicazione).

[4] K. Dan, T. Yanagita e M. Sugiyama, Prot. XXVIII, p. 66 (1937).

quello isolato dall'Arnaudi e classificato come *Flavobacterium dehydrogenans*[1].

L'interesse biologico di queste azioni microbiche non risiede soltanto nel fatto che per la prima volta venivano descritti schizomiceti capaci di attaccare le sterine, ma anche e specialmente perchè l'ossidazione da essi provocata si realizza con caratteri di alta specificità, sicchè è possibile la loro utilizzazione da un punto di vista industriale, per alcune trasformazioni che hanno luogo nel corso della sintesi degli ormoni sessuali. Essi infatti consentono, diversamente dai reattivi chimici ad azione non selettiva, l'ossidazione dei gruppi alcolici secondari e quindi permettono l'ottenimento di rendimenti assai più elevati.

I caratteri biologici di questi batteri sono per sè stessi assai interessanti e sembrano avere uno stretto legame con le loro proprietà biochimiche. Essi elaborano un pigmento giallo citrino che è racchiuso nella capsula del batterio e non diffonde nei liquidi e che si forma esclusivamente alla luce. Tale pigmento sembra legato alle capacità respiratorie ed ossidative del batterio, in quanto quelli intensamente pigmentati per esposizione alla luce diffusa, determinano un minore abbassamento del potenziale *redox* del mezzo nutritivo di quelli privi di pigmento perchè cresciuti al buio ed inoltre metabolizzano con intensità assai più accentuata il glucosio e l'alcole etilico di quelli non pigmentati. Parallelamente si può constatare che la deidrogenazione del deidroandrosterone è più debole od anche nulla se si impiegano stipiti privi di pigmento. Viceversa, culture abbondantemente pigmentate e trattate per 3 ore a 56° C si sono mostrate ancora capaci della ossidazione sopra citata, onde l'attività ossidativa risulterebbe legata al pigmento più che ai processi vitali del batterio. Va inoltre rammentata la notevole resistenza che presenta il *Flavobacterium dehydrogenans* all'alcole etilico (7%), all'acetone (9%) ed all'acqua ossigenata, che nella misura del 0,025% addizionata al brodo-lievito, non ne arresta la moltiplicazione.

Nel 1944 Ercoli e Molina[2] hanno osservato un particolare comportamento biochimico in altri due *Flavobacterium* morfologicamente assai simili al *dehydrogenans*. Uno, denominato *Flav. androstendionicum*, capace di ossidare l'androstendiolo in androstenedione, avente cioè l'attitudine a deidrogenare i due ossidrili alcoolici secondari in posizione C 3 e C 17, mentre non può ossidare l'androstenediolo a testosterone, ossidazione provocata invece dal *Flav. dehydrogenans*. Il secondo, denominato *Flav. carbonilicum*, dimostrante invece un potere ossidante intermedio fra quello esplicato dal *Flav. dehydrogenans* ed il *Flav. androstendionicum*. Esso infatti, messo ad agire sull'androstendiolo, determina la formazione di una miscela di testosterone e di androstenedione. È da tenere presente però che non sono stati determinati i livelli di potenziale *redox* ai quali vennero realizzate da Molina ed Ercoli le ossidazioni con tali batteri e non si può escludere *a priori* che le condizioni ambientali nelle quali l'ossidazione si svolge, non intervengano a determinare una più o meno intensa azione ossidante. Se così fosse, i due germi sopra citati rientrerebbero evidentemente nella specie *Flav. dehydrogenans*.

La capacità ossidante dei flavobatteri si estrinseca però anche su altre sostanze oltre alle sterine. Notevole è l'azione di alcuni di essi sopra la glicerina che viene intensamente acidificata e su diversi idrati di carbonio i quali rimangono, parimenti alla glicerina inerti dal punto di vista fermentativo e risultano invece più o meno intensamente acidificati. Anche più interessante è il fatto che alcuni idrocarburi e loro derivati possano essere utilizzati dai flavobatteri in via ossidativa. È stato possibile infatti far crescere diversi flavobatteri in soluzioni saline nutritive, nelle quali l'unica fonte di carbonio era costituita da toluolo, xilolo, paraffina, acido benzoico, acido salicilico ed acido fenico.

Alcune esperienze attualmente in corso di esecuzione ci fanno supporre che fra i commensali dei cellulosolitici aerobi ossidanti la cellulosa del terreno, siano largamente rappresentati anche i *Flavobacterium* e che la loro presenza non sia senza significato nello svolgimento di questo importante processo degradativo.

Carlo Arnaudi

Istituto di Microbiologia agraria e tecnica dell'Università di Milano, 2 marzo 1946.

Summary

A few *Flavobacterium*, among them *Flav. dehydrogenans*, present oxidative activities upon sterins from the serie of sexual hormons, acting in a selective manner. These microorganisms have a scarce fermentative capacity against carbohydrates and an eminent oxidative action upon hydrocarbures and their derivatives as toluol, xylol, paraphine, benzoic acid, salicylic acid, phenol.

The pigment of *Flav. dehydrogenans*, which is exclusively elaborated at diffusive light, is straightly fixed at oxidative processes determined by the bacterium.

[1] C. Arnaudi, Boll. I.S.M. (1942); Centr. Bakt., II. Abt. *105* (1942).

[2] L. Molina e A. Ercoli, Boll. I.S.M. (1944).

Sur certaines conditions de la transformation du type antigénique et de l'équipement enzymatique d'un colibacille, sous l'effet d'un principe inducteur de nature thymonucléique issu d'un autre colibacille (mutation «dirigée»)

Depuis les travaux de Griffith, on connaît la possibilité, pour un pneumocoque, de changer de type et récemment Avery, MacLeod et McCarty ont découvert la nature thymonucléique du principe inducteur grâce auquel un type de pneumocoque peut imposer sa propre spécificité à un autre type en voie de mutation. Berry a retrouvé des phénomènes comparables dans le domaine des virus (virus de Shope-Sanarelli). A notre tour, nous avons vu un type de colibacille imposer sa propre spécificité et quelques-uns au moins de ses caractères enzymatiques à un autre type de colibacille, et cela grâce à l'entrée en jeu d'un principe inducteur thymonucléique[1]. Il est assez vraisemblable que des transformations du même ordre sont susceptibles de se produire, *avec plus ou moins de facilité*, chez toutes les bactéries et tous les virus, et qu'elles jouent quelque rôle dans l'évolution naturelle des flores microbiennes saprophytes et pathogènes. Mais il convient d'éviter une erreur de perspective et de ne pas tomber dans un mobilisme exagéré: de pareilles mutations «dirigées» semblent garder un caractère exceptionnel et les types antigéniques demeurent usuellement stables, conservant ainsi, *en pratique*, toute leur valeur d'«espèces élémentaires». C'est ce que nous voudrions montrer à propos des colibacilles.

[1] Exper., *1*, 334 (1945).

Les colibacilles donnent lieu à un très grand nombre de types antigéniques distincts, dont chacun est caractérisé par la constitution de son polysaccharide somatique. Mais il semble exister des groupes parmi tous ces types, basés sur la spécificité des matières protéiques de la cellule bactérienne; toutefois la question est encore en pleine étude dans notre laboratoire. Les colibacilles C_1 et C_2 dont il a été question dans notre précédente note, appartiennent à un même groupe et il y a tout lieu de penser que la possibilité d'une mutation dirigée ne dépasse pas le cas de deux germes très voisins l'un de l'autre. Mais à beaucoup près, cette condition n'est pas suffisante pour qu'une pareille mutation puisse s'installer; il faut encore, selon toute probabilité, que le germe intéressé soit dans un certain état d'instabilité dont la nature intime nous échappe complètement. Ainsi, nous avons toujours échoué dans nos tentatives répétées pour transformer C_1 rough en C_2 smooth, grâce à une nucléoprotéine ou à un acide nucléique extraits de C_2. D'autre part, parmi les assez nombreuses variantes qu'il est possible de distinguer dans C_2 rough, en se basant sur l'aspect des colonies, une seule (forme à très petites colonies) se montre sensible à l'extrait provenant de C_1, et peut se transformer sous son action en C_1 smooth. Quant à C_1 rough, ce n'est qu'assez exceptionnellement qu'on obtient son passage à C_1 smooth, sous l'effet du même extrait[1]. Ces faits rappellent absolument ceux qui ont été vus dans le domaine des pneumocoques.

Il convient de noter, toutefois, que l'échec d'une tentative de mutation dirigée peut fort probablement tenir aussi à des causes autres que la trop grande stabilité du germe considéré. Notre technique de préparation de l'extrait inducteur comporte une courte autolyse du colibacille tué par le toluène, à 37° C ou mieux à la température ordinaire (quelques heures), une courte digestion pepsique vers p_H 2 et à la température ordinaire (quelques heures) et des précipitations fractionnées en milieu alcoolique et acide. Cela offre bien des possibilités d'altération de l'acide thymonucléique hautement polymérisé, tant par voie enzymatique (thymonucléase bactérienne) que par voie purement chimique (en particulier action des acides et des alcalis). AVERY et ses collaborateurs ont rencontré des difficultés du même ordre dans le cas du pneumocoque, mais ils ont pu les tourner en recourant à une extraction des germes par le désoxycholate de sodium. Il semble beaucoup moins aisé d'obtenir les nucléoprotéines du colibacille et même le procédé par autolyse échoue totalement ou presque totalement avec la grande majorité des germes: ou bien on n'extrait à peu près rien, ou bien les enzymes dégradent complètement l'acide nucléique au fur et à mesure de sa libération. Dans les cas les plus favorables (C_1 par exemple), les pertes en acide nucléique hautement polymérisé sont certainement énormes et sans doute faut-il attribuer à ce fait l'activité relativement basse de nos préparations du principe inducteur nucléique de C_1: activité limitée à des dilutions de quelques centaines de *milliers* de fois, alors que les préparations homologues d'AVERY se montrent agissantes jusqu'à des dilutions atteignant plusieurs centaines de *millions* de fois. Nous ajouterons que, chez le colibacille, le principe actif paraît nettement moins sensible aux enzymes bactériens et à l'acidité tant qu'il est encore en combinaisons protéiques.

[1] C_2 smooth passe très facilement à l'état C_2 rough, dans les cultures usuelles sur bouillon et sur gélose, en donnant lieu à de nombreuses variantes quant à l'aspect des colonies. Dans les mêmes conditions le passage de C_1 smooth à C_1 rough est assez fréquent, mais ne présente pas le spectre complexe de variantes auquel donne lieu C_2.

Nous nous sommes posé la question de savoir si quelque relation de caractère statistique ne pourrait pas être établie entre la concentration du milieu de culture en principe nucléique inducteur et la fréquence d'apparition du mutant. Nous poursuivons l'étude du problème, mais nous nous heurtons à une grosse difficulté pratique: la forme rough originelle pousse en dépôt cohérent au fond des tubes, même sur les milieux non salés, alors que le mutant smooth donne lieu à une culture diffuse envahissant le milieu. Comment, dans ces conditions, établir avec quelque exactitude et par l'artifice des repiquages sur gélose (colonies isolées) les proportions respectives des deux formes ? Ajoutons enfin que dans le cas du colibacille, la transformation est capable de s'opérer encore au sein des cultures en bouillon peptoné usuel; l'adjonction de sérum sanguin ne paraît pas présenter l'utilité fondamentale qu'elle revêt dans le cas du pneumocoque.

En résumé, nous apportons, nous semble-t-il, la preuve d'une *possibilité* de mutation dirigée sous l'action d'un principe thymonucléique, chez les colibacilles[1]. Mais la manifestation du phénomène demeure fort contingente par suite, tout à la fois, de l'inégale faculté de mutation des divers germes et de la grande imperfection de nos techniques actuelles. Venant s'ajouter aux remarquables observations sur le pneumocoque qui se sont échelonnées des travaux de GRIFFITH à ceux d'AVERY et ses collaborateurs, nos observations sur le colibacille contribuent à démontrer, de façon plus générale, la réalité même des mutations chez les bactéries — êtres dépourvus de sexualité — réalité qui ne peut plus être mise en doute dans le cas des microorganismes plus élevés en organisation et doués de sexualité, depuis les belles études récentes de BEADLE et TATUM sur la moisissure *Neurospora* et de SPIEGELMAN et LINDEGREN sur la levure.

ANDRÉ BOIVIN, ALBERT DELAUNAY,
ROGER VENDRELY et YVONNE LEHOULT

Institut Pasteur (Paris–Garches), le 6 mars 1946.

Summary

The transformation of antigenic and enzymatic properties of a bacteria can be produced by the action of an inductor thymonucleic factor, which comes from a related bacteria. However, this transformation is only possible when the bacteria which must undergo the "mutation dirigée" is in a state of pronounced instability, the determinism of which is still unknown. We find to-day the greatest difficulties to isolate, without destroying it, the inductor factor, because it is very sensitive to bacterial enzyms and to the delayed action of acids and bases.

[1] Seul l'acide nucléique provenant du germe inducteur se montre actif (acide nucléique de C_1 dans le cas du passage de C_2 à C_1). Toutes les tentatives faites pour utiliser d'autres acides nucléiques d'origine bactérienne (bactéries non apparentées), végétale ou animale, ont échoué.

Einfluß antianaphylaktisch wirkender Stoffe auf die Antikörperproduktion

Um Antiseren von möglichst hohem Titer zu gewinnen, ist es erforderlich, den Tieren das Antigen zu wiederholten Malen und in geeigneten zeitlichen Intervallen zu injizieren. Durch solche Reinjektionen kann die Antikörperbildung vermehrt angeregt werden. Leider

suite la chute est brusque et à la 25e h (blastopore en petit croissant) les réactions sont de nouveau nulles.

A ces différences statistiques correspondent également des différences dans la nature des réactions.

Au début, vers la 17e h, les formations secondaires sont intimement associées à l'embryon primaire; ce sont des vésicules cérébrales supplémentaires, une multiplication des otocystes en position orthotopique, des noyaux chordaux inclus dans le cerveau, ou même parfois dans l'organe adhésif. Ces noyaux de chorde sont toujours nettement arrondis, sans tendance à l'élongation ni gradient de différenciation. On peut leur attribuer un caractère nuqual.

Vers la 19e h, on constate une plus grande indépendance des formations secondaires. Celles-ci se placent à un endroit quelconque de l'embryon, de préférence sur la paroi ventrale. Leur extrémité antérieure se confond en avant avec le cerveau primaire. Mais on observe de plus un axe nettement troncal, possédant de la moelle, de la chorde ayant une nette tendance à l'élongation et un gradient de différenciation, parfois des somites. Les otocystes, très abondants, peuvent se trouver à n'importe quel endroit de l'embryon. Yeux et nez secondaires sont toutefois exceptionnels.

Cette indépendance des formations secondaires se précise à la 21e h. L'hétérotopie peut être telle que l'on rencontre à l'extrémité antérieure de l'hôte, formant avec la tête de celui-ci les combinaisons les plus étranges, des formations purement troncales. La proportion des somites croît sensiblement; dans quelques cas on observe des pronéphros secondaires; ceux-ci toutefois n'apparaissent qu'au niveau du pronéphros primaire.

Après ce stade optimal, les formations secondaires gardent leur caractère hétérotopique mais décroissant en importance: chorde, somites, pronéphros disparaissent rapidement et le névraxe fait place à des formations *ganglionnaires, du mésenchyme inducteur* de nageoire, des otocystes.

Il est à remarquer que dans l'ensemble des expériences, les organes sensoriels prosencéphaliques ne sont apparus que de façon exceptionnelle. A ce point de vue seulement, nos résultats diffèrent des inductions obtenues à partir de morceaux d'organes adultes. Sinon, les ressemblances sont assez frappantes, d'autant plus que les variations obtenues en fonction des stades centrifugés montrent un grand parallélisme avec les séries obtenues par CHUANG[1], (1939, 1940) au fur et à mesure de l'épuisement de l'inducteur par l'eau chaude. Cet auteur en concluait à l'existence de substances inductrices spécifiques pour les divers organes.

Cette hypothèse rencontrerait dans notre cas des difficultés singulières. Il est possible de trouver une explication plus satisfaisante en faisant intervenir la notion bien connue mais trop souvent oubliée de la compétence limitée dans le temps de l'ectoblaste.

L'évolution du métabolisme nucléinique qui est apparue dans notre travail en collaboration avec J. BRACHET doit demander un certain temps de latence avant que l'élevation du potentiel morphogénétique puisse se réaliser. Si le terme de cette évolution correspond au moment où l'ectoblaste est en période de pleine compétence, les réactions seront maximales. Si, à la suite d'une centrifugation plus précoce ou plus tardive, ce terme correspond à une compétence limitée ou nulle, les réactions seront limitées ou nulles. Dans ce cas, la *nature* des inductions ne serait pas fonction de substances spécifiques mais de l'état de compétence de l'ectoblaste. Cette hypothèse qui devra être expérimentalement vérifiée est rendue plausible par des expériences de HOLTFRETER[1] (1938), dont les résultats montrent de fortes analogies avec au moins une partie des nôtres.

Le manque de spécificité de l'influx inducteur ressort également du mode d'interaction mutuelle des systèmes primaire et secondaire, ce qui fera l'objet d'une note ultérieure.

Conclusions: 1) La centrifugation de la blastula ou de la gastrula des Amphibiens provoque au sein de l'ectoblaste, indépendamment de toute intervention de l'organisateur, l'apparition de complexes d'organes ecto- et mésoblastiques.

2) La région où se manifeste cette augmentation du potentiel morphogénétique se caractérise par la présence d'acides ribonucléiques en forte concentration (travail en collaboration avec J. BRACHET).

3) Le nombre des formations secondaires, leur nature, leur localisation dépend du stade où se fait la centrifugation. Il est supposé que la nature des organes formés est fonction de la compétence de l'ectoblaste au moment où se produit la réaction déclanchée par le traumatisme cellulaire.

J. PASTEELS

Laboratoire d'embryologie, Faculté de médecine de l'Université de Bruxelles, le 30 novembre 1946.

Summary

The centrifugation of the Amphibia blastula or gastrula produces in the ectoblastic area the appearance of complexes of ecto- and mesoblastic organs. Experiments of explantations and cultivation *in vitro* and also of grafts on a normal embryo demonstrate that those very abnormal secundary embryos are formed without any intervention of the normal organizer. The ectoblastic cells that undergo this increase of morphogenetic potential are characterized by a great concentration of ribonucleic acid in their cytoplasm (this part of work in collaboration with J. BRACHET). Those secundary embryos vary greatly in their frequence, their nature, and their location, depending on the stage in which the centrifugation was done. It is supposed that the nature of the organs formed is dependent on the degree of potency attained by the ectoblast at the time when the reaction arises that is produced by the cellular trauma.

[1] J. HOLTFRETER, Arch. Entw. Mech. *138*, 163–196 (1938).

[1] H. H. CHUANG, Arch. Entw. Mech. *139*, 556–638 (1939); *140*, 25–38 (1940).

Sur le rôle possible des deux acides nucléiques dans la cellule vivante

Les travaux d'AVERY, MACLEOD et MCCARTY[1], ainsi que nos recherches personnelles[2], sur le phénomène des mutations «dirigées» chez les bactéries, rendent très vraisemblables non seulement l'existence d'acides désoxyribonucléiques différents d'un germe à l'autre, mais encore la présence simultanée, dans un même germe, de plusieurs acides désoxyribonucléiques distincts, dont chacun serait capable d'exercer une action

[1] AVERY, MACLEOD et MCCARTY, J. exper. Med. *79*, 137 (1944); *81*, 501 (1945); *83*, 89, 97 (1946).

[2] BOIVIN et VENDRELY, Exper. *1*, 334 (1945); *2*, 139 (1946); Helv. chim. acta *29*, 1338 (1946).

inductrice propre. D'autre part, il y a actuellement tout lieu de penser que la cellule bactérienne possède des gènes (TATUM et coll.[1]) se groupant dans un vrai noyau, dont ROBINOW[2] vient d'obtenir de magnifiques photographies. Cela conduit à envisager chaque gène bactérien comme n'étant rien d'autre qu'une macromolécule particulière d'acide désoxyribonucléique et il est tout naturel d'étendre cette conception aux cellules végétales et animales. Ainsi, toutes les cellules d'un être renfermeraient, dans leur noyau, la même collection de macromolécules désoxyribonucléiques dépositaires des caractères génotypiques.

Dès qu'on songe à l'étroite parenté de constitution chimique des deux acides nucléiques, il devient logique d'admettre — par raison d'analogie et jusqu'à preuve formelle du contraire — l'existence d'acides ribonucléiques différents les uns des autres, et de leur attribuer, dans la cellule, des fonctions rappelant celles des acides désoxyribonucléiques. Dans le cas des acides ribonucléiques comme dans celui des acides désoxyribonucléiques (et par analogie avec ce qu'on admet pour les protéines), on peut envisager la possibilité de différences intéressant soit la structure «primaire» (constitution), soit plutôt la structure «secondaire» (état de plissement ou de pelotonnement) de la chaîne polynucléotidique.

Depuis les travaux de CASPERSSON et depuis ceux de BRACHET, on connaît le rapport qui existe entre la richesse du cytoplasme d'une cellule en acide ribonucléique et la capacité de synthèse de cette cellule à l'égard des protéines. CLAUDE, puis STERN, puis BRACHET et JEENER ont montré que l'acide ribonucléique entre dans la composition de minuscules granules cytoplasmiques (les microsomes de CLAUDE, d'un diamètre de l'ordre de 100 mμ); d'après BRACHET, ces organites renferment toute une série d'enzymes et ils représenteraient le lieu essentiel des synthèses cellulaires, particulièrement le lieu de la synthèse des protéines[3]. Les levûres renferment, elles aussi, de pareils granules (BRACHET). Dans le cas des bactéries, on voit parfois des granulations «métachromatiques» à base d'acide ribonucléique, mais la cellule est toujours extrêmement riche en acide ribonucléique, dont la situation intracytoplasmique ne paraît guère faire de doute (VENDRELY[4]).

Mais alors, la nature des macromolécules ribonucléiques présentes dans le cytoplasme de chaque type cellulaire ne conditionnerait-elle pas les caractères propres à ce type et tout particulièrement son équipement enzymatique? L'hypothèse paraît tentante et voici quelle forme il semble possible de lui donner, dans l'état présent de nos connaissances.

Dans le noyau de chaque cellule d'un être vivant existerait un centre directeur, identique pour toutes les cellules de cet être, et représenté par l'ensemble des gènes désoxyribonucléiques dépositaires des caractères généraux de l'espèce. Dans le cytoplasme des diverses cellules se rencontreraient des centres directeurs secondaires, constitués par des acides ribonucléiques, et qui varieraient de type cellulaire à type cellulaire dans le détail de leur composition. Ces centres secondaires tiendraient sous leur contrôle immédiat l'équipement enzymatique répondant à chaque type cellulaire et déterminant, en dernière analyse, l'ensemble des caractères de ce type. Par leurs modifications de constitution, ils conditionneraient les processus de différenciation progressive et de spécialisation des diverses cellules d'un même être à partir de la cellule-œuf initiale, cellule-œuf douée de polarité et présentant des gradients qui pourraient s'inscrire dans l'hétérogénéité de ses éléments ribonucléiques cytoplasmiques. Les gènes devraient nécessairement agir, en quelque manière, sur les microsomes; la chose pourrait se produire d'une part en mode permanent, à travers la membrane nucléaire, et d'autre part (d'une façon particulièrement active), au moment de la mitose, alors que la membrane nucléaire s'efface, que les chromosomes se dispersent dans le cytoplasme et que le nucléole, riche en acide *ribonucléique*, disparaît pour se dissoudre en quelque sorte dans ce même cytoplasme. Mais en l'état actuel de nos connaissances, tenter de préciser davantage reviendrait à quitter le domaine de la vraisemblance pour s'égarer dans celui de l'hypothèse purement gratuite.

Remarquons aussitôt que les macromolécules ribonucléiques ne sauraient, *à elles-seules*, définir de façon absolument complète l'équipement enzymatique des cellules où elles figurent. En effet, une «adaptation enzymatique» peut se produire, chez les bactéries comme chez les levûres et probablement — à un degré plus ou moins marqué — chez toutes les cellules: sur le double plan qualitatif et quantitatif, les biocatalyseurs présents dans une cellule dépendent de la nature et de la concentration des divers substrats qu'on offre à cette cellule (travaux classiques de KARSTRÖM et ceux de nombreux autres auteurs). D'autre part, de bien remarquables observations de SPIEGELMAN et LINDEGREN[1] ont révélé la possibilité, pour un enzyme, de se *multiplier* autocalytiquement au sein du cytoplasme, en l'absence du gène en cause et sous la seule influence du substrat correspondant. Ajoutons enfin que la teneur d'un élément en acides ribonucléiques totaux ne saurait prendre la valeur d'une constante cellulaire, puisqu'on voit ces substances augmenter beaucoup chez les levûres (CASPERSSON et coll., BRACHET et coll.) et chez les bactéries (BOIVIN et VENDRELY[2], CASPERSSON[3] et coll.), lorsqu'on fait passer les micro-organismes de l'état de repos à l'état d'intense multiplication.

Des observations toutes récentes de SPIEGELMAN[4] viennent parler en faveur de notre hypothèse concernant le rôle des acides ribonucléiques et leur multiplicité. En effet, cet auteur est parvenu à accélérer *spécifiquement* l'adaptation enzymatique d'une levure à un sucre, par action d'une fraction nucléoprotéidique retirée de la même levure préalablement adaptée à ce sucre. La technique de préparation de l'extrait (emploi du bicarbonate de sodium) laisse deviner l'intervention des seuls acides ribonucléiques.

Bien des points demeurent obscurs dans la théorie que nous venons d'esquisser. En particulier, on ne saurait préciser actuellement le mécanisme selon lequel les gènes désoxyribonucléiques peuvent commander l'édification des acides ribonucléiques cytoplasmiques et le mécanisme selon lequel ces acides ribonucléiques peuvent présider à l'élaboration des enzymes; sans

[1] TATUM et coll., Proc. nat. Acad. Sci. U.S.A. *30*, 404 (1944); *31*, 215 (1945); Nature *158*, 558 (1946).

[2] ROBINOW, Proc. roy. Soc. London B *130*, 299 (1942); J. Hyg. *43*, 413 (1944); *Addendum*, par ROBINOW, au livre de DUBOS: The bacterial Cell (1 vol., 1945).

[3] Voir, en particulier, BRACHET, Embryologie chimique (1 vol., 1945).

[4] VENDRELY, C. r. Acad. Sci. *222*, 1357 (1946); *223*, 342 (1946).

[1] SPIEGELMAN et LINDEGREN, Proc. nat. Acad. Sci. U.S.A. *31*, 95 (1945); Bact. Rev. *9*, 111 (1945).

[2] BOIVIN et VENDRELY, C. r. Soc. Biol. *137*, 432 (1943).

[3] CASPERSSON et coll., Nordisk Medicin *28*, 2636 (1945).

[4] SPIEGELMAN, Cold Spring Harbor Symp. quant. Biol. *11* (1946), sous presse.

3 Exper.

doute s'agit-il ici et là d'actions d'ordre catalytique... mais que dire de plus? Aussi bien, et malgré les ingénieuses explications proposées par Yudkin[1], par Lindegren[2] et par Spiegelman[3], il serait bien scabreux d'affirmer que tout est clair dans l'effet stimulant des substrats sur cette même élaboration des enzymes; tout au plus peut-on soupçonner quelque intervention de la loi d'action de masse... Quoiqu'il en soit, la théorie que nous venons de formuler nous paraît mériter de retenir — au moins à titre de possibilité — l'attention du biologiste, dans la rude tâche qui lui incombe de démêler l'écheveau des processus moléculaires responsables, en dernière analyse, de cette prodigieuse réalité qu'est l'organisation.

André Boivin et Roger Vendrely

Institut de Chimie biologique de la Faculté de Médecine de Strasbourg[4], le 15 novembre 1946.

Summary

According to the writer's theory a great number of different desoxyribonucleic and ribonucleic acids exist in each cell: desoxyribonucleic acids in the nucleus (genes) and ribonucleic acids in the cytoplasm (microsomes). Through catalytic actions the macromolecular desoxyribonucleic acids govern the building of macromolecular ribonucleic acids, and, in turn, these control the production of cytoplasmic enzymes. In truth, the enzymic equipment results simultaneously from the effect of ribonucleic acids (catalytic action) and from the effect of substrates (mass action). This hypothesis explains cellular differentiation (multicellular organism) through constitutional variations of cytoplasmic ribonucleic acids. The writer's fundamental arguments come from the study of bacterial biology, especially from the study of mutations directed by principles of desoxyribonucleic nature.

[1] Yudkin, Biol. Rev. *13*, 93 (1938).
[2] Lindegren, Proc. nat. Acad. Sci. U.S.A. *32*, 68 (1946).
[3] Spiegelman, Cold Spring Harbor Symp. quant. Biol. *11* (1946), sous presse.
[4] Antérieurement: Institut Pasteur, Garches-Paris.

Über die Auslösung der Fluchtreaktion bei Fliegen

In seinem «Grundriß der vergleichenden Physiologie» schreibt v. Buddenbrock[1] (S. 151): «Will man eine auf dem Tisch sitzende Stubenfliege mit der Hand greifen, so fliegt sie weg. Dies hat man stets auf den Gesichtssinn der Fliege bezogen, aber Gaffron wies nach, daß die Fliege nicht auf das Herannahen der Hand reagiert, wenn sie sich unter einer Glasglocke befindet. Wahrscheinlich muß also eine mechanische Luftbewegung dem optischen Reize sich zugesellen.» Diese Mitteilung veranlaßte mich, der Sache nachzugehen. Gaffron[2] beschreibt ihre diesbezüglichen Beobachtungen folgendermaßen (S. 310): «Bewegt man einen optisch ausgezeichneten Gegenstand an einem Glasbehälter mit Fliegen vorbei, so löst das nicht die geringste Reaktion aus. Auch auf eine rasche Handbewegung, als ob man sie an der Glasscheibe fangen wollte, zeigen sie keine Fluchtreaktion. Da das übliche Fliegenfangen sehr vorsichtige Bewegungen verlangt, liegt der Verdacht nahe, daß die Tiere wesentlich auf die beim Fang entstehenden Luftbewegungen reagieren.»

Es sollte nun geprüft werden, ob sich eine solche Reaktion auf Luftbewegungen und somit eine Art «Ferntastsinn» bei Fliegen tatsächlich nachweisen läßt.

Zur Wiederholung des Gaffronschen Versuches fing ich eine Schmeißfliege (*Calliphora erythrocephala*) und brachte sie unter eine Glashaube (umgekehrtes, rechteckiges Vollglasaquarium). Es zeigte sich, daß das Tier bei rascher Annäherung meiner Hand (Fangbewegung) in den meisten Fällen prompt durch Auffliegen reagierte. Das Glas wurde beim «Fangen» nicht berührt. Der Versuch wurde an anderen Exemplaren der gleichen Art und an der Stubenfliege (*Musca domestica*) mit gleichem Erfolg wiederholt. Eine an der Außenseite meines Zimmerfensters (einer dicken, unbeweglichen Glasscheibe) sitzende Fliege flog beim Herannahen meiner Hand von innen her ebenfalls prompt davon. Reizung durch Luftbewegungen ist in all diesen Fällen infolge der zwischengeschalteten Glaswand ausgeschlossen. Die Fluchtreaktion wurde also rein optisch ausgelöst.

Das negative Ergebnis des gleichen Versuches bei Gaffron beruht wohl hauptsächlich darauf, daß ihre Fliegen zu zahm bzw. zu sehr an die Versuchssituation und Reizung gewöhnt waren. Sie verwendete vermutlich gezüchtetes Material, ich stets frisch gefangene Fliegen. Ferner wäre zu bedenken, daß die Annäherung eines Gegenstandes rein von der Ventralseite her eine mehr oder weniger künstliche Reizsituation darstellt; eine glasartig durchsichtige Unterlage spielt in der natürlichen Fliegenumwelt wohl kaum eine Rolle. Deshalb wartete ich bei meinen Versuchen vorzugsweise bis die herumlaufende Fliege sich einer Kante des Aquariums genähert hatte, so daß mein Angriff von vorne oder von der Seite her erfolgen konnte.

Nachdem nun feststand, daß optische Reize allein die Fluchtreaktion auslösen können, blieb noch die Frage zu entscheiden, ob daneben auch eine Wahrnehmung von Luftbewegungen im Sinne Gaffrons von Einfluß sein kann. Ich fing dazu wiederum eine Schmeißfliege, betäubte sie leicht mit Äther und bedeckte die gesamte Oberfläche ihrer beiden Facettenaugen unter dem Binokular mit einem frisch hergestellten, schnelltrocknenden Brei aus Ruß und Firnis. Bei Verwendung der richtigen Mischung ergibt sich so ein vollkommener, lichtdichter Verschluß, der so gut haftet, daß ihn die Fliege nach dem Erwachen aus der Narkose nicht ablösen kann. Die übrigen Sinnesorgane des Kopfes (Ozellen, Fühler usw.) blieben unberührt. — Nach völliger Erholung von der Narkose flog die geblendete Fliege oft längere Zeit herum. Der Flug erfolgte etwas langsamer und war weniger zielgerichtet als zuvor; er erinnerte in seiner schwerfälligen Art etwa an den Flug einer Hummel. Das Tier verstand es noch überraschend gut, an den Zimmerwänden und anderen Gegenständen zu landen.

Es zeigte sich, daß die blinde Fliege weder auf dem Tisch sitzend noch fliegend in irgendwelcher Weise auf Fangbewegungen reagierte. Im Fluge konnte sie z. B. ohne Mühe mit der Hand gefangen werden. Auch stillsitzend reagierte sie auf normale Fangbewegungen gar nicht mehr. Sogar wenn man mit der Hand einen kräftigen Schlag dicht an der Fliege vorbei führte, so daß sie von einem starken Luftstoß getroffen und passiv bewegt wurde, duckte sie sich nur ein wenig zusammen, wie es auch normale Fliegen tun, wenn man sie anbläst.

Daß das Ausbleiben einer Reaktion nach der Blen-

[1] W. v. Buddenbrock, Grundriß der vergl. Physiol., 2. Aufl., 1. Bd., Berlin 1937.
[2] M. Gaffron, Z. vergl. Physiol. *20* (1934).

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Chemical Specificity of Nucleic Acids and Mechanism of their Enzymatic Degradation[1]

By ERWIN CHARGAFF[2], New York, N.Y.

I. *Introduction*

The last few years have witnessed an enormous revival in interest for the chemical and biological properties of nucleic acids, which are components essential for the life of all cells. This is not particularly surprising, as the chemistry of nucleic acids represents one of the remaining major unsolved problems in biochemistry. It is not easy to say what provided the impulse for this rather sudden rebirth. Was it the fundamental work of E. HAMMARSTEN[3] on the highly polymerized desoxyribonucleic acid of calf thymus? Or did it come from the biological side, for instance the experiments of BRACHET[4] and CASPERSSON[5]? Or was it the very important research of AVERY[6] and his collaborators on the transformation of pneumococcal types that started the avalanche?

It is, of course, completely senseless to formulate a hierarchy of cellular constituents and to single out certain compounds as more important than others. The economy of the living cell probably knows no conspicuous waste; proteins and nucleic acids, lipids and polysaccharides, all have the same importance. But one observation may be offered. It is impossible to write the history of the cell without considering its geography; and we cannot do this without attention to what may be called the chronology of the cell, i. e. the sequence in which the cellular constituents are laid down and in which they develop from each other. If this is done, nucleic acids will be found pretty much at the beginning. An attempt to say more leads directly into empty speculations in which almost no field abounds more than the chemistry of the cell. Since an ounze of proof still weighs more than a pound of prediction, the important genetical functions, ascribed —probably quite rightly—to the nucleic acids by many workers, will not be discussed here. Terms such as "template" or "matrix" or "reduplication" will not be found in this lecture.

II. *Identity and Diversity in High Molecular Cell Constituents*

The determination of the constitution of a complicated compound, composed of many molecules of a number of organic substances, evidently requires the exact knowledge of the nature and proportion of all constituents. This is true for nucleic acids as much as for proteins or polysaccharides. It is, furthermore, clear that the value of such constitutional determinations will depend upon the development of suitable methods of hydrolysis. Otherwise, substances representing an association of many chemical individuals can be described in a qualitative fashion only; precise decisions as to structure remain impossible. When our laboratory, more than four years ago, embarked upon the study of nucleic acids, we became aware of this difficulty immediately.

The state of the nucleic acid problem at that time found its classical expression in LEVENE's monograph[1]. (A number of shorter reviews, indicative of the development of our conceptions concerning the chemistry of nucleic acids, should also be mentioned[2].) The old tetranucleotide hypothesis—it should never have been called a theory—was still dominant; and this was characteristic of the enormous sway that the organic chemistry of small molecules held over biochemistry. I should like to illustrate what I mean by one example. If in the investigation of a disaccharide consisting of two different hexoses we isolate 0·8 mole of one sugar and 0·7 mole of the other, this will be sufficient for the

[1] This article is based on a series of lectures given before the Chemical Societies of Zürich and Basle (June 29th and 30th, 1949), the Société de chimie biologique at Paris, and the Universities of Uppsala, Stockholm, and Milan.

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[3] E. HAMMARSTEN, Biochem. Z. *144*, 383 (1924).

[4] J. BRACHET in *Nucleic Acid*, Symposia Soc. Exp. Biol. No. 1 (Cambridge University Press, 1947), p. 207. Cp. J. BRACHET, in *Nucleic Acids and Nucleoproteins*, Cold Spring Harbor Symp. Quant. Biol. *12*, 18. (Cold Spring Harbor, N.Y., 1947).

[5] T. CASPERSSON, in *Nucleic Acid*, Symp. Soc. Exp. Biol., No. 1 (Cambridge University Press, 1947), p. 127.

[6] O. T. AVERY, C. M. MACLEOD, and M. MCCARTY, J. Exp. Med. *79*, 137 (1944).

[1] P. A. LEVENE and L. W. BASS, Nucleic Acids (Chemical Catalog Co., New York, 1931).

[2] H. BREDERECK, Fortschritte der Chemie organischer Naturstoffe *1*, 121 (1938). – F. G. FISCHER, Naturwissensch. *30*, 377 (1942). – R. S. TIPSON, Adv. Carbohydrate Chem. *1*, 193 (1945). – J. M. GULLAND, G. R. BARKER, and D. O. JORDAN, Ann. Rev. Biochem. *14*, 175 (1945). – E. CHARGAFF and E. VISCHER, Ann. Rev. Biochem. *17*, 201 (1948). – F. SCHLENK, Adv. Enzymol. *9*, 455 (1949).

recognition of the composition of the substance, provided its molecular weight is known. The deviation of the analytical results from simple, integral proportions is without importance in that case. But this will not hold for high-molecular compounds in which variations in the proportions of their several components often will provide the sole indication of the occurrence of different compounds.

In attempting to formulate the problem with some exaggeration one could say: The validity of the identification of a substance by the methods of classical organic chemistry ends with the mixed melting point. When we deal with the extremely complex compounds of cellular origin, such as nucleic acids, proteins, or polysaccharides, a chemical comparison aiming at the determination of identity or difference must be based on the nature and the proportions of their constituents, on the sequence in which these constituents are arranged in the molecule, and on the type and the position of the linkages that hold them together. The smaller the number of components of such a high-molecular compound is, the greater is the difficulty of a decision. The occurrence of a very large number of different proteins was recognized early; no one to my knowledge ever attempted to postulate a protein as a compound composed of equimolar proportions of 18 or 20 different amino acids. In addition, immunological investigations contributed very much to the recognition of the multiplicity of proteins. A decision between identity and difference becomes much more difficult when, as is the case with the nucleic acids, only few primary components are encountered. And when we finally come to high polymers, consisting of one component only, e. g. glycogen or starch, the characterization of the chemical specificity of such a compound becomes a very complicated and laborious task.

While, therefore, the formulation of the tetranucleotide conception appeared explainable on historical grounds, it lacked an adequate experimental basis, especially as regards "thymonucleic acid". Although only two nucleic acids, the desoxyribose nucleic acid of calf thymus and the ribose nucleic acid of yeast, had been examined analytically in some detail, all conclusions derived from the study of these substances were immediately extended to the entire realm of nature; a jump of a boldness that should astound a circus acrobat. This went so far that in some publications the starting material for the so-called "thymonucleic acid" was not even mentioned or that it was not thymus at all, as may sometimes be gathered from the context, but, for instance, fish sperm or spleen. The animal species that had furnished the starting material often remained unspecified.

Now the question arises: How different must complicated substances be, before we can recognize their difference? In the multiformity of its appearances nature can be primitive and it can be subtle. It is primitive in creating in a cell, such as the tubercle bacillus, a host of novel compounds, new fatty acids, alcohols, etc., that are nowhere else encountered. There, the recognition of chemical peculiarities is relatively easy. But in the case of the proteins and nucleic acids, I believe, nature has acted most subtly; and the task facing us is much more difficult. There is nothing more dangerous in the natural sciences than to look for harmony, order, regularity, before the proper level is reached. The harmony of cellular life may well appear chaotic to us. The disgust for the amorphous, the ostensibly anomalous—an interesting problem in the psychology of science—has produced many theories that shrank gradually to hypotheses and then vanished.

We must realize that minute changes in the nucleic acid, e. g. the disappearance of one guanine molecule out of a hundred, could produce far-reaching changes in the geometry of the conjugated nucleoprotein; and it is not impossible that rearrangements of this type are among the causes of the occurrence of mutations[1].

The molecular weight of the pentose nucleic acids, especially of those from animal tissue cells, is not yet known; and the problem of their preparation and homogeneity still is in a very sad state. But that the desoxypentose nucleic acids, prepared under as mild conditions as possible and with the avoidance of enzymatic degradation, represent fibrous structures of high molecular weight, has often been demonstrated. No agreement has as yet been achieved on the order of magnitude of the molecular weight, since the interpretation of physical measurements of largely asymmetric molecules still presents very great difficulties. But regardless of whether the desoxyribonucleic acid of calf thymus is considered as consisting of elementary units of about 35,000 which tend to associate to larger structures[2] or whether it is regarded as a true macromolecule of a molecular weight around 820,000[3], the fact remains that the desoxypentose nucleic acids are high-molecular substances which in size resemble, or even surpass, the proteins. It is quite possible that there exists a critical range of molecular weights above which two different cells will prove unable to synthesize completely identical substances. The enormous number of diverse proteins may be cited as an example. *Duo non faciunt idem* is, with respect to cellular chemistry, perhaps an improved version of the old proverb.

III. *Purpose*

We started in our work from the assumption that the nucleic acids were complicated and intricate high-

[1] For additional remarks on this problem, compare E. CHARGAFF, in *Nucleic Acids and Nucleoproteins*, Cold Spring Harbor Symp. Quant. Biol., *12*, 28 (Cold Spring Harbor, N.Y., 1947).

[2] E. HAMMARSTEN, Acta med. Scand., Suppl. *196*, 634 (1947). – G. JUNGNER, I. JUNGNER, and L.-G. ALLGÉN, Nature *163*, 849 (1949).

[3] R. CECIL and A. G. OGSTON, J. Chem. Soc. 1382 (1948).

polymers, comparable in this respect to the proteins, and that the determination of their structures and their structural differences would require the development of methods suitable for the precise analysis of all constituents of nucleic acids prepared from a large number of different cell types. These methods had to permit the study of minute amounts, since it was clear that much of the material would not be readily available. The procedures developed in our laboratory make it indeed possible to perform a complete constituent analysis on 2 to 3 mg of nucleic acid, and this in six parallel determinations.

The basis of the procedure is the partition chromatography on filter paper. When we started our experiments, only the qualitative application to amino acids was known[1]. But it was obvious that the high and specific absorption in the ultraviolet of the purines and pyrimidines could form the basis of a quantitative ultra-micro method, if proper procedures for the hydrolysis of the nucleic acids and for the sharp separation of the hydrolysis products could be found.

IV. *Preparation of the Analytical Material*

If preparations of desoxypentose nucleic acids are to be subjected to a structural analysis, the extent of their contamination with pentose nucleic acid must not exceed 2 to 3%. The reason will later be made clearer; but I should like to mention here that all desoxypentose nucleic acids of animal origin studied by us so far were invariably found to contain much more adenine than guanine. The reverse appears to be true for the animal pentose nucleic acids: in them guanine preponderates. A mixture of approximately equal parts of both nucleic acids from the same tissue, therefore, would yield analytical figures that would correspond, at least as regards the purines, to roughly equimolar proportions. Should the complete purification—sometimes an extremely difficult task—prove impossible in certain cases, one could think of subjecting preparations of both types of nucleic acid from the same tissue specimen to analysis and of correcting the respective results in this manner. This, however, is an undesirable device and was employed only in some of the preparations from liver which will be mentioned later.

It is, furthermore, essential that the isolation of the nucleic acids be conducted in such a manner as to exclude their degradation by enzymes, acid or alkali. In order to inhibit the desoxyribonucleases which require magnesium[2], the preparation of the desoxypentose nucleic acids was carried out in the presence of citrate ions[3]. It would take us here too far to describe in detail the methods employed in our laboratory for the preparation of the desoxypentose nucleic acids from animal tissues. They represent in general a combination of many procedures, as described recently for the isolation of yeast desoxyribonucleic acid[1]. In this manner, the desoxypentose nucleic acids of thymus, spleen, liver, and also yeast were prepared. The corresponding compound from tubercle bacilli was isolated *via* the nucleoprotein[2]. The procedures leading to the preparation of desoxypentose nucleic acid from human sperm will soon be published[3]. All desoxypentose nucleic acids used in the analytical studies were prepared as the sodium salts (in one case the potassium salt was used); they were free of protein, highly polymerized, and formed extremely viscous solutions in water. They were homogeneous electrophoretically and showed a high degree of monodispersity in the ultracentrifuge.

The procedure for the preparation of pentose nucleic acids from animal tissues resembled, in its first stages, the method of CLARKE and SCHRYVER[4]. The details of the isolation procedures and related experiments on yeast ribonucleic acid are as yet unpublished. Commercial preparations of yeast ribonucleic acid also were examined following purification. As has been mentioned before, the entire problem of the preparation and homogeneity of the pentose nucleic acids, and even of the occurrence of only one type of pentose nucleic acid in the cell, urgently requires re-examination.

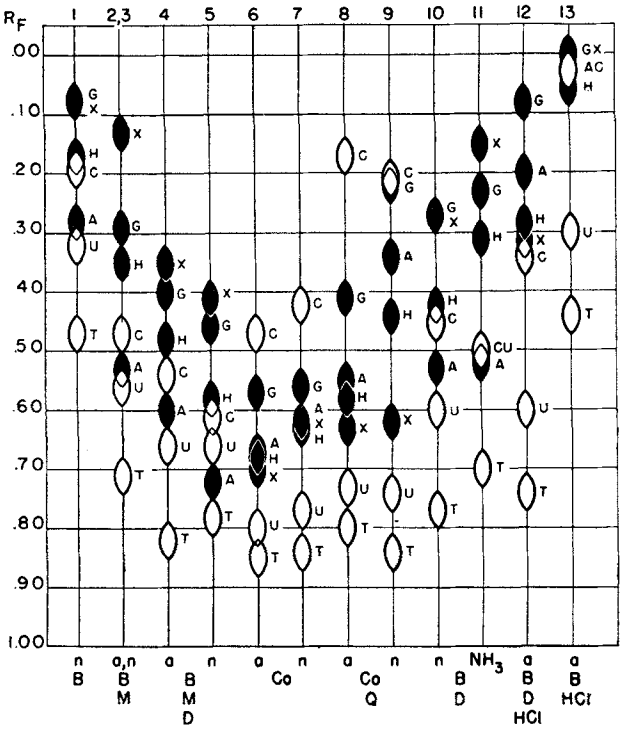
V. *Separation and Estimation of Purines and Pyrimidines*

Owing to the very unpleasant solubility and polar characteristics of the purines, the discovery of suitable solvent systems and the development of methods for their quantitative separation and estimation[5] presented a rather difficult problem in the solution of which Dr. ERNST VISCHER had an outstanding part. The pyrimidines proved somewhat easier to handle. The choice of the solvent system for the chromatographic separation of purines and pyrimidines will, of course, vary with the particular problem. The efficiency of different solvent systems in effecting separation is illustrated schematically in Fig. 1. Two of the solvent systems listed there are suitable for the separation of the purines found in nucleic acids, i. e. adenine and guanine, namely (1) n-butanol, morpholine, diethylene glycol, water (column 5 in Fig. 1); and (2) n-butanol, diethylene glycol, water in a NH_3 atmosphere (column 11). The second system listed proved particularly

[1] R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. *38*, 224 (1944).
[2] F. G. FISCHER, I. BÖTTGER, and H. LEHMANN-ECHTERNACHT, Z. physiol. Chem. *271*, 246 (1941).
[3] M. MCCARTY, J. Gen. Physiol. *29*, 123 (1946).
[1] E. CHARGAFF and S. ZAMENHOF, J. Biol. Chem. *173*, 327 (1948).
[2] E. CHARGAFF and H. F. SAIDEL, J. Biol. Chem. *177*, 417 (1949).
[3] S. ZAMENHOF, L. B. SHETTLES, and E. CHARGAFF, Nature (in press).
[4] G. CLARKE and S. B. SCHRYVER, Biochem. J. *11*, 319 (1917).
[5] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *168*, 781 (1947); *176*, 703 (1948).

convenient. The separation of the pyrimidines is carried out in aqueous butanol (column 1).

Following the separation, the location of the various adsorption zones on the paper must be demonstrated. Our first attempts to bring this about in ultraviolet light were unsuccessful, probably because of inadequate filtration of the light emitted by the lamp then at our disposal. For this reason, the expedient was



Schematic representation of the position on the paper chromatogram of the purines and pyrimidines following the separation of a mixture. *A* adenine, *G* guanine, *H* hypoxanthine, *X* xanthine, *U* uracil, *C* cytosine, *T* thymine. The conditions under which the separations were performed are indicated at the bottom, *a* acidic, *n* neutral, *B* n-butanol, *M* morpholine, *D* diethylene glycol, *Co* collidine, *Q* quinoline.

(Taken from E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 704 [1948].)

used of fixing the separated purines or pyrimidines on the paper as mercury complexes which then were made visible by their conversion to mercuric sulfide. The papers thus developed served as guide strips for the removal of the corresponding zones from untreated chromatograms that were then extracted and analyzed in the ultraviolet spectrophotometer. The development of the separated bases as mercury derivatives has, however, now become unnecessary, except for the preservation of permanent records, since there has for some time been available commercially an ultraviolet lamp emitting short wave ultraviolet ("Mineralight", Ultraviolet Products Corp., Los Angeles, California). With the help of this lamp it is now easy to demonstrate directly the position of the separated purines and pyrimidines (and also of nucleosides and nucleotides[1]) which appear as dark absorption shadows on the background of the fluorescing filter paper and can be cut apart accordingly. (We are greatly indebted to Dr. C. E. CARTER, Oak Ridge National Laboratory, who drew our attention to this instrument[2].)

The extracts of the separated compounds are then studied in the ultraviolet spectrophotometer. The measurement of complete absorption spectra permits the determination of the purity of the solutions and at the same time the quantitative estimation of their contents. The details of the procedures employed have been published[3]. In this manner, adenine, guanine, uracil, cytosine, and thymine (and also hypoxanthine, xanthine, and 5-methylcytosine[4]) can be determined quantitatively in amounts of 2–40 γ. The precision of the method is $\pm 4\%$ for the purines and even better for the pyrimidines, if the averages of a large series of determinations are considered. In individual estimations the accuracy is about $\pm 6\%$.

Procedures very similar in principle served in our laboratory for the separation and estimation of the ribonucleosides uridine and cytidine and for the separation of desoxyribothymidine from thymine. Methods for the separation and quantitative determination of the ribonucleotides in an aqueous ammonium isobutyrate-isobutyric acid system have likewise been developed[5].

VI. *Methods of Hydrolysis*

It has long been known that the purines can be split off completely by a relatively mild acid hydrolysis of the nucleic acids. This could be confirmed in our laboratory in a more rigorous manner by the demonstration that heating at 100° for 1 hour in N sulfuric acid effects the quantitative liberation of adenine and guanine from adenylic and guanylic acids respectively[6]. The liberation of the pyrimidines, however, requires much more energetic methods of cleavage. Heating at high temperatures with strong mineral acid under pressure is usually resorted to. To what extent these procedures brought about the destruction of the pyrimidines, could not be ascertained previously owing to the lack of suitable analytical procedures. The experiments summarized in Table I, which are quoted from a recent paper[6], show that the extremely robust cleavage methods with mineral acids usually employed must have led to a very considerable degradation of cytosine to uracil. Uracil and also thymine are much more resistant. For this reason, we turned to

[1] E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949).

[2] A similar arrangement was recently described by E. R. HOLIDAY and E. A. JOHNSON, Nature *163*, 216 (1949).

[3] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 703 (1948).

[4] J. KREAM and E. CHARGAFF, unpublished experiments.

[5] E. VISCHER, B. MAGASANIK, and E. CHARGAFF, Federation Proc. *8*, 263 (1949). – E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949).

[6] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 715 (1948).

Table I

Resistance of pyrimidines to treatment with strong acid. A mixture of pyrimidines of known concentration was dissolved in the acids indicated below and heated at 175° in a bomb tube. The concentration shifts of the individual pyrimidines were determined through a comparison of the recoveries of separated pyrimidines before and after the heating of the mixture.

Experiment No.	Acid	Heating time min.	Concentration shift, per cent of starting concentration		
			Uracil	Cytosine	Thymine
1	HCl (10%)	90	+62	−63	+3
2	10 N HCOOH + N HCl (1:1)	60	+ 3	− 5	0
3		120	+24	−19	0
4	HCOOH (98 to 100%)	60	0	− 1	−2
5		120	0	+ 2	+1

the hydrolysis of the pyrimidine nucleotides by means of concentrated formic acid. For the liberation of the purines N sulfuric acid (100°, 1 hour) is employed; for the liberation of the pyrimidines, the purines are first precipitated as the hydrochlorides by treatment with dry HCl gas in methanol and the remaining pyrimidine nucleotides cleaved under pressure with concentrated formic acid (175°, 2 hours). This procedure proved particularly suitable for the investigation of the desoxypentose nucleic acids. For the study of the composition of pentose nucleic acids a different procedure, making use of the separation of the ribonucleotides, was developed more recently, which will be mentioned later.

VII. *Composition of Desoxypentose Nucleic Acids*

It should be stated at the beginning of this discussion that the studies conducted thus far have yielded no indication of the occurrence in the nucleic acids examined in our laboratory of unusual nitrogenous constituents. In all desoxypentose nucleic acids investigated by us the purines were adenine and guanine, the pyrimidines cytosine and thymine. The occurrence in minute amounts of other bases, e.g. 5-methylcytosine, can, however, not yet be excluded. In the pentose nucleic acids uracil occurred instead of thymine.

A survey of the composition of desoxyribose nucleic acid extracted from several organs of the ox is provided in Table II. The molar proportions reported in each case represent averages of several hydrolysis experiments. The composition of desoxypentose nucleic acids from human tissues is similarly illustrated in Table III. The preparations from human liver were obtained from a pathological specimen in which it was possible, thanks to the kind cooperation of M. FABER, to separate portions of unaffected hepatic tissue from carcinomatous tissue consisting of metastases from the sigmoid colon, previous to the isolation of the nucleic acids[1].

Table II[1]

Composition of desoxyribonucleic acid of ox (in moles of nitrogenous constituent per mole of P).

Constituent	Thymus			Spleen		Liver
	Prep. 1	Prep. 2	Prep. 3	Prep. 1	Prep. 2	
Adenine . .	0·26	0·28	0·30	0·25	0·26	0·26
Guanine . .	0·21	0·24	0·22	0·20	0·21	0·20
Cytosine . .	0·16	0·18	0·17	0·15	0·17	
Thymine . .	0·25	0·24	0·25	0·24	0·24	
Recovery . .	0·88	0·94	0·94	0·84	0·88	

[1] From E. CHARGAFF, E. VISCHER, R. DONIGER, C. GREEN, and F. MISANI, J. Biol. Chem. *177*, 405 (1949); and unpublished results.

Table III[2]

Composition of desoxypentose nucleic acid of man (in moles of nitrogenous constituent per mole of P).

Constituent	Sperm		Thymus	Liver	
	Prep. 1	Prep. 2		Normal	Carcinoma
Adenine . . .	0·29	0·27	0·28	0·27	0·27
Guanine . . .	0·18	0·17	0·19	0·19	0·18
Cytosine . . .	0·18	0·18	0·16		0·15
Thymine . . .	0·31	0·30	0·28		0·27
Recovery . . .	0·96	0·92	0·91		0·87

In order to show examples far removed from mammalian organs, the composition of two desoxyribonucleic acids of microbial origin, namely from yeast[3] and from avian tubercle bacilli[4], is summarized in Table IV.

Table IV[5]

Composition of two microbial desoxyribonucleic acids.

Constituent	Yeast		Avian tubercle bacilli
	Prep. 1	Prep. 2	
Adenine	0·24	0·30	0·12
Guanine	0·14	0·18	0·28
Cytosine	0·13	0·15	0·26
Thymine	0·25	0·29	0·11
Recovery	0·76	0·92	0·77

The very far-reaching differences in the composition of desoxypentose nucleic acids of different species are best illustrated by a comparison of the ratios of adenine to guanine and of thymine to cytosine as given in Table V. It will be seen that in all cases where enough material for statistical analysis was available highly significant differences were found. The analytical figures on which Table V is based were derived by comparing the ratios found for individual nucleic acid hydrolysates of one species regardless of the organ from which the preparation was isolated. This procedure assumes that there is no organ specificity with

[1] Unpublished experiments.

[2] From E. CHARGAFF, S. ZAMENHOF, and C. GREEN, Nature (in press); and unpublished results.

[3] E. CHARGAFF and S. ZAMENHOF, J. Biol. Chem. *173*, 327 (1948).

[4] E. CHARGAFF and H. F. SAIDEL, J. Biol. Chem. *177*, 417 (1949).

[5] From E. VISCHER, S. ZAMENHOF, and E. CHARGAFF, J. Biol. Chem. *177*, 429 (1949); and unpublished results.

Table V

Molar proportions of purines and pyrimidines in desoxypentose nucleic acids from different species.

Species	Number of different organs	Number of different preparations	Adenine/Guanine			Thymine/Cytosine		
			Number of hydrolyses[3]	Mean ratio	Standard error	Number of hydrolyses[3]	Mean ratio	Standard error
Ox[1]	3	7	20	1·29	0·013	6	1·43	0·03
Man[2]	2	3	6	1·56	0·008	5	1·75	0·03
Yeast	1	2	3	1·72	0·02	2	1·9	
Avian tubercles bacillus	1	1	2	0·4		1	0·4	

[1] Preparations from thymus, spleen, and liver served for the purine determinations, the first two organs for the estimation of pyrimidines.

[2] Preparations from spermatozoa and thymus were analysed.

[3] In each hydrolysis between 12 and 24 determinations of individual purines and pyrimidines were performed.

respect to the composition of desoxypentose nucleic acids of the same species. That this appears indeed to be the case may be gathered from Tables II and III and even better from Table VI where the average purine and pyrimidine ratios in individual tissues of the same species are compared. That the isolation of nucleic acids did not entail an appreciable fractionation is shown by the finding that when whole defatted human spermatozoa, after being washed with cold 10% trichloroacetic acid, were analyzed, the same ratios of adenine to guanine and of thymine to cytosine were found as are reported in Tables V and VI. It should also be mentioned that all preparations, with the exception of those from human liver, were derived from pooled starting material representing a number, and in the case of human spermatozoa a very large number, of individuals.

Table VI

Molar proportions of purines and pyrimidines in desoxypentose nucleic acids from different organs of one species.

Species	Organ	Adenine/ Guanine	Thymine/ Cytosine
Ox	Thymus	1·3	1·4
	Spleen	1·2	1·5
	Liver	1·3	
Man	Thymus	1·5	1·8
	Sperm	1·6	1·7
	Liver (normal)	1·5	1·8
	Liver (carcinoma)	1·5	1·8

The desoxypentose nucleic acids extracted from different species thus appear to be different substances or mixtures of closely related substances of a composition constant for different organs of the same species and characteristic of the species.

The results serve to disprove the tetranucleotide hypothesis. It is, however, noteworthy—whether this is more than accidental, cannot yet be said—that in all desoxypentose nucleic acids examined thus far the molar ratios of total purines to total pyrimidines, and also of adenine to thymine and of guanine to cytosine, were not far from 1.

VIII. *Composition of Pentose Nucleic Acids*

Here a sharp distinction must be drawn between the prototype of all pentose nucleic acid investigations—the ribonucleic acid of yeast—and the pentose nucleic acids of animal cells. Nothing is known as yet about bacterial pentose nucleic acids. In view of the incompleteness of our information on the homogeneity of pentose nucleic acids, which I have stressed before, I feel that the analytical results on these preparations do not command the same degree of confidence as do those obtained for the desoxypentose nucleic acids.

Table VIII[1]

Composition of pentose nucleic acids from animal tissues.

Constituent	Calf liver	Ox liver	Sheep liver	Pig liver	Pig pancreas
Guanylic acid . . .	16·3	14·7	16·7	16·2	22·5
Adenylic acid . . .	10	10	10	10	10
Cytidylic acid . . .	11·1	10·9	13·4	16·1	9.8
Uridylic acid . . .	5·3	6·6	5·6	7·7	4·6
Purines: pyrimidines	1·6	1·4	1·4	1·1	2·5

Three procedures, to which reference is made in Tables VII and VIII, were employed in our laboratory for the analysis of pentose nucleic acids. In *Procedure 1*, the pentose nucleic acid was hydrolysed to the nucleotide stage with alkali, at p_H 13·5 and 30°, and the nucleotides, following adjustment to about p_H 5, separated by chromatography with aqueous ammonium isobutyrate-isobutyric acid as the solvent. Under these conditions, guanylic acid shares its position on the chromatogram with uridylic acid; but it is possible to determine the concentrations of the two components in the eluates by simultaneous equations based on the ultraviolet absorption of the pure nucleotides[2]. The very good recoveries of nucleotides obtained in terms of both nucleic acid phosphorus and nitrogen show the cleavage by mild alkali treatment of pentose nucleic acids to be practically quantitative.—In

[1] Unpublished results.

[2] E. VISCHER, B. MAGASANIK, and E. CHARGAFF, Federation Proc. *8*, 263 (1949). – E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949).

Table VII[1]
Composition of yeast ribonucleic acid (in moles of nitrogenous constituent per mole of P).

Constituent	Preparation 1			Preparation 2			Preparation 3		
	Procedure 1	Procedure 2	Procedure 3	Procedure 1	Procedure 2	Procedure 3	Procedure 1	Procedure 2	Procedure 3
Adenylic acid	0·29	0·26	0·26	0·27		0·24	0·25	0·23	0·24
Guanylic acid	0·28	0·29	0·26	0·25		0·25	0·26	0·28	0·26
Cytidylic acid	0·18	0·17	0·24	0·20			0·21	0·21	
Uridylic acid	0·20	0·20	0·08	0·18	0·19		0·20	0·25	
Recovery	0·95	0·92	0·84	0·90			0·92	0·97	

[1] From E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 715 (1948). – E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949); and unpublished results.

Procedure 2, the purines are first liberated by gaseous HCl in dry methanol and the evaporation residue of the reaction mixture is adjusted to p_H 13·5 and then treated as in Procedure 1. In this manner, uridylic and cytidylic acids, adenine and guanine are separated and determined on one chromatogram.—The determinations of free purines and pyrimidines in acid hydrolysates of pentose nucleic acids, following the methods outlined before for the desoxypentose nucleic acids, are listed as *Procedure 3*. It will be seen that it is mainly uracil which in this procedure escapes quantitative determination. This is due to the extreme refractoriness of uridylic acid to complete hydrolysis by acids, a large portion remaining partially unsplit as the nucleoside uridine. As matters stand now, I consider the values for purines yielded by Procedures 1 and 3 and those for pyrimidines found by Procedures 1 and 2 as quite reliable.

A survey of the composition of yeast ribonucleic acid is provided in Table VII. Preparations 1 and 2, listed in this table, were commercial preparations that had been purified in our laboratory and had been subjected to dialysis; Preparation 3 was isolated from baker's yeast by B. MAGASANIK in this laboratory by procedures similar to those used for the preparation of pentose nucleic acids from animal tissues and had not been dialyzed. It will be seen that the results are quite constant and not very far from the proportions required by the presence of equimolar quantities of all four nitrogenous constituents.

An entirely different picture, however, was encountered when the composition of pentose nucleic acids from animal cells was investigated. A preliminary summary of the results, in all cases obtained by *Procedure 1*, is given in Table VIII. Here guanylic acid was the preponderating nucleotide followed, in this order, by cytidylic and adenylic acids; uridylic acid definitely was a minor constituent. This was true not only of the ribonucleic acid of pancreas which has been known to be rich in guanine[1], but also of all pentose nucleic acids isolated by us from the livers of three different species (Table VIII).

In the absence of a truly reliable standard method for the isolation of pentose nucleic acid from animal tissue, generalizations are not yet permitted; but it would appear that pentose nucleic acids from the same organ of different species are more similar to each other, at least in certain respects (e. g. the ratio of guanine to adenine), than are those from different organs of the same species. (Compare the pentose nucleic acids from the liver and the pancreas of pig in Table VIII.)

IX. *Sugar Components*

It is deplorable that such designations as desoxyribose and ribose nucleic acids continue to be used as if they were generic terms. Even the "thymus nucleic acid of fish sperm" is encountered in the literature. As a matter of fact, only in a few cases have the sugars been identified, namely, *d*-2-desoxyribose as a constituent of the guanine and thymine nucleosides of the desoxypentose nucleic acid from calf thymus, D-ribose as a constituent of the pentose nucleic acids from yeast, pancreas, and sheep liver.

Since the quantities of novel nucleic acids usually will be insufficient for the direct isolation of their sugar components, we attempted to employ the very sensitive procedure of the filter paper chromatography of sugars[1] for the study of the sugars isolated from minute quantities of nucleic acids. It goes without saying that identifications based on behavior in adsorption or partition are by no means as convincing as the actual isolation, but they will at least permit a tentative classification of new nucleic acids. Thus far the pentose nucleic acids of pig pancreas[2] and of the avian tubercle bacillus[3] have been shown to contain ribose, the desoxypentose nucleic acids of ox spleen[4],

[1] E. HAMMARSTEN, Z. physiol. Ch. *109*, 141 (1920). – P.A. LEVENE and E. JORPES, J. Biol. Chem. *86*, 389 (1930). – E. JORPES, Biochem. J. *28*, 2102 (1934).

[1] S.M. PARTRIDGE, Nature *158*, 270 (1946). – S.M. PARTRIDGE and R.G. WESTALL, Biochem. J. *42*, 238 (1948). – E. CHARGAFF, C. LEVINE, and C. GREEN, J. Biol. Chem. *175*, 67 (1948).

[2] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 715 (1948).

[3] E. VISCHER, S. ZAMENHOF, and E. CHARGAFF, J. Biol. Chem. *177*, 429 (1949).

[4] E. CHARGAFF, E. VISCHER, R. DONIGER, C. GREEN, and F. MISANI, J. Biol. Chem. *177*, 405 (1949).

yeast and avian tubercle bacilli[1] desoxyribose. It would seem that the free play with respect to the variability of components that nature permits itself is extremely restricted, where nucleic acids are concerned.

X. *Depolymerizing Enzymes*

Enzymes capable of bringing about the depolymerization of both types of nucleic acids have long been known; but it is only during the last decade that crystalline ribonuclease[2] and desoxyribonuclease[3] from pancreas have become available thanks to the work of KUNITZ. Important work on the latter enzyme was also done by MCCARTY[4].

Table IX[5]

Enzymatic degradation of calf thymus desoxyribonucleic acid.

	Digestion *hours*	Dialysis *hours*	Distribution of fractions % of original	Composition of fractions (molar proportions)			
				Adenine / Guanine	Thymine / Cytosine	Adenine / Cytosine	Pyrimidines / Purines
Original . . .	0	0	100	1·2	1·3	1·6	1·2
Dialysate . . .	6	6	53	1·2	1·2	1·2	1·0
Dialysis residue	24	72	7	1·6	2·2	3·8	2·0

We were, of course, interested in applying the chromatographic micromethods for the determination of nucleic acid constituents to studies of enzymatic reaction mechanisms for which they are particularly suited. The action of crystalline desoxyribonuclease on calf thymus desoxyribonucleic acid resulted in the production of a large proportion of dialyzable fragments (53 per cent of the total after 6 hours digestion), without liberation of ammonia or inorganic phosphate. But even after extended digestion there remained a non-dialyzable core whose composition showed a significant divergence from both the original nucleic acid and the bulk of the dialyzate[6]. The preliminary findings summarized in Table IX indicate a considerable increase in the molar proportions of adenine to guanine and especially to cytosine, of thymine to cytosine, and of purines to pyrimidines. This shows that the dissymmetry in the distribution of constituents, found in the original nucleic acid (Table II), is intensified in the core. The most plausible explanations of this interesting phenomenon, the study of which is being continued, are that the preparations consisted of more than one desoxypentose nucleic acid or that the nucleic contained in its chain clusters of nucleotides (relatively richer in adenine and thymine) that were distinguished from the bulk of the molecule by greater resistance to enzymatic disintegration.

In this connection another study, carried out in collaboration with S. ZAMENHOF, should be mentioned briefly that dealt with the desoxypentose nuclease of yeast cells[1]. This investigation afforded a possibility of exploring the mechanisms by which an enzyme concerned with the disintegration of desoxypentose nucleic acid is controlled in the cell. Our starting point again was the question of the specificity of desoxypentose nucleic acids; but the results were entirely unexpected. Since we had available a number of nucleic acids from different sources, we wanted to study a pair of desoxypentose nucleic acids as distant from each other as possible, namely that of the ox and that of yeast, and to investigate the action on them of the two desoxypentose nucleases from the same cellular sources. The desoxyribonuclease of ox pancreas has been thoroughly investigated, as was mentioned before. Nothing was known, however, regarding the existence of a yeast desoxypentose nuclease.

It was found that fresh salt extracts of crushed cells contained such an enzyme in a largely inhibited state, due to the presence of a specific inhibitor protein. This inhibitor specifically inhibited the desoxypentose nuclease from yeast, but not that from other sources, such as pancreas. The yeast enzyme depolymerized the desoxyribose nucleic acids of yeast and of calf thymus, which differ chemically, as I have emphasized before, at about the same rate. In other words, the enzyme apparently exhibited inhibitor specificity, but not substrate specificity. It is very inviting to assume that such relations between specific inhibitor and enzyme, in some ways reminiscent of immunological reactions, are of more general biological significance. In any event, a better understanding of such systems will permit an insight into the delicate mechanisms through which the cell manages the economy of its life, through which it maintains its own continuity and protects itself against agents striving to transform it.

XI. *Concluding Remarks*

Generalizations in science are both necessary and hazardous; they carry a semblance of finality which conceals their essentially provisional character; they drive forward, as they retard; they add, but they also take away. Keeping in mind all these reservations, we arrive at the following conclusions. The desoxypentose nucleic acids from animal and microbial cells contain varying proportions of the same four nitrogenous constituents, namely, adenine, guanine, cytosine, thymine. Their composition appears to be characteristic of the species, but not of the tissue, from which

[1] E. VISCHER, S. ZAMENHOF, and E. CHARGAFF, J. Biol. Chem. *177*, 429 (1949).
[2] M. KUNITZ, J. Gen. Physiol. *24*, 15 (1940).
[3] M. KUNITZ, Science *108*, 19 (1948).
[4] M. MCCARTY, J. Gen. Physiol. *29*, 123 (1946).
[5] From S. ZAMENHOF and E. CHARGAFF, J. Biol. Chem. *178*, 531 (1949).
[6] S. ZAMENHOF and E. CHARGAFF, J. Biol. Chem. *178*, 531 (1949).
[1] S. ZAMENHOF and E. CHARGAFF, Science *108*, 628 (1948); J. Biol. Chem. *180*, 727 (1949).

they are derived. The presumption, therefore, is that there exists an enormous number of structurally different nucleic acids; a number, certainly much larger than the analytical methods available to us at present can reveal.

It cannot yet be decided, whether what we call the desoxypentose nucleic acid of a given species is one chemical individual, representative of the species as a whole, or whether it consists of a mixture of closely related substances, in which case the constancy of its composition merely is a statistical expression of the unchanged state of the cell. The latter may be the case if, as appears probable, the highly polymerized desoxypentose nucleic acids form an essential part of the hereditary processes; but it will be understood from what I said at the beginning that a decision as to the identity of natural high polymers often still is beyond the means at our disposal. This will be particularly true of substances that differ from each other only in the sequence, not in the proportion, of their constituents. The number of possible nucleic acids having the same analytical composition is truly enormous. For example, the number of combinations exhibiting the same molar proportions of individual purines and pyrimidines as the desoxyribonucleic acid of the ox is more than 10^{56}, if the nucleic acid is assumed to consist of only 100 nucleotides; if it consists of 2,500 nucleotides, which probably is much nearer the truth, then the number of possible "isomers" is not far from 10^{1500}.

Moreover, desoxypentose nucleic acids from different species differ in their chemical composition, as I have shown before; and I think there will be no objection to the statement that, as far as chemical possibilities go, they could very well serve as one of the agents, or possibly as the agent, concerned with the transmission of inherited properties. It would be gratifying if one could say—but this is for the moment no more than an unfounded speculation—that just as the desoxypentose nucleic acids of the nucleus are species-specific and concerned with the maintenance of the species, the pentose nucleic acids of the cytoplasm are organ-specific and involved in the important task of differentiation.

I should not want to close without thanking my colleagues who have taken part in the work discussed here; they are, in alphabetical order, Miss R. DONIGER, Mrs. C. GREEN, Dr. B. MAGASANIK, Dr. E. VISCHER, and Dr. S. ZAMENHOF.

Zusammenfassung

Die Betrachtung der Nukleinsäuren, sowohl der Desoxypentosen als der Pentosen enthaltenden Verbindungen, als organische Makromoleküle macht eine Auseinandersetzung mit den Problemen notwendig, welche sich auf die Bestimmung von Identität oder Verschiedenheit solcher aus verschiedenen Zellen isolierten hochpolymeren Substanzen beziehen. Dies führt zu einer kritischen Besprechung der sicherlich nicht haltbaren Tetranukleotidhypothese und zur Formulierung eines Arbeitsprogramms für die Aufklärung der Zusammensetzung individueller Nukleinsäuren.

Die mikrochromatographischen und spektrophotometrischen Methoden zur Trennung und quantitativen Bestimmung der stickstoffhaltigen Nukleinsäurebestandteile werden kurz geschildert. Sie ermöglichen die quantitative Analyse der Purine Adenin, Guanin, Hypoxanthin und Xanthin und der Pyrimidine Cytosin, Uracil und Thymin im Bereiche von 2 bis 40 γ. An die Beschreibung der Verfahren zur Isolierung der als Analysenmaterial dienenden hochpolymerisierten Nukleinsäurepräparate aus verschiedenen Zellen schließt sich eine Besprechung der Hydrolysen- und Analysenmethoden, die auf sehr geringe Nukleinsäuremengen (2 bis 3 mg) anwendbar sind.

Alle bis jetzt untersuchten Desoxypentosenukleinsäuren enthielten 2-Desoxyribose als Zucker und Adenin, Guanin, Cytosin und Thymin als Stickstoffkomponenten in für die betreffende Zelle konstanten, von der Tetranukleotidhypothese weit abweichenden Proportionen. Ihre Zusammensetzung ist spezifisch für die Spezies, die als Ausgangsmaterial dient, jedoch nicht für das Ausgangsgewebe. Weit auseinanderliegende Arten, wie z. B. Säugetiere gegenüber Mikroorganismen, enthalten völlig verschieden zusammengesetzte Desoxypentosenukleinsäuren. In manchen Fällen lassen sich jedoch auch bei näherliegenden Nukleinsäuren, z. B. denen des Ochsen und des Menschen, ins Gewicht fallende Verschiedenheiten aufzeigen. Die Untersuchung der Pentosenukleinsäuren, die auf einer quantitativen Bestimmung der sie zusammensetzenden Mononukleotide beruht, ist noch nicht so weit gediehen. Sie hat vorläufig gezeigt, daß sich die Verbindungen aus Säugetiergewebe von denen aus Hefe durch einen relativ sehr hohen Gehalt an Guanylsäure unterscheiden, und hat Anhaltspunkte dafür gegeben, daß die Pentosenukleinsäuren eher organ- als spezies-spezifisch sind.

Als Beispiele für den Beitrag, den die Untersuchung enzymatischer Reaktionsmechanismen zum Problem der chemischen Spezifizität der Nukleinsäuren leisten kann, werden schließlich Versuche mit der kristallisierten Desoxyribonuklease aus Pankreas und mit einer durch interessante Hemmungsstoffspezifizität ausgezeichneten Desoxypentosenuklease aus Hefe geschildert. Zum Abschluß werden einige der Probleme gestreift, die sich aus der hier nachgewiesenen Existenz vieler verschiedener Nukleinsäuren ergeben.

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Effects of Ultra-Violet Light on Nucleic Acid and Nucleoproteins and Other Biological Systems[1]

By J. A. V. BUTLER[2], London

It may be helpful to start with a rather general picture of the chemical and physico-chemical background of U.V. processes and also to discuss in a rather general way the possible links between the chemical phenomena and the biological effects observed. I think it will be clear that there is at present a very considerable gap between the chemistry and the biology, i.e. it is impossible to say precisely what the biological effects will be, even if we know what chemical actions were produced.

The action of light on simple chemical compounds is fairly well understood. The general result of the absorption of light by a system of nuclei and electrons is an electronic excitation, i.e. the displacement of one or more electrons from the levels they occupy in the unexcited molecule to excited states of higher energy. The energy so gained may be used in the following ways:

(1) Re-radiation (fluorescence; phosphorescence).

(2) Degradation to thermal vibrations of atoms.

(3) In bringing about chemical changes in the excited molecule, e.g. a breakage of co-valent or other bonds resulting in dissociation of the excited molecule. This happens when the energy level of the excited state is above that of the dissociated molecule.

(4) By collision with other atoms or molecules: the transfer of excitation energy to colliding molecules may occur and chemical reactions may as a consequence take place in these.

Many examples of such processes are known. In applying these concepts to biological systems two kinds of difficulties arise:

(1) Many of the absorbing molecules, e.g. proteins, nucleic acids, are extremely complex and it is usually impossible to decide what is the activated state and to trace its history.

(2) Of the many compounds which are capable of absorbing radiation, it is not known and can only be decided by indirect evidence, which are important and which are secondary in bringing about the biological effect.

[1] Lecture given to the Congrès International de Photobiologie, Amsterdam, August 1954.

[2] Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London.

Much of the biological work has been concerned specifically with mutation. It must not be forgotten that this is a highly specialised result of u.v. radiation, since of the many actions which occur, most are lethal if the dose is sufficient and very few result in mutation. In the study of mutations produced by light we are dealing with a *non-typical* process. The lethal and other effects of light are equally important as the genetical effects. They are probably due to action of light on the *enzyme systems*. This is a little explored field which would repay examination–as many enzymes have prosthetic groups which absorb in u.v. or visible. It is probable that many of the effects of u.v. are due to disorganisation of the metabolism in the cell by inactivating or modifying critical metabolic systems.

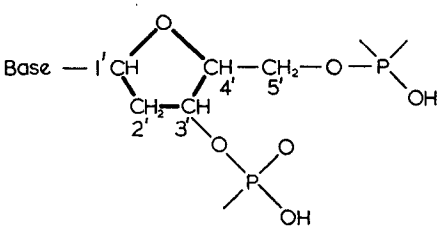


Fig. 1.–Structural formula of DNA.

It has been found that in many cases the action spectrum of light corresponds to the u.v. spectrum of nucleic acids. Since chromosomes contain DNA it is inferred that the light producing mutations is that absorbed by DNA. There are exceptions, however, e.g. the observation of MCAULEY and FORD that the maximum efficiency of irradiation of *Chaetomium* corresponds with absorption spectrum of proteins[1]. There are also the observations of HOLLAENDER of two peaks in the action spectrum[2], one of which corresponds to absorption by proteins. I shall deal here principally with the action of u.v. on desoxyribonucleic acids. The formula of a typical part of the DNA chain is shown in Figure 1.

Direct action of u.v. on nucleic acids.–Although the absorption coefficients of nucleotides and of DNA are high, the quantum efficiency of chemical changes

[1] T. M. MCAULAY and A. L. FORD, Heredity *1*, 247 (1947).

[2] A. HOLLAENDER, K. B. RAPER, and R. D. COGHILL, Amer. J. Botany *32*, 160 (1940).

produced is apparently low, but very few accurate measurements of chemical changes produced by u.v. light on these compounds have been made. ERRERA[1] gives values of the order of 10^{-2}–10^{-5} in certain cases. It has been found that purines are very stable to u.v.; pyrimidines are less so, the pyrimidine ring being decomposed with the formation of urea[2], but it is doubtful if this occurs to any appreciable extent when the pyrimidine is present in DNA. There is a loss of viscosity of DNA (Fig. 2), but it is not certain that this is the result of the breakage of nucleotide chains[3]. It is difficult to be sure of this as only one break per particle will produce a considerable decrease of viscosity; however, no great change of molecular weight has been detected as a result of u.v. radiation. It is difficult to be sure, as the molecular weight methods can not be

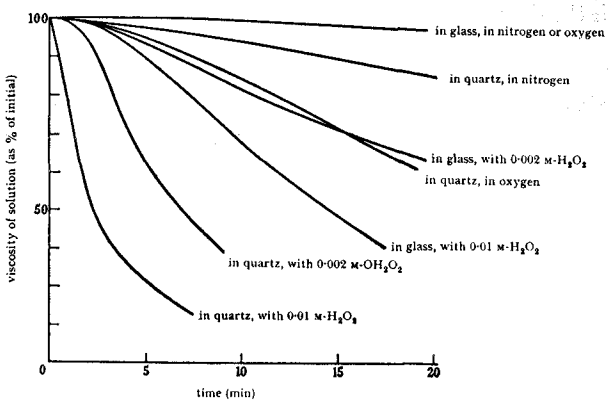


Fig. 2.–Change of viscosity of thymonucleic acid (0·1%) solutions with time when irradiated under various conditions, by means of mercury u.v. arc. at constant intensity [J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953)].

applied easily to mixtures. It must be admitted that this conclusion may have to be reconsidered when more precise methods of measurement are available. However, it is more likely that the loss of viscosity is due to a partial collapse of the stiff DNA particle due to breakage of the hydrogen bonds which maintain the original configuration. The amount of energy absorbed is considerable. Much of the energy absorbed may be degraded to vibrational energy and lost as heat. One quantum of wavelength 2500 Å = 4·96 e.v. = 115 kcals/mol, is sufficient to break at least 20 hydrogen bonds.

In case of proteins there is fairly clear evidence that u.v. radiation is capable of producing denaturation by breakage of H bonds e.g. CLARK[4] (1937–1945) found that exposure to u.v. light lowers the flocculation temperature of serum albumen.

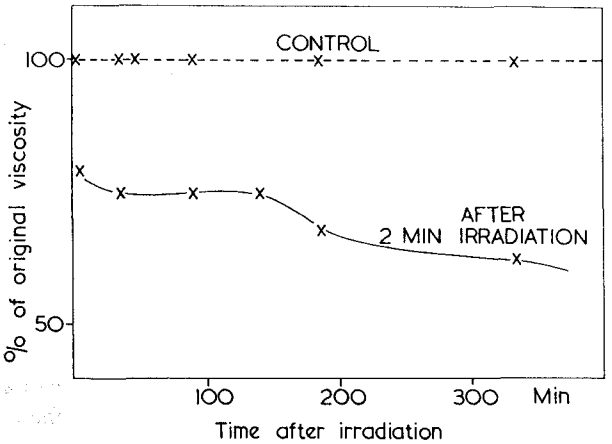


Fig. 3.–Change of viscosity of 0·05% DNA solution when kept at 40°C after 2 min u.v. irradiation in quartz tube in nitrogen.

We have found some indication that after u.v. irradiation, DNA is also more sensitive to heat degradation than originally (Fig. 3). This indicates that u.v. brings about changes which predispose the particle to heat denaturation and it is therefore probable that some hydrogen bonds are broken during the irradiation.

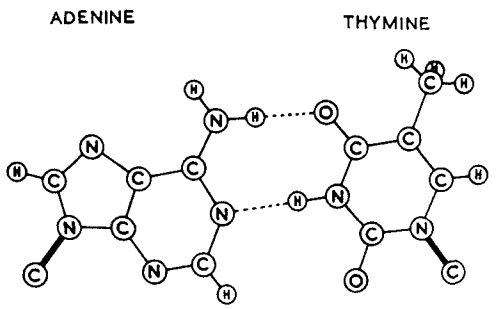


Fig. 4.–Hydrogen bonding of bases of DNA. [F. H. C. CRICK and J. D. WATSON, Nature *171*, 737 (1953).]

In the case of DNA it is possible to give a more precise interpretation of the hydrogen bond breakage in terms of the WATSON and CRICK model[1], according to which two nucleotide threads are hydrogen-bonded together (Fig. 4). It is however, not at all clear, as we shall see later, why breakage of such hydrogen bonds should produce any genetical effects as they could easily be remade, and can only be remade in one way. However, it would be very difficult to assert that u.v. does not also cause minute amounts of deamination or dehydroxylation which would have a permanent effect. I shall discuss the biological effects of such changes later.

Indirect actions of u.v. and visible light.– We turn now to cases in which light is absorbed by other molecules in the presence of nucleic acids.

[1] M. ERRERA, Biochem. Biophys. Acta *8*, 30, 115 (1952); Progress in Biophysics *3*, 88 (1952).

[2] M. M. STIMSON and T. R. LOOFBOUROW, J. Chem. Soc. *8*, 44 (1940).

[3] J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953).

[4] J. B. CLARK, Amer. J. Physiol. *113*, 538 (1935); J. Gen. Physiol. *19*, 199 (1935); *27*, 101 (1943). – E. V. RAJEWSKI, Biochem. Z. *227*, 272 (1930).

[1] J. D. WATSON and F. H. C. CRICK, Nature *171*, 737 (1953).

(1) The dissociation of oxygen itself can be brought about by wavelengths < 2530 Å. The oxygen atoms formed are, of course, powerful oxidizing agents easily producing OH radicals, which can react directly with nucleic acid. The can also give rise to hydrogen peroxide in the solution, which will have effects discussed below. It is also possible that O_2 will be activated at rather lower wavelengths by light absorbed by the nucleic acid, although this has not been proved. DNA is certainly more rapidly degraded by u.v. light (2500 Å) in the presence than in the absence of oxygen (see Fig. 1).

(2) Hydrogen peroxide when present is dissociated with practically unit efficiency by light of wavelength < 3100 Å, and the main products is hydroxyl radicals: $H_2O_2 \rightarrow 2\,OH$. There are certain secondary reactions possible e.g.

$$H_2O_2 + OH = H_2O + O_2H$$

but these are probably unimportant in the presence of an oxidizable substrate. The action of OH radicals on DNA is quantitatively very similar to that of X-rays. The loss of viscosity is of the same order as that produced by a quantity of X-rays which yield an equal number of OH radicals. Among the actions of photochemically formed hydroxyl radicals, which have been observed[1] or inferred are:

(1) deamination of the bases,
(2) dehydroxylation of the bases,
(3) partial destruction of pyrimidine ring,
(4) breakage of bond between the base and the sugar,
(5) oxidation of alkyl groups (in the case of ethyl phosphates),
(6) the breakage of the nucleotide chain and the liberation of phosphate.

It might be noted that the latter seems to be a consequence of the oxidation of the sugar moiety. This has not been proved directly, but in the case of the ethyl phosphates it is found[2] that an equivalent oxidation of the ethyl group always accompanies the liberation of the free phosphate. It is also found that with DNA the phosphate liberated increases approximately as the square of the time (Fig. 5)–which is due to the fact that in order to liberate a phosphate group, *two* adjacent sugar-phosphate links have to be broken. This explains why the yield of free PO_4 from small doses of OH radicals is very small, although the efficiency of the liberation of free phosphate from alkyl phosphates and simple nucleotides by the action of OH radicals is remarkably high, about one phosphate being liberated with adenosine for each H_2O_2 decomposed. It may be that a short chain reaction is occurring. But at least we can infer from this that the hydroxyl radicals produced by irradiation of hydrogen and other peroxides are very efficient in breaking the nucleotide chain, as well as bringing about other degradative reactions. This is in strong contrast to the effect of u.v. alone.

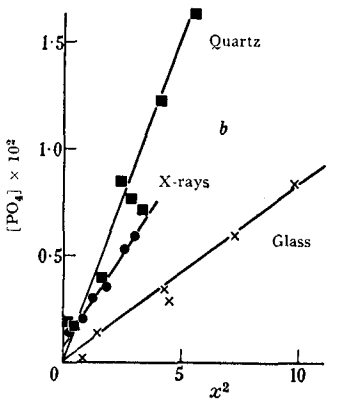


Fig. 5.–Amount of free phosphate liberated by irradiation under different conditions from thymonucleic acid, plotted against square of time. The unit of time is that which corresponds to the decomposition of 0·167% of 0·01 M-hydrogen peroxide; or in the case of the X-irradiation to 10^4 *R*. The X-ray data are from J. WEISS and M. E. SCHOLES, Exp. Cell. Res. Suppl. *2*, 219 (1952). [J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] 141, 562 (1953).]

(3) *After-effects of irradiation of DNA.*– I might refer here to the after-effects of irradiating DNA. As mentioned above, u.v. irradiation causes some after-effect on the viscosity in the absence of oxygen. It is much more marked in presence of oxygen. In the presence of hydrogen peroxide the loss of viscosity initiated by u.v. light continues in the dark until it is practically complete (Fig. 6). It is difficult to distinguish a genuine "after-effect" from the slow-continued action of hydrogen peroxide, because although the peroxide does not act on purified DNA, it is activated by and can then degrade DNA which has been damaged by X-rays[1] and the same might possibly be true of u.v. It is also possible that DNA which has been irradiated

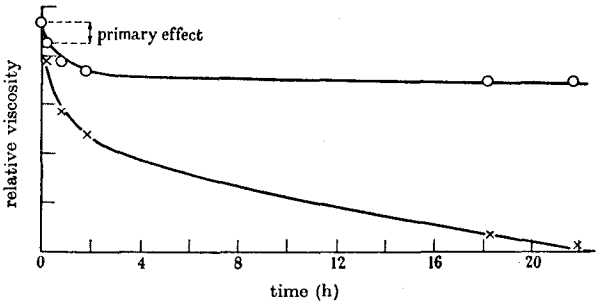


Fig. 6.–"After-effects" of irradiation of thymonucleic acid with hydrogen peroxide in quartz tube. –O– Effect of hydrogen peroxide (5×10^{-3} M). –×– Effect of ultra-violet (0·5 min) in same solution. [J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953).]

[1] J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953). – B. E. CONWAY and J. A. V. BUTLER, J. Chem. Soc. *1952*, 834.

[2] J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. *141*, 562 (1953).

[1] This may not take place in the course of the enzymic decomposition of H_2O_2 by catalase. It is possibly due to imparities in the catalase.

by u.v. can initiate a radical decomposition of H_2O_2 which continues in the dark. However, the question of whether H_2O_2 can act on DNA in the dark is largely an academic one, as tissues contain substances, e.g. ascorbic acid and cysteine, which are capable of activating H_2O_2[1].

In view of the fact that catalase is sometimes added to remove H_2O_2 in experiments with viruses it might also be noted that the addition of catalase to remove hydrogen peroxide does not diminish the amount of phosphate liberated e.g. a solution of adenosine 5-phosphate, which, after illumination in the presence of H_2O_2 contained 3·9 γ per ml of free phosphate, on standing for 24 h with catalase (which reduced the concentrations of H_2O_2 to zero), had 5·4 γ; but without catalase, 4·9 γ of free phosphate. This is probably due to the formation of small amounts of radicals in the reaction between H_2O_2 and catalase[2]. Even without any illumination, catalase-H_2O_2 releases a significant amount of phosphate from adenosine-5-phosphate.

I must also mention the possibility, discussed by WEISS and SCHOLES[3], that phosphate is "labilized" by OH radicals owing to the oxidation of the O_4 carbon of the ribose, the keto-phosphate so formed being unstable and slowly hydrolysed by water. That such an effect probably occurs to some extent is shown by the following experiment with adenosine (ribose) 5-phosphate. 1 h treatment at 70° with 0·5 N H_2SO_4 hydrolyses very little phosphate from the unirradiated adenosine 5-phosphate (Table) but an appreciably greater amount after treatment with H_2O_2 + u.v.

It can be seen that the amount of "labile" phosphate formed is of the same magnitude as that liberated during the irradiation[4].

Table–Liberation of phosphate from adenosine 5-phosphate

	Free PO_4 (initial) γ per ml	Free PO_4 (After 1 h hydrolysis with 0·5 NH_2SO_4) γ per m
1. Adenosine 5-phosphate . . .	0·26	0·34
2. Same after 1 h u.v. + H_2O_2 .	3·3	6·8
3. Same after standing 24 h . .	4·4	6·5

(4) *Formation of peroxides by u.v.-light.* There is also the related subject of peroxides produced by the action of u.v. on broth, etc., by which, as WYSS and STONE have shown[1], mutations are produced, as easily as by direct exposure to u.v. These peroxides can be formed:

(a) by the action of H_2O_2, formed as described above, on organic compounds,

(b) by the direct action of oxygen atoms on organic compounds,

(c) by the reaction of the activated compound on molecular oxygen, e.g. $R \rightarrow \overset{*}{R}$; $\overset{*}{R} + O_2 \rightarrow RO_2$.

Such peroxidic substances will behave similarly to hydrogen peroxide; they can produce OH radicals by spontaneous decomposition and by reacting with activating substances such as cysteine; radicals are also produced to some extent, probably, in the action of peroxidases. Peroxides like butyl hydroperoxide in presence of Fe^{++} easily cause radical degradation of DNA[2].

(5) *Action of other absorbing molecules*, e.g. dyes. This is a very large subject which has been very little explored. Dyes like methylene blue, when activated by light, can dehydrogenate the prosthetic groups of enzymes, and as result cause marked interference with metabolic processes. More deep seated changes may also be brought about e.g. in the presence of O_2, H_2O_2 may be formed, which may react on genetic elements in the ways I have discussed above. The photodegradation of DNA by illumination in presence of dyes and carcinogen substances has been reported[3]. In general a large amount of illumination is required. In our own experiments it was found to be difficult to get consistent results owing to denaturation effects, but in some cases at least oxygen has acted as an inhibitor of degradation process, so that the primary action did not appear to be the activation of molecular oxygen.

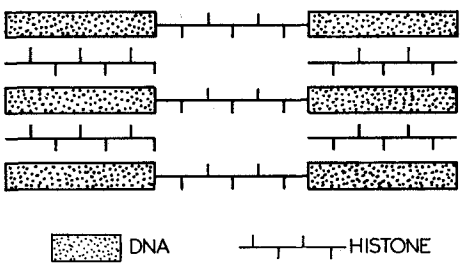


Fig. 7.–A possible structure of chromosomes: DNA particles joined by histone links.

Biological Consequences.–We can now ask, to what extent can these chemical actions, produced by u.v. and other radiations, be used to explain the biological effects e.g. chromosome breaks? The Watson and Crick model[4] provided a possible explanation of how the duplication

[1] B. E. CONWAY, Brit. J. Radiol. *27*, 42 (1954); Nature *173*, 579 (1954).
[2] B. E. CONWAY and J. A. V. BUTLER, J. Chem. Soc. *1952*, 834.
[3] J. WEISS and M. E. SCHOLES, Exp. cell Research, suppl. *2*, 219 (1952); Nature *171*, 920 (1953).
[4] For further experiments see J. WEISS and M. E. SCHOLES, Exp. cell Research, suppl. *2*, 219 (1952); Nature *171*, 920 (1953), and J. A. V. BUTLER and P. SIMSON, Liège, Symp. on Radiobiology, *1955*, 46.

[1] W. S. STONE, O. WYSS, and F. HAAS, Proc. Nat. Acad. Sci. *33*, 59 (1947).
[2] J. A. V. BUTLER and K. A. SMITH, Nature *165*, 847 (1950).
[3] G. OSTER and A. D. MCLAREN, J. Gen. Physiol. *33*, 215 (1950). – H. KOFFLER and I. L. MARKEDT, Proc. Soc. Exp. Biol. Med. *76*, 90 (1951).
[4] J. D. WATSON and F. H. C. CRICK, Nature *171*, 737, 964 (1953).

of a DNA fibre with its bases in a specific order can occur. One half of the double fibre is exactly complementary to the other half, so that each half can act as a template for the other. It is obvious that if one base is damaged by loss of $-NH_2$ or $-OH$, or more drastic ways, the reproduction of the fibre at this point could not occur and this would result in a break in the newly synthesized fibre. We have seen that hydrogen and other peroxides, both with and without u.v. illumination, can bring about such reactions and also cause complete breaks in the nucleotide chain.

There is little evidence that u.v., in the absence of oxygen or peroxides, can bring about such effects, at least on a detectable scale, although it may cause a kind of denaturation of the DNA as the result of breakage of hydrogen bonds. It is, as I pointed out, difficult to see how the mere breakage of hydrogen bonds between the two halves of the double fibre could produce permanent effects. However, what happens to nucleic acid cannot be the whole story, as there are also peptides and proteins which are formed by processes at present unknown. The DNA present in the cell as a complex with basic proteins–either protamines or histones, which may have equally important functions. It must also be remembered that the chromosome is a much larger entity than the nucleic acid particle and chromosome breaks are a phenomenon belonging to a much larger order of magnitude than the separate DNA particles. In the rat chromosome there are approximately 10^5 DNA particles. If we identify these particles with the genes, they must, according to the results of genetics, be combined in a linear order. How can this occur? It is possible, of course, that the DNA particles are united directly with each other; but they undoubtedly come apart quite easily in the presence of 2M salt, and are then found to be present in solution as distinct particles. If I am permitted to speculate a little, one possibility is that they are joined by histone links. We have found[1] that in beef thymus nucleoprotein there are at least two distinct histones, which are distinguishable electrophoretically and have different compositions. One contains a larger proportion of lysine in place of the usual arginine as the principal basic amino-acid. It is possible that one of these histones provides longitudinal links between the DNA particles while the other provides lateral links either within a single particle determine the way in which the fibre is folded; or between parallel fibres (see Fig. 7)[1]. The junctions of the nucleic acid with histone, being probably made by a "salt-like" bond, will probably represent weak points at which breakage more easily occurs.

It is thus possible for the excitation, produced by the u.v. light absorbed in the nucleotide thread, to be transmitted down the column of parallel nucleotide plates, until it produces strong vibrations at the end which are able to break the bond which unites the DNA to the histone. The continuity of the chromosome would thus be broken. However, as I said above, the biological properties may depend on a higher degree of complexity than that carried by a single nucleic acid fibre. The activity of the transforming principle is greatly reduced by quantities of u.v. which have no effect on the viscosity of DNA[2], u.v. therefore causes damage which is not immediately apparent in the viscosity, although as we have seen it may become so on heating.

I have discussed briefly the kinds of chemical effects which may be expected to occur when u.v. light acts on living cells, and particularly on the chromosomes. It will be evident that much remains to be done before the circumstances in which the photochemical reactions occur are clearly defined. Still less is known about the biological consequences of chemical changes and it is probable that the most urgent task at the present time is the study of ways of distinguishing and defining the different kinds of radiation damage.

I am indebted to Mr. F. S. FEATES and Mr. JOHNS for assistance in the experiments quoted.

Résumé

L'auteur a examiné les conséquences chimiques de l'absorption de radiations ultra-violettes par les substances présentes dans les cellules vivantes (en particulier l'acide désoxyribonucléique).

Les effets observés sont dus: 1° à l'absorption directe des radiations; 2° à une action directe – ou activée photodynamiquement – sur l'oxygène présent. Les conséquences de cette dernière sont très semblables à celles produites par les rayons X.

En ce qui concerne l'absorption directe des radiations U.V., il se pourrait que la rupture des chromosomes soit due à celle des liens existant entre les chaînes de DNA et l'histone.

[1] P. F. DAVISON, D. W. F. JAMES, K. V. SHOOTER, and J. A. V. BUTLER, Biochim. Biophys. Acta *1954*.

[1] It has since been found that different DNA fractions are associated with the different histones [J. LUCY and J. A. V. BUTLER, Biochim. Biophys. Acta *16*, 431 (1955)]. The general picture of DNA-histone interactions given here remains possible.

[2] S. ZAMENHOF, H. E. ALEXANDER, and G. LEIDY, J. Exp. Med. *98*, 373 (1953).

ausmünzen lassen. In dieser Beziehung muß an die Eiskeimtheorie von BERGERON — ausgebaut durch FINDEISEN[1] — für die Niederschlagsbildung erinnert werden, die eine der wichtigsten Bedingungen für die Niederschlagsbildung zu unserer Kenntnis gebracht und eine Erklärung dafür gegeben hat, warum einmal Regen fällt, während ein anderes Mal bei scheinbar gleicher Wetterlage Regen und Schnee ausbleiben. Noch läßt sich aber nicht absehen, wann diese Erkenntnisse zu einer wesentlichen Verbesserung unserer Niederschlagsvorhersagen im täglichen Dienst führen werden.

Wenn die letzten Jahrzehnte seit Ende des ersten Weltkrieges zu großen Fortschritten in der Prognostik geführt haben, so ist dieser Fortschritt einerseits dem gewaltig anschwellenden, täglich einlaufenden Beobachtungsmaterial, andererseits den Ergebnissen der wissenschaftlichen Forschung zuzuschreiben. Man kann dabei fragen, ob und wann denn die Meteorologen imstande sein werden, wenigstens die alltäglichen Kurzfristprognosen mit absoluter Sicherheit auszuarbeiten bzw. das künftige Wetter mit Sicherheit vorauszuberechnen, da ja die Theoretiker bereits ein Gleichungssystem von genügendem Umfang zur Durchführung derartiger Berechnungen ausgearbeitet haben. V. BJERKNES[2] zum Beispiel hat an die grundsätzliche Möglichkeit einer exakt rechnerischen Lösung des Problems in der Zukunft geglaubt, während SCHMAUSS[3] schon vor langer Zeit die Unmöglichkeit absolut richtiger Prognosen betont hat. Erst ERTEL[4] hat aber nachgewiesen, warum selbst bei streng kausaler Auffassung der meteorologischen Vorgänge eine eindeutige Wettervorhersage für Teilgebiete der Atmosphäre prinzipiell unmöglich sei, weil für die mathematische Behandlung des Problems natürliche Randbedingungen für die willkürlichen Grenzen der Teilgebiete vorhanden sein müßten, was nicht der Fall ist. Jede brauchbare Prognose kann daher grundsätzlich nur eine mit einer Unbestimmtheit behaftete Approximation sein, die natürlich trotzdem ein brauchbares Ergebnis liefert, sofern die Unbestimmtheit unterhalb der Erfordernisse des praktischen Lebens bleibt.

Die Arbeit des Prognostikers wird also nie ein ganz zweifelsfreies Ergebnis liefern können. Mit dieser Einschränkung werden sich die Meteorologen bescheiden und ihr Augenmerk darauf richten müssen, die Approximation immer genauer zu gestalten. Aber nicht nur die Meteorologen, sondern auch die Öffentlichkeit, die absolut richtige Prognosen wünscht, muß sich mit dieser Sachlage abfinden.

[1] FINDEISEN, Meteor. Z. *55*, 121 (1938); *56*, 365 (1939).
[2] V. BJERKNES, Meteor. Z. *36*, 68 (1919).
[3] A. SCHMAUSS, Das Problem der Wettervorhersage (Leipzig 1937).
[4] H. ERTEL, Methoden und Probleme der dynamischen Meteorologie (Berlin 1938).

Summary

Since the first world war the reliability of weather forecasts based on synoptic maps has been greatly enhanced, owing on the one hand to the extraordinarily increased number of wireless weather reports, on the other hand to new scientific insight into weather developments gained up to now. Of especial importance were the data from high levels of the free atmosphere obtained by radio sounding, by which the construction of weather maps with contours was made possible. Scientific research after the first world war was based on the polar front theory of BJERKNES and his followers and was greatly furthered in the course of time especially by the understanding of the great importance of the occurrences in the stratosphere for weather development.

New Investigations on Enzymatic Glycolysis and Phosphorylation

By OTTO MEYERHOF[1], Philadelphia, Pa.

The achievements of the last decades in the fields of enzymology, intermediary metabolism, hormones, and vitamins are so overwhelming that one could be tempted to quote the ironic lines of GOETHE, spoken by the famulus Wagner in *Faust II*, as a serious interpretation of these endeavours:

Was man an der Natur Geheimnisvolles pries,
Das wagen wir verständig zu probieren,
Und was sie einst organisieren ließ,
Das lassen wir kristallisieren[2].

However, simultaneously with this progress, with the elucidation of the self-sustaining cycles of intermediary enzymatic reactions, and with the purification and crystallization of many enzymes, hormones, vitamins, and viruses, much self-restraint and sobriety has returned. The biochemists and physiologists now repudiate the materialistic fantasies and boastings of their predecessors. In our view the organization of the cell, the living unity itself can not be explained by purely physical and chemical methods. We restrict ourselves, therefore, to the study of the physical and chemical reactions of these organized, self-perpetuating living units.

About 1900 BUCHNER was successful in separating from the living yeast cell the enzymatic system which

[1] Department of Physiological Chemistry, School of Medicine, University of Pennsylvania.

[2] GOETHE'S *Faust II:*

The mystery which for man in Nature lies
We dare to test, by knowledge led,
And that which she was wont to organize
We crystallize instead.

converts sugar to alcohol and CO_2. The same separation was accomplished in 1926 for the glycolytic enzyme system of muscle[1]. Here glycogen is split into two mols of lactic acid per unit of hexose. Later on the same system was brought into solution from many other cells or organs, from brain, chicken embryo, malignant tumors, erythrocytes, and different kinds of bacteria.

The oxidative system is less easily brought into solution, some of its enzymatic components strongly adhering to structural elements—but many enzymes responsible for individual oxidative steps of sugar metabolism were also obtained in extracts.

Besides the insolubility of some components of the oxidative system (cytochromes a and b and cytochrome oxidase) it differs also in another sense from the glycolytic system, viz: there seems to be no common pathway of sugar oxidation in all cells and tissues, although the so-called tricarboxylic acid cycle of KREBS is prevalent in many of them[2]. We restrict our discussion in the following to the anaerobic breakdown, to glycolysis in the stricter sense of this term. Here the pathway is uniform.

Even if the end products of various forms of sugar fermentation are different, and consist instead of lactic acid as in animal tissue, of alcohol and CO_2 as in yeast, of H_2, acetic acid, lactic acid and CO_2 in coli bacteria, or of propionic or butyric acid as in propionic or butyric acid bacteria, all the steps leading from sugar to pyruvate are none the less identical, and only the fate of the pyruvate differs. Moreover, if the pyruvate remains as an end product, glycerol is formed simultaneously as a reduction product[3]. In the case of glycolysis of animal tissues (and also in lactic acid bacteria) the pyruvate reaction is a single reduction step:

$$\underset{\text{(pyruvic acid)}}{CH_3 \cdot CO \cdot COOH} + H_2 \rightarrow \underset{\text{(lactic acid)}}{CH_3 \cdot CHOH \cdot COOH}$$

Furthermore, the steps leading from sugar to pyruvic acid are also the initial steps of the prevalent form of sugar oxidation. (The oxidation of sugar to hexonic acids or of phosphohexoses to phosphohexonic acid is apparently of minor biological importance.) Since pyruvic acid can also be reversibly aminated, forming alanine, and also plays a role in the turnover of fat, it occupies a central position in intermediary cell metabolism.

I shall discuss the subject under three headings:

I. The scheme of intermediaries of glycolysis.
II. The phosphorylating mechanisms.
III. The isolated enzymes.

Our attention will be devoted mainly to the progress in the last eight years, before the Nazi regime in Germany and the second World War disrupted international science and scientific intercourse[1].

I. *The Scheme of Intermediaries of Glycolysis*

This scheme was fully developed between 1932–1939 and has undergone no further change since. All intermediaries from glucose-1-phosphate to phosphopyruvic acid are phosphorylated compounds. Most of them are true phosphoric acid esters, formed by condensation of an alcoholic (OH) group with the third valency H of phosphoric acid; some of them have the phosphate group linked to a carboxyl or carbonyl group. During the years of the development of this scheme some investigators and critics obstinately clung to the idea that this scheme was not ubiquitous, but that some organs were able to glycolyze in a different way by a so-called non-phosphorylating glycolysis. Even now the ghost of this non-phosphorylating glycolysis is still haunting some biochemical laboratories and the desks of some scientific writers. Actually, it never existed. It could be refuted in every instance where an experimental approach was feasible. Mostly the arguments in favor of it were fallacies in themselves: in many enzymatic systems, separated from the cell, the reactivity of glucose and hexosediphosphate (HDP) is altered. Often hexosediphosphate reacts with a much lower speed than sugar or not at all. This was used as an argument against this diphosphate as a common thoroughfare of sugar breakdown. But because of the transphosphorylation mechanism, which will be discussed in the next section, the speed of the global reaction can be much higher than the isolated single reaction steps; hexosediphosphate decomposes in such a system in the presence of free sugar much faster than in the absence of it[2]. The opposite can also be observed; viz.: that hexosediphosphate forms lactic acid under conditions where free sugar does not. Here the very sensitive enzyme which phosphorylates sugar (hexokinase) is destroyed by the extracting procedure.

There is also a positive argument: methyl glyoxal ($CH_3 \cdot CO \cdot COH$) is transformed in the presence of glutathione to lactic acid by a very widespread enzyme[3]. But methyl glyoxal itself, contrary to earlier assumptions, is not a physiological intermediary product of sugar breakdown. It arises from triosephosphate by a non-enzymatic decomposition[4]. In the living cell this reaction surely plays a subordinate role, if a role at all.

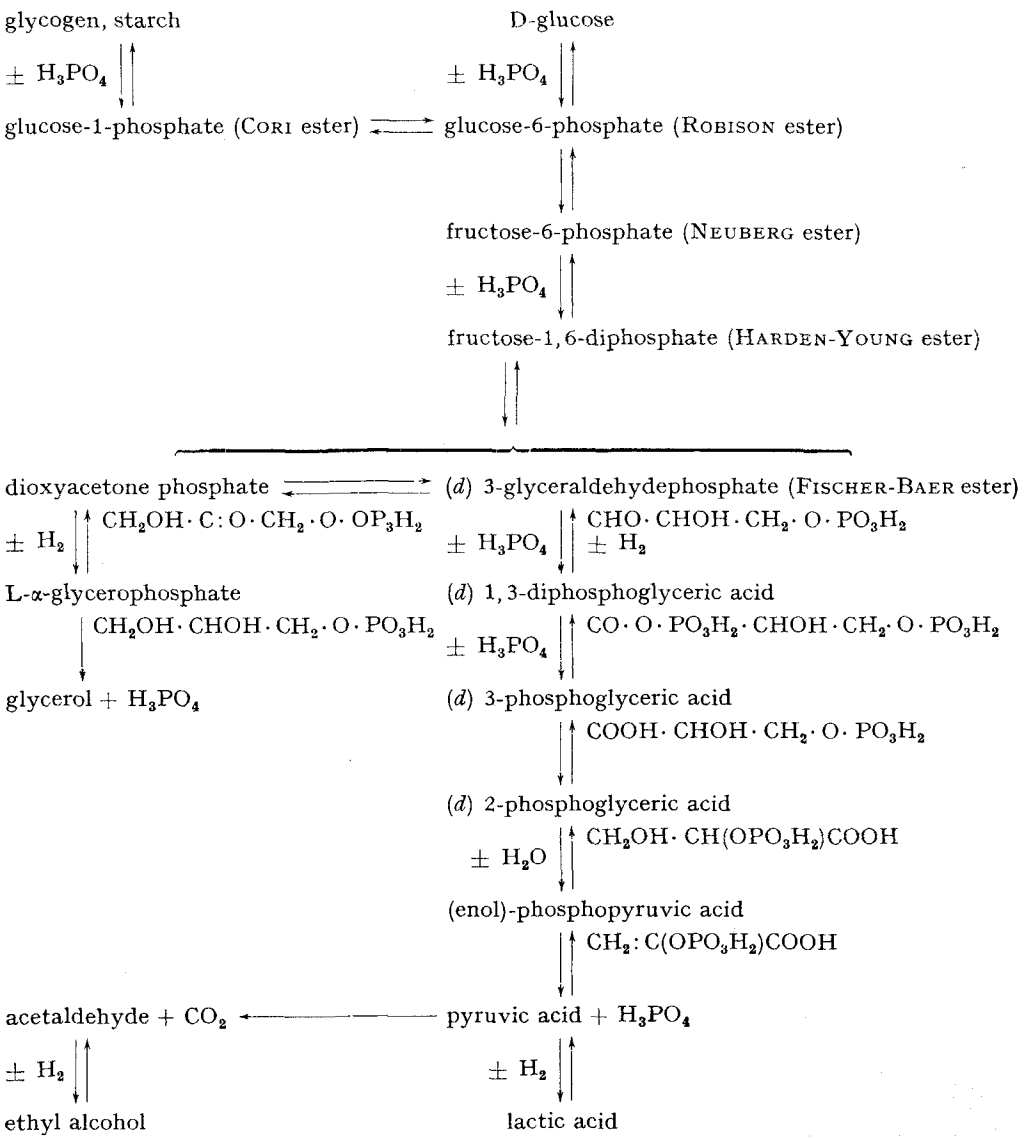
Several hexosemonophosphates other than those given in the scheme are synthesized and metabolized by the living cell. Mannose-6-phosphate is formed from

[1] O. MEYERHOF, Naturwiss. *14*, 1175 (1926).
[2] H. A. KREBS, Advances in Enzymology *3*, 191 (1943).
[3] C. NEUBERG and E. REINFURTH, Biochem. Z. *92*, 234 (1918).

[1] Former reviews: O. MEYERHOF, Erg. Physiol. *39*, 10 (1937). – J. K. PARNAS, Erg. Enzymforschung *6*, 57 (1937).
[2] O. MEYERHOF, W. KIESSLING, and W. SCHULZ, Biochem. Z. *292*, 25 (1937).
[3] K. LOHMANN, Biochem. Z. *254*, 332 (1932); *262*, 152 (1933).
[4] O. MEYERHOF and K. LOHMANN, Biochem. Z. *271*, 89 (1934).

Scheme

Two changes are introduced into the scheme as given in my publications of 1941 and 1942[1]. 1,3-diphosphoglyceraldehyde, supposed to be an intermediary by O. WARBURG and W. CHRISTIAN[2], is left out, because no such substance is formed[3]. The reaction between phosphopyruvic acid and pyruvic acid + phosphate, which was supposed to be irreversible[4], was proved to be reversible[5] like all the other intermediary steps and is now designated by a double arrow.



mannose as glucose- and fructose-phosphates from the respective sugars[6]. Galactose-1-phosphate[7], the analogue of glucose-1-phosphate, is formed in the liver during the assimilation of galactose and is probably also an intermediary in yeast if the latter is adapted to the fermentation of galactose. Fructose-1-phosphate, another analogue of glucose-1-phosphate, was first obtained by splitting off one phosphate group from hexosediphosphate[1]. It was also obtained by enzymatic synthesis from *d*-glyceraldehyde and dioxyacetonephosphate. The enzyme responsible for this synthesis was called aldolase, because this is an aldol-condensation; moreover, the exact chemical nature of the reversible splitting and synthesis of hexosediphosphate to and from triosephosphates was proved in this way[2]. Quite recently it was shown that fructose-1-phosphate may be formed by enzymatic phosphorylation of fructose by means of ATP[3].

[1] O. MEYERHOF, Biol. Symposia *5*, 143 (1941); Symposium on Respiratory Enzymes (Univ. of Wisconsin Press, Madison, 1942), p. 3.

[2] O. WARBURG and W. CHRISTIAN, Biochem. Z. *301*, 221 (1939); *303*, 40 (1939).

[3] O. MEYERHOF and P. OESPER, J. Biol. Chem. *170*, 1 (1947).

[4] O. MEYERHOF, P. OHLMEYER, W. GENTNER, and M. MEIER-LEIBNITZ, Biochem. Z. *298*, 396 (1938).

[5] H. A. LARDY and J. A. ZIEGLER, J. Biol. Chem. *159*, 343 (1945). – O. MEYERHOF and P. OESPER, Federation Proc. *7*, 174 (1948).

[6] C. M. JEPHCOTT and R. ROBISON, Biochem. J. *28*, 1844 (1934).

[7] H. W. KOSTERLITZ, Biochem. J. *37*, 319 (1943).

[1] B. TANKO and R. ROBISON, Biochem. J. *29*, 961 (1935).

[2] O. MEYERHOF, K. LOHMANN, and P. SCHUSTER, Biochem. Z. *286*, 301 (1936).

[3] G. T. CORI and M. W. SLEIN, Federation Proc. *6*, 246 (1947).

The last compound discovered in the scheme, 1,3-diphosphoglyceric acid (or phosphoglycerylphosphate) was found by NEGELEIN and BRÖMEL in 1939[1]. A similar compound, acetylphosphate,

$$\begin{array}{l} CH_3CO \\ | \\ OH_2PO_3 \end{array}$$

where the phosphate is likewise bound to a carboxyl group, was discovered by LIPMANN in 1940[2]. It is formed by lactic acid bacteria in the oxidation of pyruvate and was synthesized in the laboratory. Similar acylphosphates are probably also formed from fatty acids and may play a role in the oxidation of fat[3].

II. *Phosphorylating mechanisms*

The special meaning of the phosphorylation of the intermediaries is revealed by their interaction with the coenzyme systems. The coferment of fermentation, discovered by HARDEN in 1900, consists actually of three different coenzymes (besides inorganic ions like Mg^{++}):

(1) the hydrogen transferring cozymase, chemically, diphosphopyridinenucleotide (DPN);

(2) the phosphorylating coenzyme or adenylic system containing the three stages adenosintriphosphate (or adenylpyrophosphate or ATP), adenosindiphosphate (ADP), and adenylic acid (or adenosinmonophosphate or AA);

(3) the cocarboxylase, diphosphothiamine.

We will concern ourselves mainly with the second system. Phosphate is taken up or received from the intermediaries by transphosphorylation with the adenylic system. With the help of radioactive inorganic phosphate it was shown definitely that this phosphate transfer occurred without intermediary liberation of inorganic phosphate[4]. Phosphorylation of glucose, therefore, in the presence of the specific enzyme "hexokinase", goes on according to the equation:

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose-6-phosphate} + \text{ADP}$$

Dephosphorylation of phosphopyruvic acid, accordingly, obeys the equation:

$$\text{phosphopyruvic acid} + \text{ADP} \rightarrow \text{pyruvate} + \text{ATP}$$

Taking both equations together we have the phosphate transfer:

$$\text{phosphopyruvate} + \text{glucose} \xrightarrow{\text{ATP}} \text{pyruvate} + \text{glucose-6-phosphate.}$$

This transfer in the stationary state of glycolysis therefore goes on with minute amounts of ATP, which acts as a true catalyst in the presence of a specific transphosphorylase.

The phosphate bond in the adenylic system is "energy rich"; this fact is of primary importance. With the splitting of ordinary ester-phosphate linkages, 1,200 to 1,500 cal heat per mol are liberated; with the splitting of each single P group of the labile phosphate of ATP, 12,000 cal are liberated[1]. The free energy change is similar. In the phosphorylation process of glycolysis, phosphate is first taken up in an "energy poor" bond of the hexosephosphate, and by the transformation of the molecule, especially with the help of the oxidative step, it is changed into an energy rich bond. This high energy phosphate is transferred as such to the adenylic system and eventually to other substrates, which preserve the energy rich bond. The phosphorylating mechanism, therefore, serves for the generation and storage of energy rich phosphate bonds. How does the oxidative step of glycolysis generate such a bond? Glyceraldehyde-3-phosphate by itself does not react with inorganic phosphate. But if both are brought together with the purified oxidizing enzyme of O. WARBURG and DPN, a reaction takes place in which DPN is reduced and 1,3-diphosphoglyceric acid originates[2]. Apparently a loose addition product forms between glyceraldehyde-phosphate and phosphate on the surface of the enzyme with the expenditure of an exceedingly small amount of energy[3]. But when by reaction with DPN the carbonyl group is oxidized to the carboxyl group the acylphosphate now has a bond with the free energy of nearly 15,000 cal. Thus the energy of oxidation of the aldehyde to carboxyl is captured in this phosphate group. By transphosphorylation with the adenylic system it is stored in ATP:

$$\text{1,3-diphosphoglyceric} + \text{ADP} \rightleftarrows \text{3-phosphoglyceric} + \text{ATP.}$$

In consequence of the internal rearrangement of 3-phosphoglyceric acid by way of 2-phosphoglyceric acid to enol-phosphopyruvate, a second energy-rich phosphate bond is created (enol-phosphate), which likewise transphosphorylates with ATP[4].

The free energy (ΔF), as distinct from the total energy or heat (ΔH), amounts to 11,000 cal for each of the labile P groups of ATP[5]. This available store of phosphate bond energy can be used either for biochemical synthesis or for the transformation into mechanical or electrical energy. Indeed, the new work of SZENT-GYÖRGYI gives us every reason to believe that ATP reacts directly with the contractile protein

[1] O. WARBURG and W. CHRISTIAN, Biochem. Z. *301*, 221 (1939); *303*, 40 (1939).
[2] F. LIPMANN, J. Biol. Chem. *134*, 463 (1940).
[3] A. LEHNINGER, J. Biol. Chem. *161*, 432 (1945); *162*, 333 (1946). – H. J. KOEPSELL, M. J. JOHNSON, and J. S. MEEK, J. Biol. Chem. *154*, 535 (1944).
[4] O. MEYERHOF, P. OHLMEYER, W. GENTNER, and M. MEIER-LEIBNITZ, Biochem. Z. *298*, 396 (1938).

[1] O. MEYERHOF and K. LOHMANN, Biochem. Z. *253*, 431 (1932).
[2] O. WARBURG and W. CHRISTIAN, Biochem. Z. *301*, 221 (1939); *303*, 40 (1939).
[3] O. MEYERHOF and P. OESPER, J. Biol. Chem. *170*, 1 (1947).
[4] J. K. PARNAS, P. OSTERN, and T. MANN, Biochem. Z. *272*, 64 (1934).
[5] O. MEYERHOF, Ann. New York Acad. Sci. *45*, 9, 377 (1944). – F. LIPMANN, Advances in Enzymology *1*, 99 (1941).

of muscle ("actomyosin") and causes such modifications in its physical structure that it can contract or relax, depending on slight changes in concentration of K^+ and Mg^{++} ions[1].

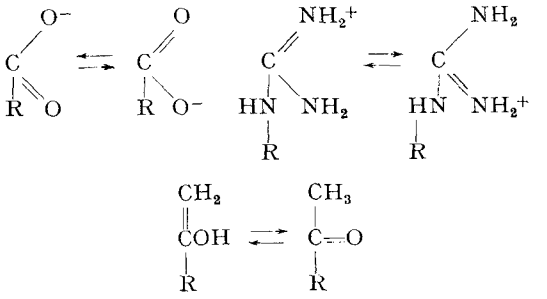
There exists another large store of high phosphate bond energy in those organs and cells which need this energy for transformation (by way of ATP) into mechanical or electrical energy. These cells are: voluntary muscle, heart, spermatozoa, the electric organs of electric fishes, and the central nervous system. All these cells and tissues are rich in phosphocreatine (vertebrates) or phosphoarginine (invertebrates). The phosphate bond energy is stored in these compounds without loss by means of the reversible reaction:

$$\text{ATP} + 2 \text{ creatine} \rightleftarrows \text{AA} + 2 \text{ phosphocreatine}.$$

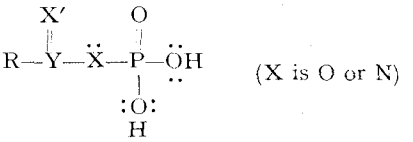
The same reaction applies to phosphoarginine.

The phosphate bond energy of ATP can also be used for endothermic (and endergonic[2]) syntheses, i.e., syntheses which need an outside source of free energy in order to occur. Especially interesting in this respect is the finding of VOGLER and UMBREIT that the energy of an inorganic chemical reaction, the oxidation of sulfur, serving as a source of energy for autotrophic Thiobacteria, *Thiobacillus thio-oxidans*, can be stored in the energy rich phosphate bond of ATP and used afterwards for the reduction of CO_2[3]. Less substantiated seems the hypothesis that a similar mechanism may be involved in the CO_2 assimilation of the green plants.

The question may be asked: what are the characteristics of an energy rich phosphate bond as distinguished from an ordinary ester bond? All the energy rich phosphates, such as acylphosphates, guanidinophosphates, enolphosphates, and pyrophosphates are formed with organic groups of high resonance. The resonance in such groups as carboxyl or guanidyl or enol (in equilibrium with keto) is produced by the oscillation of two valency electrons (or a hydrogen atom) between two otherwise identical configurations:

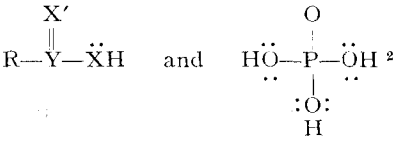


Moreover, because the phosphate molecule exhibits resonance between its different hydroxyl groups, the bridge of the ester linkage $-O-$ or $-N-$ is influenced by "opposing resonance" of the organic group and the phosphate group[1]. This makes such a structure unstable:



(X is O or N)

It leads to a great liberation of free energy when it is hydrolyzed to



and

If no endothermic syntheses occur, as for instance in the enzymatic extract of yeast, the labile phosphate of ATP, regenerated by the two steps of glycolysis already mentioned (the acylphosphate in 1,3-phosphoglyceric acid and the enolphosphate of phosphopyruvate) is transferred to new hexose molecules by the reactions:

$$\text{hexose} + \text{ATP} \rightarrow \text{hexosemonophosphate} + \text{ADP}$$

$$\text{hexosemonophosphate} + \text{ATP} \rightarrow \text{hexosediphosphate} + \text{ADP}$$

Every mol of HDP formed in this way gives rise to 2 mols of triosephosphate, each of which again produces 2 energy rich phosphate bonds, or together four. On the other hand, only two are needed to form hexosediphosphate. This leads to the autocatalytic increase of hexosediphosphate, or "HARDEN-YOUNG ester", in yeast extract, a reaction which was originally discovered (although misinterpreted) by HARDEN and YOUNG in 1905. In the living cell a very active ATP-ase counteracts this increase by decomposing the excess of ATP. But this enzyme is strongly bound to the structural elements of the cell and only present in traces in the enzymatic extracts[3, 4]. In the living cell phosphorylation and dephosphorylation are kept in step by the ATP-ase.

Another group-transferring enzymatic mechanism may be briefly described: the "transglucosidation", which is also involved in the remarkable reversible phosphorolysis of glycogen. CORI[5], who had discovered the splitting of glycogen to glucose-1-phosphate, showed later on that the reversal of this reaction (observed by KIESSLING[6] as well as by the school of CORI) does not start with completely pure (or crystallized) en-

[1] A. SZENT-GYÖRGYI, Muscular Contraction (Acad. Press, New York, 1947).
[2] C. D. CORYELL, Science *92*, 380 (1940).
[3] K. G. VOGLER and W. UMBREIT, J. Gen. Physiol. *26*, 157 (1942).
[1] M. KALCKAR, Chem. Rev. *28*, 72 (1941).
[2] For organic pyrophosphate a similar reasoning applies.
[3] O. MEYERHOF, J. Biol. Chem. *157*, 105 (1945).
[4] This is the origin of the somewhat puzzling "HARDEN-YOUNG equation", stating that for the fermentation of one mol of hexose a second mol of hexose is esterified to hexosediphosphate.
[5] C. F. CORI, G. T. CORI, and S. P. COLOWICK, J. Biol. Chem. *121*, 465 (1937).
[6] W. KIESSLING, Naturwiss. *27*, 129 (1939).

zyme, from the side of glucose-1-phosphate, unless some glycogen or soluble starch is already present as a "primer". The reaction, therefore, must be formulated[1]:

glucose-1-phosphate + glucosidic linkage ⇄ glucose-glucosidic linkage + phosphate

Because adenylic acid is a necessary part of this enzymatic mechanism (either firmly bound to the protein or as a dissociable coenzyme[2]) the uptake of phosphate occurs probably by way of intermediate phosphorylation of adenylic acid. On the other hand the main reaction here is not a transphosphorylation but a "transglucosidation". This was recently proved by a group of California workers[3]. E.g., in *Pseudomonas sacharophila*, an enzyme exists which catalyzes the reaction:

glucose-1-phosphate + fructose → glucosefructoside (sucrose) + phosphate.

Different ketoses and also aldopentoses can replace fructose, while a glucose-1-linkage (carbonyl bond) is always needed to synthesize a disaccharide. That the glucose unit is transferred in these cases, is neatly demonstrated when the phosphorylase is added to a solution of glucose-1-phosphate in the presence of inorganic phosphate, which contains radioactive P, but in the absence of any other sugar, which could act as "acceptor". Then a free exchange of radioactive P takes place between inorganic phosphate and glucose-1-phosphate without any glucose appearing. The reaction is[4]:

glucose-1-phosphate + enzyme → glucose-enzyme + phosphate.

The glucose-enzyme compound retains the free energy of about 2,000 cal of the carbonyl phosphate and can use it, if a ketose is added to it, for the formation of a disaccharide. Such a transglucosidation, moreover, can take place in the absence of bound and free phosphate, when a mixture of glucose-1-fructose (sucrose) and a second ketose is present. Here, reversible exchange reactions of the following type occur:

glucose-1-fructoside + sorbose ⇄ glucose-1-sorboside + fructose.

Still another type of enzymatic phosphorolysis, very similar to those described, was recently discovered by KALCKAR[5]. Here ribose, the aldopentose which is a constituent of the nucleotides, takes the place of glucose in forming the carbonylphosphate, ribose-1-phosphate. This reaction may be formulated:

ribose-1-purine + phosphate ⇄ ribose-1-phosphate + purine.

The purine may be hypoxanthine or guanine. This phosphorolysis, which replaces the glucosidic bond with the imidazol nitrogen, is probably an important step in the metabolism of the nucleic acids.

III. *Studies of individual enzymes*

From the host of intermediary reactions, which are connected either with the transformation of the intermediaries themselves or with their interaction with the various coenzymes, many pure enzymes were isolated, and in some cases crystallized. Of these purified enzymes, I shall discuss three where especially interesting information was gained in the last years.

A. *Enolase*

It was found in the Heidelberg laboratory (LOHMANN and MEYERHOF, and KIESSLING[1]) that enolphosphopyruvate was formed from 2-phosphoglyceric acid by an enzyme called enolase. This enzyme was also responsible for the inhibition of glycolysis by NaF. Although many dephosphorylating enzymes are also sensitive to fluoride, they need much higher concentrations for the same inhibition. In consequence, phosphoglyceric acid accumulates in glycolyzing enzyme extracts in the presence of NaF. By blocking the enolase, fluoride inhibition acts as a barrier behind which the last intermediary piles up because it cannot be further transformed. But this barrier remains tight only if enough inorganic phosphate is present; phosphate is therefore also a component of the inhibiting system[2]. This situation was further cleared up in 1941 by WARBURG and CHRISTIAN[3]. Enolase, obtained in pure form from the crystallized Hg salt, needed a bivalent cation for its action, preferentially Mg or Mn. If Mg is present (this must be regarded as the physiological cation of the reaction) and inorganic phosphate, a Mg–F-phosphate complex forms, which deprives the enzyme protein of its necessary Mg. This explains the fluoride inhibition. Probably the same explanation holds for the inhibition of ATP-ase by fluoride, since this enzyme likewise needs Mg. The fact that Mg is a necessary coenzyme factor of glycolysis and fermentation was already discovered in 1931 by LOHMANN[4].

B. *Hexokinase*

Hexokinase, the enzyme necessary for initiating the turnover of glucose or fructose, was separated from yeast and partially purified in 1927[5]. In 1935 it was demonstrated that this initiating reaction was the

[1] C. F. CORI, G. T. CORI, and A. A. GREEN, J. Biol. Chem. *151*, 39 (1943); Federation Proc. *4*, 234 (1945).
[2] G. T. CORI and A. A. GREEN, J. Biol. Chem. *151*, 31 (1943). – G. T. CORI and C. F. CORI, J. Biol. Chem. *158*, 321 (1945).
[3] M. DOUDOROFF, J. Biol. Chem. *151*, 351 (1943).
[4] W. Z. HASSID, M. DOUDOROFF, and H. A. BAKER, Arch. Biochem. *14*, 29 (1947).
[5] H. M. KALCKAR, J. Biol. Chem. *167*, 477 (1947).

[1] K. LOHMANN and O. MEYERHOF, Biochem. Z. *273*, 60 (1934). – W. KIESSLING, Chem. Ber. *68*, 597 (1935).
[2] O. MEYERHOF and W. SCHULZ, Biochem. Z. *297*, 66 (1938).
[3] O. WARBURG and W. CHRISTIAN, Naturwiss. *29*, 589 (1941).
[4] K. LOHMANN Biochem. Z. *237*, 445 (1931).
[5] O. MEYERHOF, Biochem Z *183*, 176 (1927).

phosphorylation of the hexoses by ATP[1]. KALCKAR proved in 1942 that the total reaction induced by hexokinase in a muscle extract:

2 glucose + ATP = 2 glucose-6-phosphate + AA (adenylic acid)

is the combined effect of two different enzymes[2]. Pure hexokinase is able to catalyze only the reaction:

glucose + ATP = glucosemonophosphate + ADP

A heat stable enzyme in muscle, called myokinase, completes the reaction by dismutating the ADP:

$$2\ \text{ADP} \rightleftarrows \text{ATP} + \text{AA}.$$

In this way ATP is formed anew and reacts again with a sugar molecule, if hexokinase is present, until practically all is finally transformed into adenylic acid.

Hexokinase was recently brought into the focus of interest on account of its specific sensitivity in the animal body. During the war the high sensitivity of this enzyme to vesicants, like mustard gas and similar substances, was discovered by English investigators[3]. Since the blistering effect on the skin was exactly parallel to the inhibition of the hexokinase in the skin, they assumed this enzyme to be the locus of attack of these poisons. Although this interpretation is not generally accepted, it stimulated the study of this enzyme and led to its crystallization from yeast.

The physiological importance of hexokinase was still more underscored by recent work in CORI's laboratory on the insulin effect[4]. The known antagonism in the living animal between the hyperglycemic principle in the anterior pituitary gland (HOUSSAY factor) and the adrenal cortical hormone on the one side and insulin on the other, can be duplicated with purified hexokinase from muscle. The activity of purified hexokinase of an alloxan diabetic rat or from the normal rat treated with anterior pituitary hormone, is lower than from the controls and still more so if adrenal cortical extract is added to the hexokinase. This inhibition is relieved by insulin, which has no effect in the absence of the antagonists. This finding undoubtedly has an important bearing on the role of insulin in the normal and diabetic organism, even if this were not the only enzymatic mechanism influenced by insulin[5].

Finally, the different reactivity of glucose and fructose in biological systems is caused by their affinity to hexokinase. Whether we have to assume generally the presence of two hexokinases, glucokinase and fructokinase, is still not definitely known[6]. Independently of this problem a puzzling discrepancy was observed between glycolysis in the intact tissue, in tissue slices, in homogenates (disintegrated cells suspended in Ringer solution) and glycolysis in extracts free of structures[1]. In the first named types of tissue preparations from brain or malignant tumor glucose is much more quickly metabolized than fructose but the difference disappears in extracts from the same tissues; here, the total reactivity of both sugars is much greater and almost equal. The distinguishing factor is, as has recently been shown, the different concentration of ATP. The ATP splitting enzyme, the ATP-ase, is strongly adsorbed on the cell structures and so long as these are present, ATP is kept down by the activity of this enzyme. In this low range of ATP the affinity of fructose is much less than that of glucose, but in a higher range their affinity is equal. If these structures are removed, the same difference can be obtained in the extract with concentrations of added ATP much lower than those found by analysis in the living cell. Incidentally, it follows from these results that the *active* concentration of ATP in the living cell where the difference in the rates of fructose and glucose turnover is very conspicuous must be a small fraction of the total ATP found by analysis. The bulk of it is either separated by cell structures from the enzymes or otherwise bound to inert proteins.

C. *Carboxylase*

K. LOHMANN showed in 1937 that cocarboxylase, separable from the enzyme carboxylase by washing yeast with slightly alkaline solutions, is nothing else but diphosphothiamine, the diphosphoric ester of vitamin B_1[2]. The carboxylase reaction of yeast:

$$\text{pyruvic acid} \rightarrow \text{acetaldehyde} + CO_2$$

does not occur in the animal. But the important observation of PETERS[3], that in vitamin B_1 deficient animals pyruvate metabolism is inhibited and pyruvate concentration in the blood is strongly increased, proves that here also the breakdown of pyruvate needs cocarboxylase. The effectiveness of free thiamin in relieving the symptoms of B_1 deficiency in animals is easily explained by the reversible reaction[4]:

$$\text{thiamin} + 2\ \text{ATP} \rightleftarrows \text{thiaminediphosphate} + 2\ \text{ADP}.$$

The difference of the reaction of pyruvate in yeast and in the animal must be attributed to the specific enzyme proteins[5]. In the latter case the decarboxylation is always coupled with oxidation:

$$\text{pyruvate} + O = \text{acetate} + CO_2.$$

[1] O. MEYERHOF, Naturwiss. *23*, 850 (1935).
[2] H. M. KALCKAR, J. Biol. Chem. *148*, 127 (1943); *153*, 385 (1944).
[3] M. DIXON and D. M. NEEDHAM, Nature *158*, 432 (1946).
[4] W. H. PRICE, C. F. CORI, and S. P. COLOWICK, J. Biol. Chem. *160*, 633 (1945).
[5] S. P. COLOWICK, G. T. CORI, and M. W. SLEIN, J. Biol. Chem. *168*, 583 (1947).
[6] G. T. CORI and M. W. SLEIN, Federation Proc. *6*, 246 (1947).

[1] O. MEYERHOF and N. GELIAZKOWA, Arch. Biochem. *12*, 405 (1947). – O. MEYERHOF, Arch. Biochem. *13*, 485 (1947). – O. MEYERHOF and J. R. WILSON, Arch. Biochem. *14*, 71 (1947).
[2] K. LOHMANN and P. SCHUSTER, Biochem. Z. *294*, 183 (1947).
[3] R. PASSMORE, R. A. PETERS, and H. M. SINCLAIR, Biochem. J. *27*, 842 (1933). – R. A. PETERS, Lancet *230*, 1161 (1936). – I. BANGA, S. OCHOA, and R. A. PETERS, Biochem. J. *33*, 1109 (1939).
[4] S. OCHOA, Biochem. J. *33*, 1262 (1939).
[5] D. E. GREEN, W. W. WESTERFELD, R. VENNESLAND, and W. E. KNOX, J. Biol. Chem. *140*, 683 (1941). – S. OCHOA, Evans' Biol. Action of Vitamins (Univ. of Chicago Press, 1942), p. 17.

F. LIPMANN discovered[1] that as intermediate acetylphosphate was formed, the phosphate group was taken up from inorganic phosphate and attached to the carboxyl group in the same way as in 1,3-phosphoglyceric acid. Because this enzyme has not yet been obtained in pure state and flavine protein and possibly diphosphopyridinenucleotide are also components of the system, it cannot be stated definitely whether these oxidations are one step reactions or not. Even in the case where the final decomposition of pyruvate is achieved by the way of synthetic reactions involving the tricarboxylic acid cycle of KREBS, cocarboxylase is a necessary component of the system.

The characteristic feature which we have already encountered with the other coenzymes, the adenylic system and cozymase, is likewise true for cocarboxylase: viz. the exact specifities of the reactions are bound to the enzyme proteins while the coenzymes exhibit only a sort of class specifity, as H_2 transporting, phosphate transferring, or decarboxylating agents. This is also true for other coenzymes like riboflavin or pyridoxal, etc.

Conclusions

Carbohydrate metabolism furnishes some very striking examples of the biological function of vitamins and hormones. As is now more and more revealed by the studies of intermediary metabolism, vitamins must be generally regarded as constituents of the effective groups of enzymes or coenzymes. Nicotinamide, the pellagra vitamin, is the reacting group of the cozymase, thiamin of the cocarboxylase, and adenylic acid plays also some role as a sort of vitamin. Quite recently it was found by LIPMANN and his group[2] that pantothenic acid (dihydroxydimethylbutyryl-β-alanide), another B-vitamin, is a constituent of the coenzyme of acetylation and acetate oxidation. In this way a great part of the acetic acid arising during the oxidation of carbohydrate and fat is disposed of.

Hormones on the other hand combine with enzymes, forming compounds of an increased or diminished activity, thereby controlling or regulating the speed of turnover of the metabolites. This is exemplified by the role of the cortical hormone and insulin on hexokinase.

It should therefore be acknowledged that the approach to intermediary metabolism described here, which tries to separate the global metabolic reaction into its constituting elements and to study the behavior and kinetics of the individual enzymatic steps, is not only of theoretical or academic interest but has yielded also results of great practical importance for general medicine, agriculture, and nutrition.

Résumé

Le schéma des étapes du dédoublement des glucosides dans la glycolyse des tissus animaux est exposé ici conformément aux résultats obtenus de 1932 à 1939. Toutes les substances intermédiaires sont phosphorylées. La transphosphorylation s'accomplit selon le système adénylique (acides adénosinetri-, di- et monophosphoriques). La grande énergie libre de ce groupe phosphorique n'est pas perdue, mais transmise aux intermédiaires de la glycolyse et s'accumule dans la phosphocréatine. Un autre type de phosphorylation est représenté par la phosphorolyse réversible du glycogène, avec formation du glucose-1-phosphate. Ce type est en réalité une «transglucosidation» comme on peut le démontrer dans la formation semblable du disaccharide à partir du glucose-1-phosphate et de la lévulose. En l'absence de la lévulose, le glucose-1-phosphate réagit avec l'enzyme en proportion stœchiométrique, et conserve l'énergie du groupe phosphorique dans la combinaison du glucose avec l'enzyme.

L'article traite des nombreuses réactions enzymatiques des enzymes purifiées et cristallisées et discute plus en détail la fonction des trois enzymes, soit: la transformation de l'acide 2-phosphoglycérique en phosphoénolpyruvique par l'énolase, la phosphorylation de la glucose par l'adénosinetriphosphate en présence de l'hexokinase, et le dédoublement de l'acide pyruvique par la carboxylase. Le rôle des vitamines comme groupes essentiels des coenzymes, et celui des hormones comme activateurs et inhibiteurs des enzymes sont mis en évidence dans les réactions métaboliques des glucosides.

[1] F. LIPMANN, J. Biol. Chem. *134*, 463 (1940).

[2] F. LIPMANN, N. O. KAPLAN, G. D. NOVELLI, L. C. TUTTLE, and B. M. GUIRARD, J. Biol. Chem. *167*, 869 (1947). – F. LIPMANN and G. D. NOVELLI, Arch. Biochem. *14*, 23 (1947).

La biologie des hématinoprotéides oxygénables[1]

Par MARCEL FLORKIN[2], Liège

On trouve dans toutes les cellules aérobies une série de biocatalyseurs de la famille des hématinoprotéides: les trois cytochromes, la peroxydase, la catalase. Souvent aussi, on y trouve la substance que KEILIN appelle «unspecific cell hematin». Selon STERN et MELNICK[3] les cellules aérobies contiennent un autre enzyme hématinoprotéidique, «l'enzyme de la réaction de Pasteur» qui catalyse l'action inhibitrice de l'oxygène sur la fermentation et la glycolyse.

Ces différentes substances fonctionnelles interviennent dans des processus biochimiques mettant primordialement en jeu la liaison chimique existant entre deux atomes d'oxygène, que ces atomes soient liés entre eux comme dans la molécule d'oxygène, ou

1 Conférence principale, présentée à la Société suisse de biologie médicale lors de la 127e Assemblée générale de la Société helvétique des sciences naturelles à Genève, le 31 août 1947.

2 Laboratoires de biochimie de l'Université de Liège.

3 K. G. STERN et J. L. MELNICK, J. biol. Chem. *139*, 301 (1941).

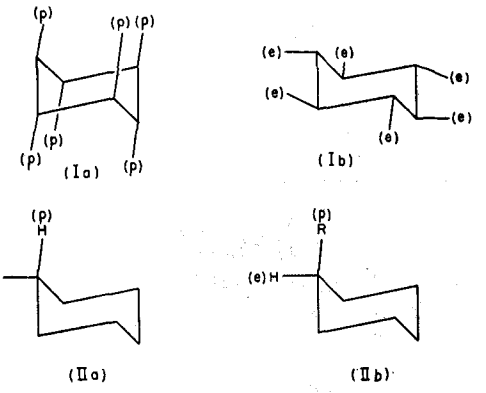
STUDIORUM PROGRESSUS

The Conformation[1] of the Steroid Nucleus

By D. H. R. BARTON[2], Cambridge, Mass.

In recent years it has become generally accepted that the chair conformation of cyclohexane is appreciably more stable than the boat. In the chair conformation it is possible[3,4] to distinguish two types of carbon-hydrogen bonds; those which lie as in (Ia) perpendicular to a plane containing essentially the six carbon atoms and which are called[3] *polar* (p), and those which lie as in (Ib) approximately in this plane. The latter have been designated[3] *equatorial* (e).

The notable researches of HASSEL and his collaborators[5,6] on the electron diffraction of cyclohexane derivatives have thrown considerable light on these more subtle aspects of stereochemistry. Thus it has been shown[5] that monosubstituted cyclohexanes adopt the equatorial conformation (IIa) rather than the polar one (IIb). This is an observation of importance for it indicates that the equatorial conformations are thermodynamically more stable than the polar ones. It should perhaps be pointed out here that although one conformation of a molecule is more stable than other



possible conformations, this does *not* mean that the molecule is *compelled* to react as if it were in this conformation or that it is rigidly fixed in any way. So long as the energy *barriers* between conformations are small, separate conformations cannot be distinguished by the classical methods of stereochemistry. On the other hand a small difference in free energy content (about one kilocal. at room temperature) between two possible conformations will ensure that the molecule appears by physical methods of examination and by thermodynamic considerations to be substantially in only *one* conformation.

[1] The word conformation is used to denote differing strainless arrangements in space of a set of bonded atoms. In accordance with the tenets of classical stereochemistry, these arrangements represent only one molecular species.

[2] Harvard University Visiting Lecturer, 1949–50, Harvard University, Cambridge 38, Mass.

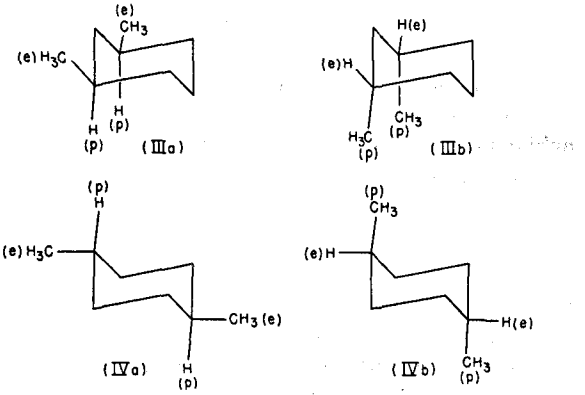
[3] C. W. BECKETT, K. S. PITZER, and R. SPITZER, J. Amer. Chem. Soc. *69*, 2488 (1947).

[4] O. HASSEL's nomenclature[5] is different, but the distinction remains the same.

[5] O. HASSEL and H. VIERVOLL, Acta Chem. Scand. *1*, 149 (1947).

[6] See O. HASSEL and B. OTTAR, Acta chem. Scand. *1*, 929 (1947) for a summarizing paper and references to earlier work.

The equatorial conformations are also the more stable in both cis-1:3- and trans-1:4- disubstituted cyclohexanes[1]. Thus cis-1:3-dimethylcyclohexane adopts the diequatorial conformation (IIIa) rather than the dipolar one (IIIb), whilst trans-1:4-dimethylcyclohexane exists as (IVa) rather than (IVb).



Thermodynamic calculations[1] show that trans-1:2-dimethylcyclohexane takes up the diequatorial conformation (V; R=CH_3) rather than the dipolar one (VI; R=CH_3). For cis-1:2-disubstituted cyclohexanes there are two possible conformations. In both of these one of the substituents forms an equatorial bond, the other a polar one. Since these differences in thermodynamic stability between equatorial and polar conformations are presumably of steric origin[1], it would appear logical to make the larger substituent form the equatorial bond.

Considerations of the same type can be extended to 2-substituted cyclohexanols. Thus[2,3] the cis-alcohols (VII; R= alkyl), on equilibration by heating with sodium, furnish almost entirely the trans-isomers (VIII; R= alkyl). In the former one substituent is polar, one equatorial; in the latter both are equatorial. The same conclusion on relative stability is reached from a consideration of thermochemical data[4]. Similarly[5] the 2:6-disubstituted cyclohexanol (IX), with two equatorial and one polar substituents, is isomerized to (X) on equilibration. The situation is the same[3] with the bicyclic trans-α-decalol. Here the isomer (XI) is isomerized to (XII) on equilibration.

A consideration of the conformations[6] (XIII) and (XIV), assumed by the steroid nucleus when the A/B ring fusion is respectively trans- and cis-, provides a striking illustration of the usefulness of the concept of

[1] C. W. BECKETT, K. S. PITZER, and R. SPITZER, J. Amer. Chem. Soc. *69*, 2488 (1947).

[2] G. VAVON, Bull. Soc. Chim. [4], *49*, 937 (1931).

[3] W. HÜCKEL, Ann. Chem. *533*, 1 (1937).

[4] A. SKITA and W. FAUST, Ber. Dtsch. Chem. Ges. *64*, 2878 (1931).

[5] G. VAVON and P. ANZIANI, Bull. Soc. Chim. [5], *4*, 1080 (1937). In connection with the conformations of poly-substituted cyclohexanes it should be mentioned that O. BASTIANSEN, O. ELLERSEN, and O. HASSEL, (Acta chem. Scand. *3*, 918 [1949]) have recently shown that the five stereoisomeric benzene hexachlorides assume, in agreement with our general argument, those conformations which have the maximum possible number of equatorial carbon-chlorine bonds.

[6] Conformations (XIII) and (XIV) are unambiguous representations of the steroid nucleus provided that rings A, B, and C are chairs. This is almost certainly true for a trans-A/B ring fusion (compare the X-ray evidence of C. H. CARLISLE and D. CROWFOOT (Proc. Roy. Soc. A *184*, 64 [1945]) on the conformation of cholesteryl iodide) and a similar situation, at least in solution, probably holds for a cis-A/B fusion. The justification for the latter has been more

Table I

Observation	Exptl. Method	References
Cholestane Series		
2α (e) more stable than 2β (p)	Reduction of 2-one	L. RUZICKA, P. A. PLATTNER, and M. FURRER, Helv. chim. acta *27*, 524 (1944).
3β (e) more stable than 3α (p)	Equilibration	pp. 98, 636*
4α (e) more stable than 4β (p)	Reduction of 4-one**	R. TSCHESCHE and A. HAGEDORN, Ber. *68*, 2247 (1935). – L. RUZICKA, P. A. PLATTNER, and M. FURRER, loc. cit.
6α (e) more stable than 6β (p)	Reduction of 6-one	pp. 223, 653.
7β (e) more stable than 7α (p)	Reduction of 7-one***	I. M. HEILBRON, W. SHAW, and F. S. SPRING, Rec. Trav. Chim. *57*, 529 (1938).
5β (e, p), 6α (e)-dibromide more stable than 5α (p), 6β (p)-dibromide	Equilibration	D. H. R. BARTON and E. MILLER, J. Amer. Chem. Soc. *72*, 1066 (1950).
Coprostane Series		
3α (e) more stable than 3β (p)	Equilibration	pp. 99, 636.
11α (e) more stable than 11β (p)	Equilibration	L. F. FIESER, preceding paper.
12β (e) more stable than 12α (p)	Equilibration	pp. 461, 657.

* All references reported in this way are to L. F. FIESER and M. FIESER, *Natural Products Related to Phenanthrene* (3rd Edition, 1949, Reinhold Publishing Corp.).

** The configurations are assigned (*vide infra*).

*** According to the standard tables of D. H. R. BARTON and W. KLYNE (Chem. and Ind. 755 [1948]) 7β-hydroxycholestane should have $[\alpha]_D$ *ca.* +52°, whilst the 7α-isomer should exhibit $[\alpha]_D$ *ca.* +8°. I. M. HEILBRON, W. SHAW, and F. S. SPRING, *loc. cit.*, observed $[\alpha]_D$ +51° and therefore the configuration of their alcohol must be 7β-.

polar and equatorial bonds. The relationship between the α- and β-nomenclature introduced by FIESER[1] and the occurrence of polar and equatorial bonds is also summarized in (XIII) and (XIV).

Thermodynamic Considerations. In a number of cases equilibration of hydroxyl groups at secondary positions in the steroid nucleus has been carried out. At other positions the corresponding ketones have been reduced by sodium and alcohol, a process which (in cyclohexane derivatives) is well established to give the thermodynamically more stable alcohols in approximately the same proportions as from equilibration experiments[2]. It is possible therefore to see how well the concept of more stable equatorial conformations is obeyed. As set out in Table I the expected relationships are observed. Also included in this Table is a reference to the equilibration of 5α:6β-dibromocholestane with the 5β:6α-isomer, for this is clearly relevant to the issue under discussion.

Elimination Evidence. Reactions whose mechanisms require concerted 1:2-elimination should proceed more readily when the four centres involved (the two carbon atoms and the two substituents) lie in one plane. For concerted ionic elimination reactions in cyclohexane derivatives the optimum arrangement of the substituents for the minimization of the activation energy is that in which both are polar[3,4]. There is much evidence in the literature which confirms this. Thus[1] cis-2-substituted cyclohexanols (VII) undergo acid catalysed dehydration [elimination of H(*p*) and OH(*p*)] more readily than the trans-isomers (VIII). In the menthol series[2] neomenthol (XV; R=CH_3, R′=H) loses water easily relative to menthol (XVI; R=CH_3, R′=H) and neoisomenthol (XV; R=H, R′=$CH)_3$ dehydrates easily relative to isomenthol (XVI; R=H, R′=CH_3)[3]. There are a number of interesting examples of this sort of phenomenon in steroid compounds. A summary is given in Table II.

Table II

Observation Easy elimination of	References
Cholestane Series	
6β-OH (p) and 5α-H (p)	D. H. R. BARTON and W. J. ROSENFELDER, J. Chem. Soc. 2459 (1949).
6β-H (p) and 5α-Cl (p)	D. H. R. BARTON and E. MILLER, J. Amer. Chem. Soc. *72*, 1066 (1950).
6β-Br (p) and 5α-Br (p)	D. H. R. BARTON and E. MILLER, loc. cit.
7α-OH (p) and 8β-H (p)	pp. 241, 242, 631
Coprostane Series	
7α-OH (p) and 8β-H (p)	pp. 118, 631
11β-OH (p) and 9α-H (p)	pp. 408, 630
11β-Br (p) and 9α-H (p)	pp. 460, 631

extensively presented elsewhere[4,5]. See also the discussion by SOBOTKA[6].

[1] L. F. FIESER, *The Chemistry of Natural Products Related to Phenanthrene* (1st Ed. 1936, Reinhold Publishing Corporation).

[2] See footnotes 2 and 3 on p. 316, 2nd column.

[3] E. D. HUGHES and C. K. INGOLD *et al.*, J. Chem. Soc. 2117 (1948).

[4] D. H. R. BARTON and E. MILLER, J. Amer. Chem. Soc. *72*, 1066 (1950).

[5] O. BASTIANSEN and O. HASSEL, Nature *157*, 765 (1946). – O. HASSEL and H. VIERVOLL, Acta chem. Scand. *1*, 149 (1947) and papers there cited. – D. H. R. BARTON, J. Chem. Soc. 340 (1948).

[6] H. SOBOTKA, *The Chemistry of the Steroids*, 1938, p. 48 *et seq.* (The Williams and Wilkins Co.).

[1] G. VAVON, Bull. Soc. Chim. [4], *49*, 937 (1931).

[2] For summary see J. L. SIMONSEN and L. N. OWEN, *The Terpenes*, Vol. I (Cambridge University Press, 1947).

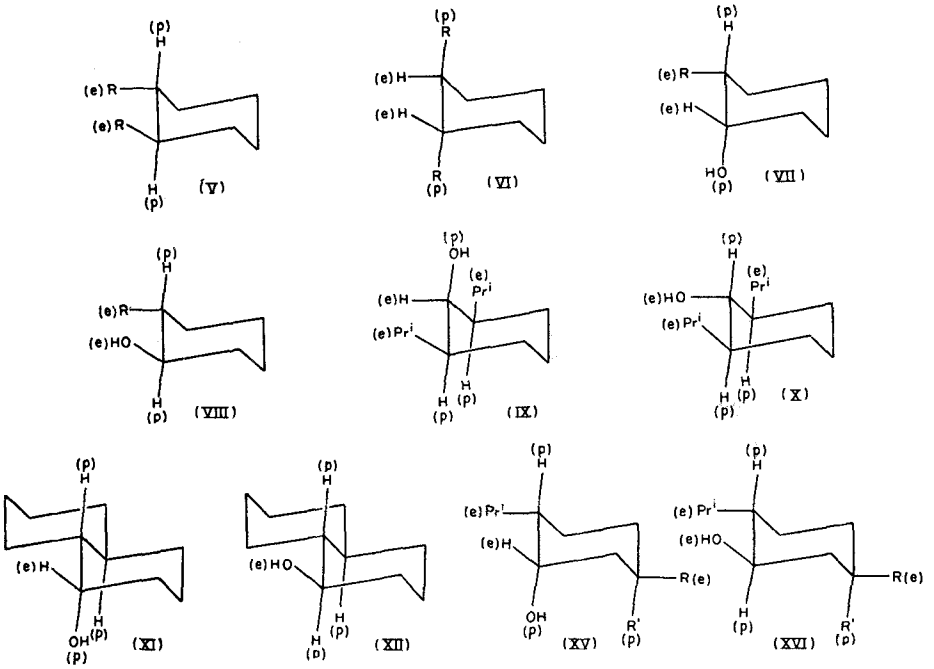
[3] Of course for pyrolytic elimination of substituents by "unimolecular" mechanisms (see D. H. R. BARTON, J. Chem. Soc. 2174 [1949]) cis-elimination is the rule and the discussion given here is no longer relevant.

Table III

Observation	References
Cholestane Series	
2β-OH (p) more hindered than 2α-OH (e)	A. FÜRST and P. A. PLATTNER, Helv. chim. acta *32*, 275 (1949).
3α-OH (p) more hindered than 3β-OH (e)	pp. 635, 636
6β-OH (p) more hindered than 6α-OH (e)	p. 223
6α-H (e) more easily oxidized than 3α-H (p)	L. F. FIESER and S. RAJAGOPALAN, J. Amer. Chem. Soc. *71*, 3938 (1949).
3β-H (e) more easily oxidized than 3α-H (p)	G. VAVON and B. JACUBOWICZ, Bull. Soc. Chim. [4], *53*, 581 (1933).
Coprostane Series	
3β-OH (p) more hindered than 3α-OH (e)	pp. 635, 636
6β-OH (p) more hindered than 6α-OH (e)	p. 652
11β-OH (p) more hindered than 11α-OH (e)	p. 408
12α-OH (p) more hindered than 12β-OH (e)	p. 658
7α-OH (p) and 12α-OH (p) more hindered than 3α-OH (e)	p. 125
7β-H (e) and 12β-H (e) more easily oxidized than 3β-H (p)	p. 126; L. F. FIESER and S. RAJAGOPALAN, J. Amer. Chem. Soc. *71*, 3935 (1949).

Steric Hindrance Evidence. The applicability of steric hindrance evidence in the assignment of configuration has long been recognised, although such assignments are not always reliable[1]. It seems possible to explain the relative magnitudes of many of the phenomena of steric hindrance in cyclohexane derivatives on the basis that polar bonds are more hindered than the corresponding equatorial bonds. An inspection of models makes this reasonable for a polar bond is always close in space to two other polar bonds each attached to the next but one carbon atom, whereas there is no similar relationship for equatorial bonds.

In support[2] of this generalization it has been observed that cis-2-substituted cyclohexanols (VII) with polar hydroxyls are more difficult to esterify, and their esters more difficult to hydrolyse, than the corresponding trans-alcohols and their esters. The same effects are observed with trans-α-decalol[1]. The esters of the alcohol (XI) (polar hydroxyl) are more difficult to hydrolyse than those of the alcohol (XII) (equatorial hydroxyl). In the menthol series[2] menthol (XVI; R=CH_3, R′=H) is more easily esterified than neomenthol (XV; R=CH_3, R′=H) and a similar relationship holds for isomenthol (XVI; R=H, R′=CH_3) and neoisomenthol (XV; R=H, R′=CH_3).



However, a reverse relationship holds[3] for chromic acid oxidation of 2-substituted cyclohexanols. Here the cis-alcohols are oxidized more rapidly than the trans-.

[1] W. HÜCKEL, Ann. Chem. *533*, 1 (1937). – Compare the preceding paper in which L. F. FIESER has discussed steric effects under the headings intraradial and extraradial.

[2] G. VAVON, Bull. Soc. Chim. [4], *49*, 937 (1931).

[1] W. HÜCKEL *et al.*, Ann. Chem. *533*, 128 (1937).

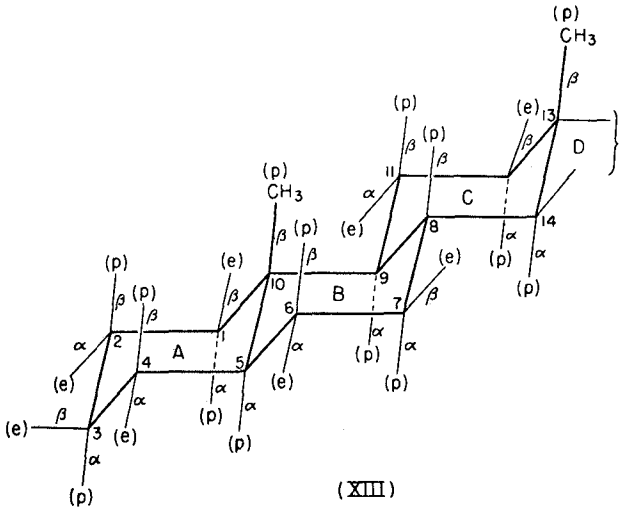
[2] For summary see: J. L. SIMONSEN and L. N. OWEN, *The Terpenes*, Vol. I (Cambridge University Press, 1947).

[3] G. VAVON, Bull. Soc. Chim. [4], *49*, 937 (1931).

This observation is adequately accommodated by the present theory if the rate determining step is attack upon the carbon-hydrogen bond rather than upon the carbon-hydroxyl linkage.

The situation in the steroid field is summarized in Table III. In every case the expected order of hindrance holds good. Also included are data for oxidations of alcohols by Br^+ to give the corresponding ketones. If such oxidations are assumed to involve attack upon the carbon-hydrogen bond then the results are in agreement with the other observations summarized in the Table.

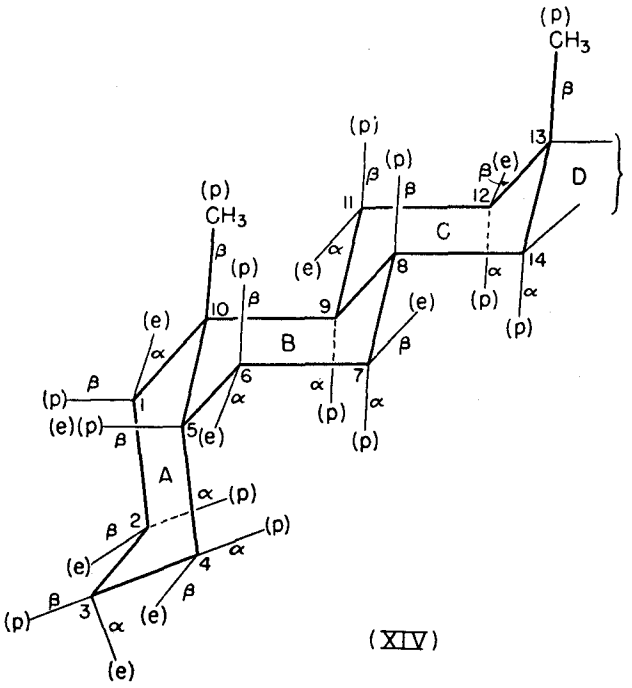
Although the concept of polar and equatorial bonds is not, of course, applicable to cyclopentane, it is of interest to note that the 17α-bond in the steroid nucleus has, because of the ring fusion to a six-membered ring, the character of a polar bond with respect to that ring. Also the 17β-bond has in its relationship to ring C the aspect of an equatorial bond. These facts are in agreement with the greater thermodynamic stability of 17β-substituents and the greater degree of steric hindrance shown by 17α-substituents[1].



(XIII)

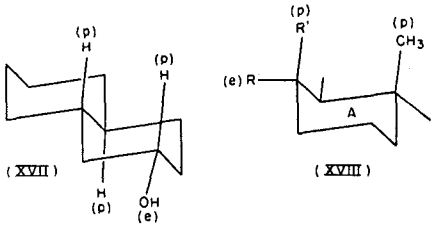
Use of the Concept. It will be clear that it is possible to assign configurations on the basis of the concept of polar and equatorial bonds. One such example has already been given in Table I. An additional illustration is provided by trans-β-decalol[2]. The more stable epimer m.p. 75° must have the hydroxyl in the equatorial conformation as in (XVII); this is in agreement with the fact that its esters are more rapidly hydrolysed than those of the epimeric (polar hydroxyl) alcohol. Other examples are mentioned below.

Extension to di- and tri-terpenoids. It would seem reasonable to extend the concept of equatorial and polar bonds to the correlation of the stereochemistry of other ring systems built up from fused cyclohexane units. Thus ring A of the diterpenoid abietic acid may be represented[3] by (XVIII; $R=CO_2H$, $R'=CH_3$) with the carboxyl occupying an equatorial conformation. It is understandable then that the esters of this acid should be more easily hydrolysed than those of (say) podocarpic acid where ring A is as shown in (XVIII; $R—CH_3$, $R'=CO_2H$), for in the latter the carboxyl occupies the more hindered polar conformation.

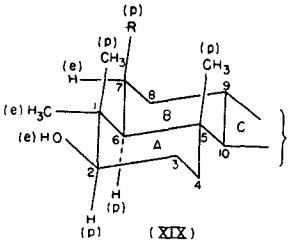


(XIV)

Now that it is recognised[1] that rings A and B of the α- and β-amyrin groups of triterpenoids and also[2] those of the lupeol group are trans-fused, it is possible to make a tentative representation of their stereochemistry



(XVII) (XVIII)



(XIX)

as shown in (XIX; R=H). Placing the hydroxyl in the equatorial conformation explains the more facile hydrolysis of β-amyrin acetate relative to epi-β-amyrin

[1] L. F. Fieser, Exper. *6*, 312 (1950).

[2] W. Hückel, Ann. Chem. *533*, 1 (1937). – W. Hückel *et al.*, Ann. Chem. *533*, 128 (1937).

[3] D. H. R. Barton, Quart. Rev. *3*, 36 (1949).

[1] D. H. R. Barton, Quart. Rev. *3*, 36 (1949).

[2] T. R. Ames and E. R. H. Jones, Nature *164*, 1090 (1949).

acetate[1] and of lupanol relative to epi-lupanol[2]. It also accounts for the easy elimination of water accompanied by molecular rearrangement, which is induced in these compounds or their derivatives by treatment with phosphorus pentachloride[3]. Such a reaction then becomes comparable to the very easy dehydration of isoborneol to give camphene, in that all the four atomic centers of importance in the reaction lie in one plane. The marked hindrance of the 7-hydroxyl group in sumaresinolic acid and its easy elimination under acid dehydrating conditions[4] are best explained if it has the polar conformation as in the part expression (XIX; R=OH).

In connection with the nomenclature of triterpenoids it would appear desirable to extend FIESER's α-, β-convention for steroids to cover triterpenoid stereochemistry also. A convenient reference point is the C_5 methyl group. Substituents on the same side of the main- ring plane as this methyl group should be regarded as having the β-configuration, those on the opposite side as having the α-configuration. Thus sumaresinolic acid would be designated 2β:7β-dihydroxyolean-12-ene-17-carboxylic acid.

Zusammenfassung

Beim Cyclohexan und seinen Abkömmlingen kann man die nicht an der Ringbildung beteiligten Valenzen der Kohlenstoffatome in «äquatoriale» und «polare» einteilen. Jedes Ringkohlenstoffatom hat eine polare und eine äquatoriale Bindung.

An einem gegebenen Kohlenstoffatom ist ein äquatorial gebundener Ligand thermodynamisch stabiler als ein polar gebundener. Zwei benachbarte Substituenten werden, wenn es sich um Ionenreaktion handelt, leichter abgespalten, wenn sie beide «polar» gebunden sind, als wenn einer von ihnen oder beide «äquatoriale» Bindungen besetzen. Ein Ligand in der polaren Stellung an einem gegebenen Kohlenstoffatom unterliegt stärkerer sterischer Hinderung als in der äquatorialen Anordnung.

Die Anwendung dieser allgemeinen Regeln auf die Steroidchemie wird kurz beschrieben. Dabei wird die Abhängigkeit dieser Anwendung von der für den Sterinkern angenommenen Konfiguration betont.

Die Ausdehnung dieser Ideen auf das Gebiet der Di- und Triterpenoide wird angedeutet.

[1] L. RUZICKA and H. GUBSER, Helv. chim. acta *28*, 1054 (1945); these authors assigned the opposite configuration at C_2.

[2] R. NOWAK, O. JEGER, and L. RUZICKA, Helv. chim. acta, *32*, 323 (1949). The equatorial conformation for the hydroxyl group in these compounds is also indicated by the fact that β-amyrin is more stable thermodynamically than epi-ß-amyrin (L. RUZICKA and W. WIRZ, *ib.*, *24*, 248 (1941).

[3] L. RUZICKA, M. MONTAVON, and O. JEGER, Helv. chim. acta *31*, 819 (1948); and earlier papers from the same laboratory.

[4] L. RUZICKA, O. JEGER, A. GROB, and H. HÖSLI, Helv. chim. acta, *26*, 2283 (1943).

Congress

ENGLAND

International Congress on Analytical Chemistry in 1952

Considerable progress has been made in connection with the arrangements for the International Congress on Analytical Chemistry which is to be held in Britain in 1952.

It has been decided that the meetings shall be held in Oxford, commencing on 4th September. Accommodation will normally be provided in Colleges but some Hotel accommodation will also be available. The technical sessions will take place in one of the main University buildings.

The period of the Congress will include a week-end and excursions and visits will be planned to take place during this period.

The arrangements for the Congress are in the hands of a General Committee representing a wide variety of interests and under the Chairmanship of the President of the Royal Society, Sir ROBERT ROBINSON, O.M.

The scope of the Congress is under active consideration by an Executive Committee, under the Chairmanship of the President of the Society of Public Analysts and Other Analytical Chemists, Mr. G. TAYLOR, M.B.E., F.R.I.C., and further details of this and other matters will be published in due course.

It is expected that a meeting of the Board of Section V., Analytical Chemistry, of the International Union of Pure and Applied Chemistry, will be held in Oxford during the same week. Sir IAN HEILBRON, F.R.S., is Honorary President and Professor C. J. VAN NIEUWENBURG President, of this Section of the International Union.

Sir WALLACE AKERS, C.B.E., is Honorary Treasurer of the Congress and the Honorary Secretary is Mr. R. C. CHIRNSIDE, F.R.I.C., Research Laboratories, The General Electric Co. Ltd. Wembley, England.

Corrigendum

J. R. BILLETER und K. MIESCHER, *Darstellung von 4-Ring-Ketonen aus dem trizyklischen Keton von Köster und Logemann*, Exper. *6*, 261 (1950):

Die Autoren machen uns darauf aufmerksam, daß die Fußnote 1 in der linken Kolonne heißen muß: *97.* Mitteilung der Reihe «Über Steroide» (*96.* Mitt. siehe Helv. chim. acta *33*, 388 [1950]) anstatt 96. und 95. Mitteilung.

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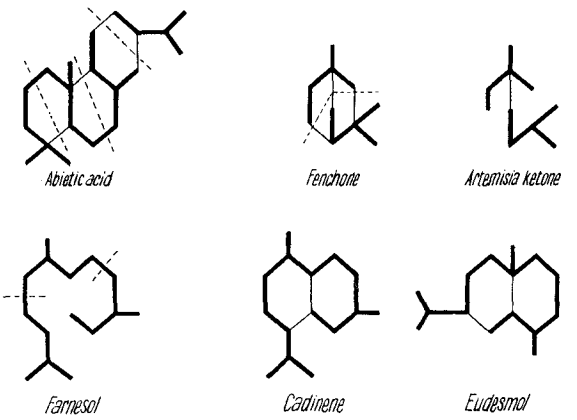
The Isoprene Rule and the Biogenesis of Terpenic Compounds[1]

By L. RUZICKA, Zurich[2]

The systematic study of the higher terpenes was started in Zurich in the early 1920's. At that time work was begun simultaneously on the diterpene abietic acid and on those sesquiterpenes which, like abietic acid, contain a hydroaromatic ring system.

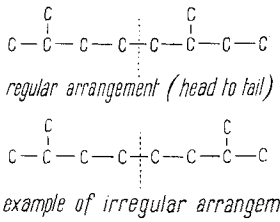
The Carbon Skeleton of the Sesqui- and Polyterpenes[3]

The importance of abietic acid (I)[4] for the development of the chemistry of the polyterpenes lies in the fact that it was soon realised that its carbon skeleton is composed of 4 isoprene units. Likewise the elucidation of the structure of hydroaromatic sesquiterpenes (I) such as cadinene and eudesmol was considerably facilitated by the observation that their carbon skeleton can formally be deduced from farnesol. Farnesol and the sesquiterpenes derived from it contain three isoprene units in regular head-to-tail arrangement (II). In abietic acid, on the other hand, the carbon skeleton is irregular, with three isoprene units arranged head-to-tail as in farnesol, while the fourth is attached the other way round (I).



I.—Regular and irregular terpenic carbon skeletons.

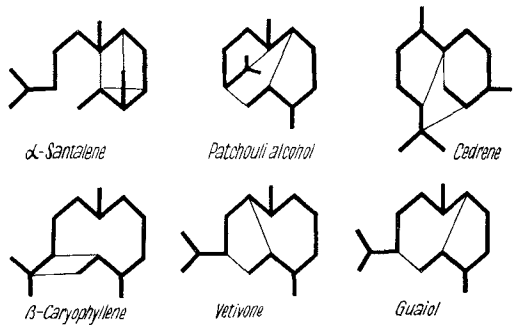
The monoterpenes are also made up of isoprene units, and this represents the common structural feature which links the various compounds of the group, even those in which the carbon skeleton is not derived from p-cymene, as for example fenchone and artemisia ketone (I).



II.—Regular and irregular arrangement of isoprene units.

This led to the formulation of the *isoprene rule*, which states that the carbon skeleton of the terpenes is composed of isoprene units linked in regular or irregular arrangement.

The isoprene rule has successfully been applied in the elucidation of the structure of terpenic compounds. A brief review of the carbon skeletons of the sesqui-, di-, and triterpenes will serve to prove the validity of the isoprene rule, and will also show that *each group has its own special variations of the fundamental rule.*



III.—Sesquiterpene carbon skeletons deduced from farnesol.

In the *sesquiterpene* group almost all compounds have regular carbon skeletons (III). Even the somewhat peculiar looking carbon skeletons of such compounds as α-santalene (SEMMLER), patchouli alcohol

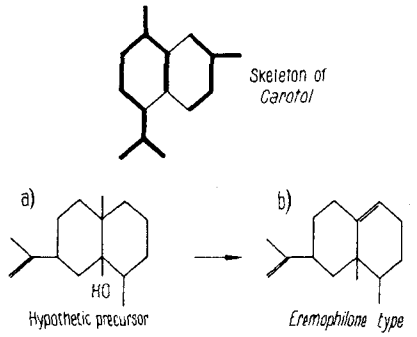
[1] From a lecture held at the XIII[th] International Congress of Pure and Applied Chemistry, Stockholm, 29[th] July, 1953.

[2] Laboratory of Organic Chemistry, E.T.H., Zurich.

[3] In this section only the carbon skeletons are considered (Tables I–VI). The precise formulae are given in the following sections.

[4] *The formulae are grouped in 23 Tables designated by roman numbers (I–XXIII). In the text reference is made to the Table by its roman number and to the formula in the Table by a small latin letter, e.g. "IXb" indicates formula b in Table IX. When the names of compounds are indicated in the Table, only the roman number of the Table is given in the text.*

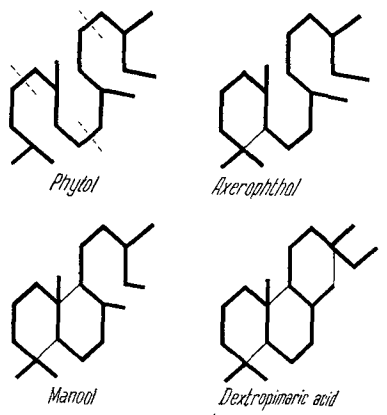
(TREIBS), cedrene[1], β-caryophyllene (ŠORM)[2], vetivone (PFAU and PLATTNER), and guaiol (PLATTNER) can be deduced from farnesol. The wide validity of this structural relationship led to the formulation of the "farnesol rule" which is a special case of the isoprene rule.



IV.—Irregular carbon skeletons of sesquiterpenes.

Of the sesquiterpenes, only carotol (ŠORM) does not follow the farnesol rule but is instead built of 3 isoprene units in irregular arrangement (IV).

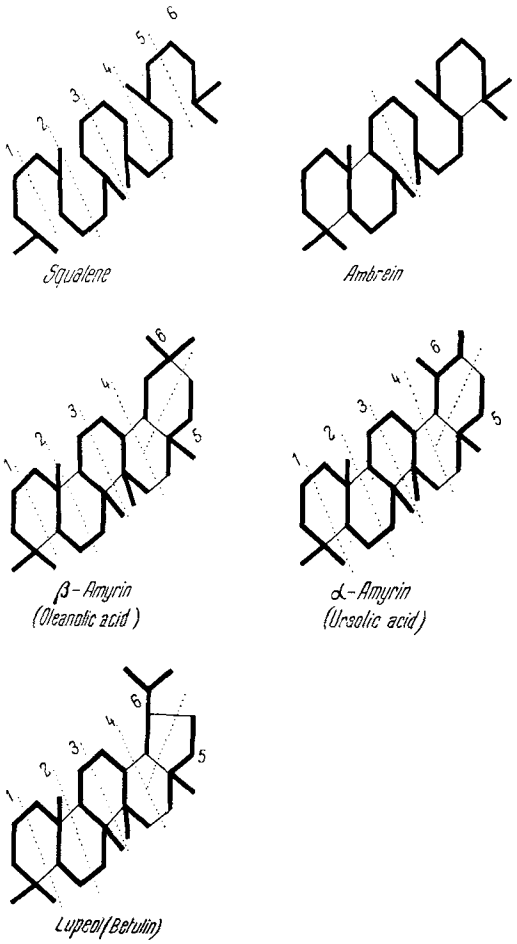
The one exception to the isoprene rule in the sesquiterpene field is eremophilone (IV) (PENFOLD and SIMONSEN). However, according to ROBERT ROBINSON, the carbon skeleton of eremophilone (IV *b*) may be derived from a eudesmol type precursor (IV *a*) by migration of a methyl group.



V.—Regular carbon skeletons of diterpenes.

In the *diterpene* group, apart from the carbon skeleton of abietic acid with an irregular sequence of four isoprene units (I), the carbon skeletons of all[3] diterpenes (V) are derived from a regular isoprene tetramer consisting of 4 isoprene units connected head-to-tail. This is best seen in four typical representatives of the diterpenes: the open chain compound phytol (F. G. FISCHER), monocyclic axerophthol (KARRER), bicyclic manool (HOSKING), and tricyclic dextropimaric acid. This structural relationship may be termed the "phytol rule", in analogy to the farnesol rule of the sesquiterpenes.

The majority of the diterpenes derivable from phytol, as for instance sclareol and agathic acid, possess the same bicyclic structure as manool. All bicyclic and tricyclic diterpenes have an identical carbon skeleton in rings *A* and *B*. It will be shown that this structural pattern is also present in rings *A* and *B* of the triterpenes.



VI.—Symmetrical and irregular carbon skeletons of triterpenes.

The *triterpenes* may be classified on the basis of several structural types. However, a higher analogue of farnesol and of phytol, with a regular head-to-tail arrangement of 6 isoprene units, is not known. A new type is encountered, represented by the aliphatic hydrocarbon squalene (KARRER), which is built of two farnesyl chains symmetrically joined end-to-end (VI).

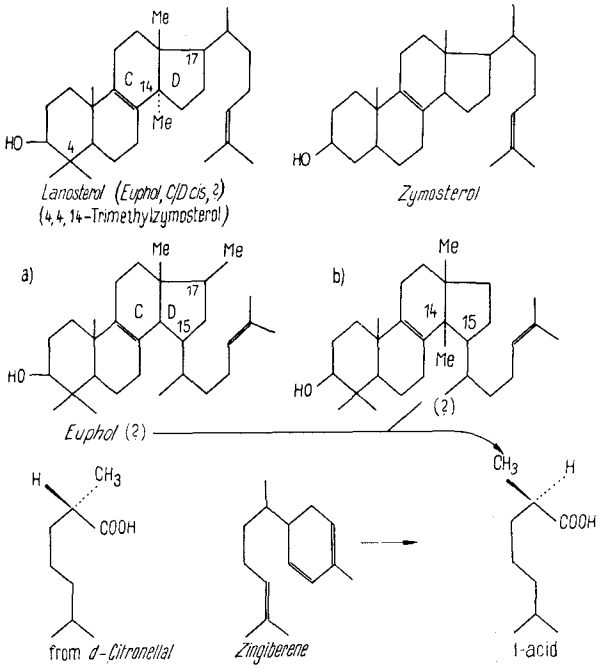
The symmetrical structure of squalene is reminiscent of the C_{40} carotenoids, which may be considered, on the basis of their carbon skeleton, as typical representatives of the tetraterpenes.

[1] The cedrene formula (III, *cf.* also XVIII) was proposed by A. ESCHENMOSER in his Ph.-D. Thesis, E.T.H., Zurich, 1952. Its correctness was demonstrated experimentally by PLATTNER *et al.* *Cf.* the paper by PL. A. PLATTNER, XIII[th] Internat. Congress of Pure and Applied Chemistry, Stockholm, 29[th] July, 1953.

[2] ŠORM was the first to propose a many-membered ring structure for a terpenic compound. The elucidation of the structural details of the caryophyllenes was completed by BARTON, CLEMO, DAWSON, and RAMAGE.

[3] α-Camphorene is not considered here, because, though the compound is obtained by dimerisation of myrcene, its structure is not definitely established.

Only one other triterpene belongs to the squalene type, the tricyclic alcohol ambrein (VI). Both squalene and ambrein are of animal origin. Almost all triterpenes of plant origin, on the other hand, have irregular pentacyclic carbon skeletons (VI) which partially deviate from the squalene type. They may be subdivided in three subgroups, the α-amyrin, the β-amyrin, and the lupeol subgroup. These three fundamental carbon skeletons of the pentacyclic triterpenes differ from each other only in the arrangement of one isoprene unit, which is attached at one end of the molecule (isoprene unit 6 in the formulae). The first four isoprene units (1–4 in the formulae) have the same arrangement as the corresponding units in squalene.



VII[1].—Lanosterol and euphol.

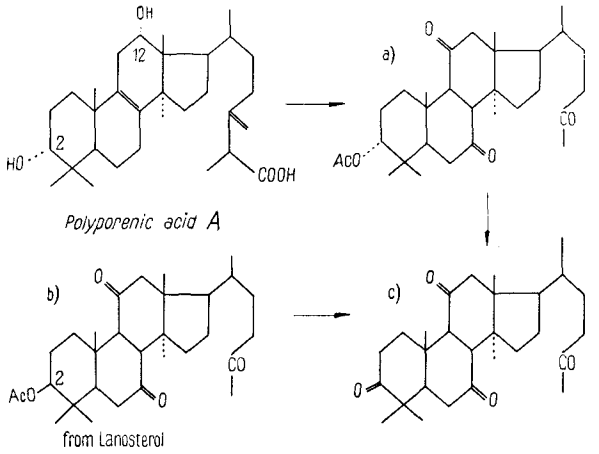
There remains to be mentioned a last group of compounds, which on the basis of their carbon skeleton may be assigned an *intermediate position between the steroids and the triterpenes*. These compounds possess the tetracyclic nucleus typical of the steroids and contain 30 carbon atoms. Two typical representatives of this group are lanosterol and euphol (JEGER).

Lanosterol (VII) was at first assumed to be a triterpene. In the course of degradative studies, however, JEGER found that the carbon skeleton is not built in accordance with the isoprene rule. Lanosterol was therefore defined as the first[2] representative of the group of the C_{30} steroids. The relationship between lanosterol and the steroids is evident at first sight, when one compares lanosterol with zymosterol. Lanosterol may in fact be considered a trimethylzymosterol.

Euphol (VII) and lanosterol differ either in the position or in the configuration of the long side chain and of a methyl group in ring *D*. Formula VII*a* was originally proposed for euphol, but a structure stereoisomeric with lanosterol, *i.e.* having a *cis C/D* junction, is not excluded. For reasons which will be discussed later, formula VII*b* should also be considered[1].

One of the latest results in the degradation of euphol should be mentioned here (VII). The 8–9 double bond of dihydroeuphol shifts easily from rings *B/C* into ring *D*. If the euphol formula differs from VII*a*, it has to be assumed that one or two methyl groups in ring *D* also migrate. In lanosterol, with a probable *trans*-junction of rings *C* and *D*, such a rearrangement does not occur. If in euphol rings *C* and *D* are *cis*-joined, methyl group migration would perhaps be explainable. The isomerisation product obtained from dihydroeuphol yields a diketone on oxidation. Stepwise degradation of this diketone leads to the dextrorotatory 2,6-dimethylheptanoic acid, which has the L configuration as the corresponding acid deducible from the sesquiterpene zingiberene (VII) and is the antipode of the acid obtained from d-citronellal, for which the *D* configuration is established.

Mention should also be made of *polyporenic acid A* (VIII), because of its close relation to lanosterol. With 31 carbon atoms, polyporenic acid *A* is something of a curiosity.



VIII.—Relation between polyporenic acid *A* and lanosterol.

The elucidation of the structure of lanosterol was of decisive assistance in the investigation of polyporenic acid *A*, and made it possible for HALSALL, JONES, and LEMIN to propose a partial formula. JEGER, HEUSSER, *et al.* have recently succeeded in establishing a *direct experimental relation between polyporenic acid A and lanosterol*. Polyporenic acid *A* can be converted in several steps to an acetoxytriketone (VIII*a*), which was supposed to be closely related to an acetoxydiketonic acid formerly obtained from lanosterol. However, the acetoxytriketone (VIII*b*) prepared from this acid differed from the degradation product obtained from polyporenic acid *A*. The close relationship between the two com-

[1] *Erratum:* In the formulae of the C_9 acids H and CH_3 should be interchanged and l should read d.

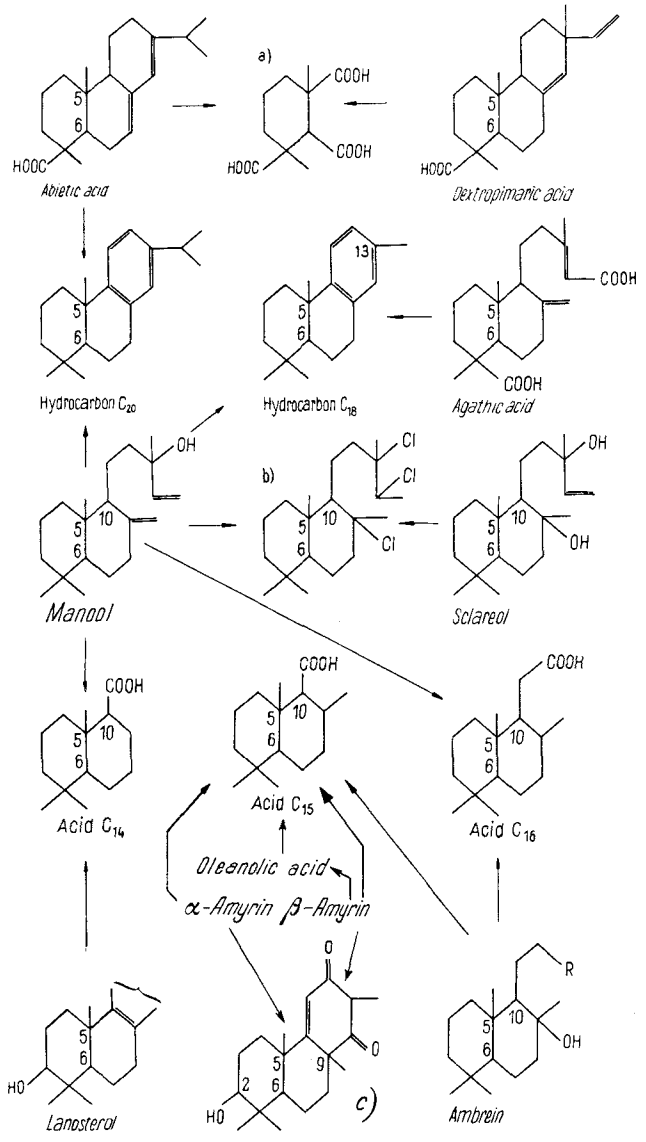
[2] The second representative is cycloartenol (SPRING; BARTON).

[1] The dehydrogenation of euphadiene with selenium to 1,2,8-trimethylphenanthrene supports the two formulae with the methyl groups in 13 and 14.

pounds was finally established when it was found that both yield the same tetraketone (VIII*c*). Since the hydroxyl group of lanosterol in position 3 has β configuration, it is possible to conclude from this result that the corresponding hydroxyl group of polyporenic acid *A* has α configuration. The configuration of the hydroxyl group in position 12 is according to HALSALL *et al.* also α[1].

Configurational Correlations in the Diterpene and Triterpene Series

As has been mentioned before, the first two rings of the diterpenes and of the triterpenes have the same structure. It can be shown that these rings also have identical configuration.



IX.—Configurational relationship between diterpenes and triterpenes.

Abietic acid and dextropimaric acid both yield the same C_{11} tricarboxylic acid on oxidative degradation (IX*a*). Manool (JEGER) and abietic acid (CAMPBELL and TODD) have been correlated through transformation into an identical C_{20} hydrocarbon. The relationship between manool and sclareol is established by the fact that both give an identical trihydrochloride (IX*b*) (HOSKING). Finally manool and agathic acid can both be converted into the same C_{18} hydrocarbon (JEGER).

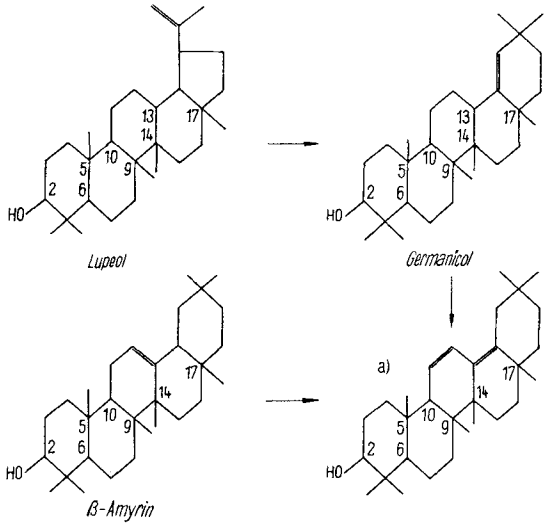
Thus it has been shown that all of these bicyclic and tricyclic diterpenes have the same configuration at the *A*/*B* ring junction. This is of course also true of the other diterpenes which have been experimentally correlated with those just discussed.

Manool has also been correlated with the tricyclic triterpene ambrein (JEGER; LEDERER) by degradation of both to an identical C_{16} acid still containing the asymmetric carbons 5, 6, and 10 (IX). Starting from ambrein (LEDERER), a C_{15} acid can be prepared, in which the three asymmetric carbons of the C_{16} acid still are present. This C_{15} acid is particularly important, because it has also been obtained both from α-amyrin and from β-amyrin (IX) (JEGER).

Manool, in turn, has been correlated with lanosterol. An identical C_{14} acid can be prepared by degradative reactions both from manool and from lanosterol (IX) (JEGER; HOSKING), showing that the two compounds have the same configuration at carbon atoms 5 and 6.

The reactions just discussed do more than merely establish the configurational identity of carbons 5, 6, and 10 in manool, sclareol, α-amyrin, β-amyrin, and ambrein, and of carbons 5 and 6 in lanosterol, abietic acid, and dextropimaric acid. By way of manool they connect lanosterol and the amyrins with the diterpenes, and via the amyrins they embrace all the numerous pentacyclic triterpenes, belonging to the α-amyrin subgroup (*e.g.* ursolic acid) and to the β-amyrin subgroup (*e.g.* oleanolic acid).

Within the triterpene group, α-amyrin and β-amyrin have been degraded (JEGER) to an identical tricylic hydroxydiketone (IX*c*), thus proving that the two



X.—Relationship between lupeol and β-amyrin.

[1] T. G. HALSALL and E. R. H. JONES in a paper presented at the XIII[th] Intern. Congress of Pure and Applied Chemistry, Stockholm, 29[th] July, 1953, also proposed formula VIII for polyporenic acid *A*.

compounds have the same configuration at carbon atoms 2, 5, 6, and 9. The identity in configuration at position 10 has already been mentioned.

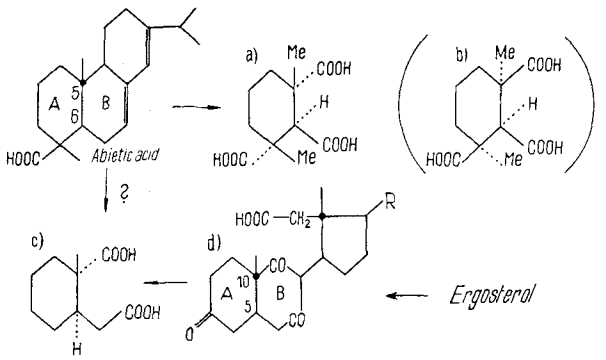
It is mostly through the work of JONES that a third subgroup of the pentacyclic triterpenes, the one of lupeol, has been correlated with β-amyrin (X). JONES obtained germanicol by rearrangement of ring *E* of lupeol. Germanicol itself has been converted (BARTON) into a doubly unsaturated triterpene alcohol (X*a*) formerly obtained from β-amyrin (JEGER).

The triterpenes of the β-amyrin subgroup contain 7 asymmetric carbon atoms in positions 2, 5, 6, 9, 10, 14, and 17, which have the same configuration as the corresponding carbon atoms in lupeol (X). Five of these seven asymmetric centers, carbon atoms 2, 5, 6, 9, and 10, have been shown to have the same configuration as in the α-amyrin subgroup (IX).

Relative and absolute Configuration of Diterpenes and Triterpenes

The first evidence regarding the relative configuration in the diterpene and triterpene series was provided for carbon atoms 5 and 6. The already mentioned C_{11} tricarboxylic acid (IX*a*), which is obtained from abietic acid and from dextropimaric acid, is optically inactive. This means that its asymmetry must be internally compensated. Two configurations (XI*a* and *b*) satisfy this condition. By studying the effect of configuration on the dissociation constants of polycarboxylic acids, BARTON was able to assign configuration XI*a*. As a consequence, *trans*-junction of rings *A* and *B* follows for all di- and triterpenes.

Another proof of the configuration at the *A*/*B* ring junction would be possible by correlation of a suitable degradation product of abietic acid with one derived from rings *A*/*B* of a steroid. Starting from ergosterol the dicarboxylic acid XI*c* has been obtained through the intermediate XI*d*. The two asymmetric carbon atoms of this acid possess the same configuration as carbons 5 and 10 in cholestanol. The preparation of the corresponding dicarboxylic acid from abietic acid is being carried out in Zürich.



XI.—Steric identity at ring A/B junction in diterpenes, triterpenes and steroids.

It is impossible to report in detail all the fundamental work which has led to an almost complete elucidation of the stereochemistry of the triterpenes. Our present day knowledge is mainly due to BARTON, CURTIS *et al.*, JEGER, JONES, KLYNE, MILLS, and PRELOG. The methods used are fundamentally the same as those applied in the steroid field and are based on the following studies:

(1) Studies of the dissociation constants of polycarboxylic acids.

(2) Studies of the relative stability of stereoisomeric 1,2-condensed ring systems, the question being in each case whether the *trans* or the *cis* configuration is more stable.

(3) Studies of the possibility of formation of bridged ring systems, in particular of lactones.

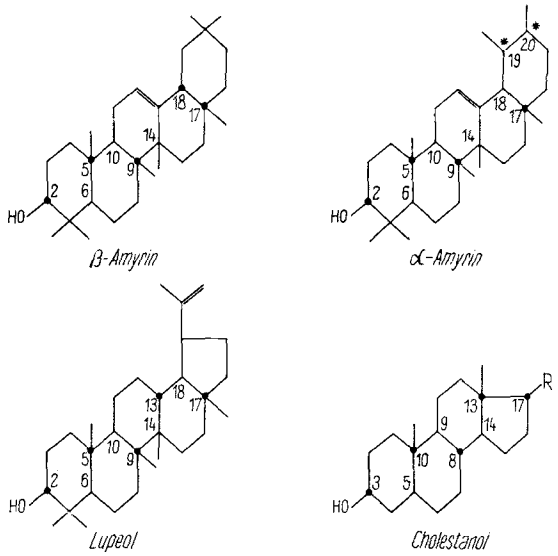
(4) Studies of the steric course of elimination and addition reactions.

(5) Studies of the contribution of individual asymmetric carbon atoms to the molecular rotation.

(6) Studies of the molecular structure by X-ray diffraction.

(7) Studies of the absolute configuration by PRELOG's method using asymmetric synthesis.

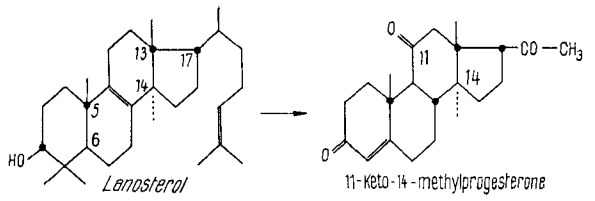
Of these methods, two provide information about the absolute stereochemical configuration: the method of MILLS, based on the contribution of the single asymmetric carbon atoms to the total optical activity, and the method of PRELOG, based on asymmetric synthesis. Combination of the results obtained by these two methods with those achieved by the other methods mentioned above leads to the assignement of the absolute configuration of the asymmetric carbons in the triterpenes.



XII.—Configurational relationship between triterpenes and steroids.

The resulting information regarding the absolute configuration of the triterpenes is presented in the formulae of α-amyrin, β-amyrin, and lupeol (XII). The

configurational formula of cholestanol is also shown for comparison purposes. In the formulae of the three triterpenes the carbon atoms which have β configuration are marked by heavy dots, according to LINSTEAD's notation. All other asymmetric carbons in α-amyrin, β-amyrin, and lupeol have α-configuration, as do the corresponding atoms in cholestanol. The carbons 19 and 20 in α-amyrin are of unknown configuration and are marked by asterisks in the formula. In the case of lupeol (and of β-amyranol) there are 7 asymmetric carbons (2, 5, 6, 9, 10, 13, and 14) having *the same configuration as the corresponding carbons in cholestanol*, while in the case of α- and β-amyrin there are 6 (carbons 2, 5, 6, 9, 10, and 14).



XIII.—Hormone from lanosterol.

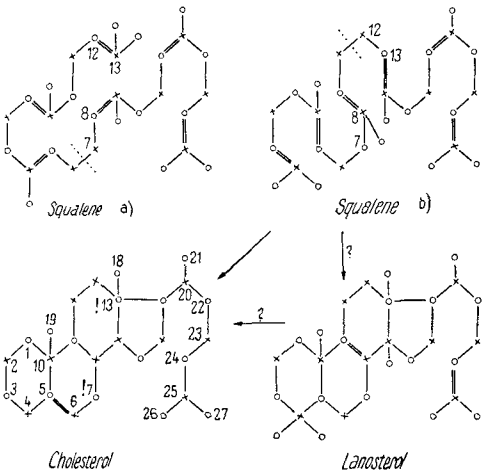
Lanosterol has been shown to have the same absolute configuration at the *A/B* ring-junction as the triterpenes and cholestanol. Mention should also be made of a tentative deduction of the configuration of carbon atoms 13, 14, and 17 from physiological properties (JEGER). A compound prepared starting from lanosterol, 11-keto-14-methylprogesterone (XIII), has been found to have the same qualitative and quantitative activity in the CORNER-ALLEN test as 11-keto-progesterone. It is a well known fact that the hormonal activity of progesterone is highly dependent on configuration, and it may be deduced from the activity of 11-keto-14-methylprogesterone that the 4 asymmetric carbons of lanosterol, which are present in 11-keto-14-methylprogesterone (XIII), have probably the same configuration as the corresponding 4 atoms in progesterone, and consequently in cholesterol.

The same conclusion has been reached by CURTIS *et al.* by X-ray diffraction study of lanosterol. CURTIS' results lead him to assign to the lanosterol molecule a flat structure, analogous to the one established by BERNAL 20 years ago for cholesterol.

Biogenesis of Steroids and Terpenic Compounds
(together with A. ESCHENMOSER[1] and H. HEUSSER)

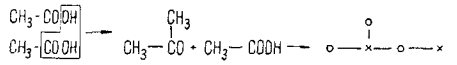
Once the structure and the configuration of a natural compound has been established, the question arises how the compound is synthesized in nature.

The first definite answer to such a question in regard to the carbon skeleton of the steroids and the triterpenes was given by BLOCH through a biological synthesis of cholesterol from acetic acid labelled with C^{14}. BLOCH has also provided evidence indicating that in all probability squalene is an intermediate in this synthesis. The two ways by which squalene may be transformed into cholesterol, are shown in schemes XIV*a* and XIV*b*. Scheme *a* represents the cyclisation of squalene as proposed by ROBINSON. Scheme *b* is the mechanism proposed by WOODWARD and BLOCH.



XIV.—Biogenesis of cholesterol and lanosterol from squalene.
° = Carbon from acetate methyl,
× = Carbon from acetate carboxyl.

In both schemes the carbon atoms originating from the methyl group of acetic acid are indicated by a small circle, and those originating from the carboxyl group of acetic acid by a cross, in accordance with the arrangement of the isoprene units in squalene (VI) and with the formation of the isoprene skeleton from acetic acid (XV).



XV.—Distribution of acetate carbons in the biogenesis of the isoprene skeleton.

Comparison of the two schemes XIV*a* and *b* shows that the cholesterol carbons at positions 7, 8, 12, and 13 are derived from different acetate carbons, depending on whether scheme *a* or scheme *b* is followed. An experimental decision between these two cyclisation schemes was reached by appropriate degradation of the biosynthetically labelled cholesterol. By degradation of cholesterol obtained from methyl labelled acetate, BLOCH has been able to prove that carbon atoms 7[1] and 13 are radioactive[2]. It follows that scheme *b* represents the cyclisation mechanism, if squalene really is the intermediate in the biosynthesis of cholesterol.

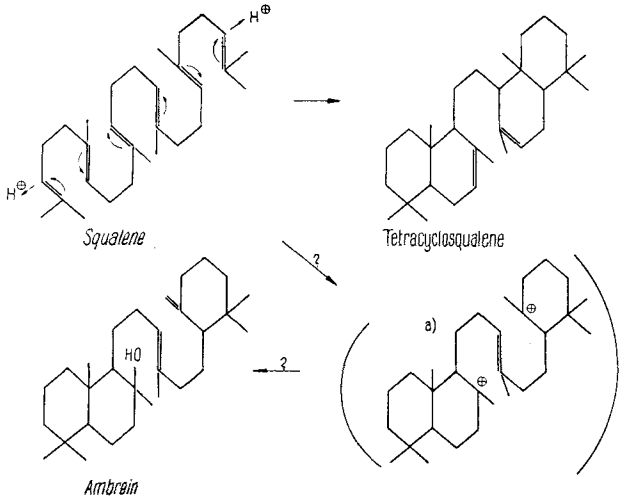
It had been shown previously that carbons 20–27 of the side chain (WÜRSCH, HUANG, and BLOCH) and 1–6,

[1] *Cf.* A. ESCHENMOSER, Ph.-D. Thesis, E.T.H., Zurich, 1952, p. 16–38.

[1] Private communication.

[2] A. MONDON, Angew. Chemie *65*, 333 (1953), has recently published a paper in which he proposes a hypothetical iso-squalene as the biological precursor of cholesterol. In this hypothesis the rearrangement required in the deduction of the cholesterol formula from squalene is avoided. However, MONDON's proposal is in disagreement with BLOCH's experimental results, as it does not account for the presence of a carbon atom derived from an acetate methyl group in position 13 of cholesterol.

10, and 19 of rings A/B of cholesterol (HUNTER, POPJAK, and CORNFORTH) are arranged according to the



XVI.—Acid-catalysed cyclisation of squalene.

alternation principle required by both cyclisation schemes (XIV).

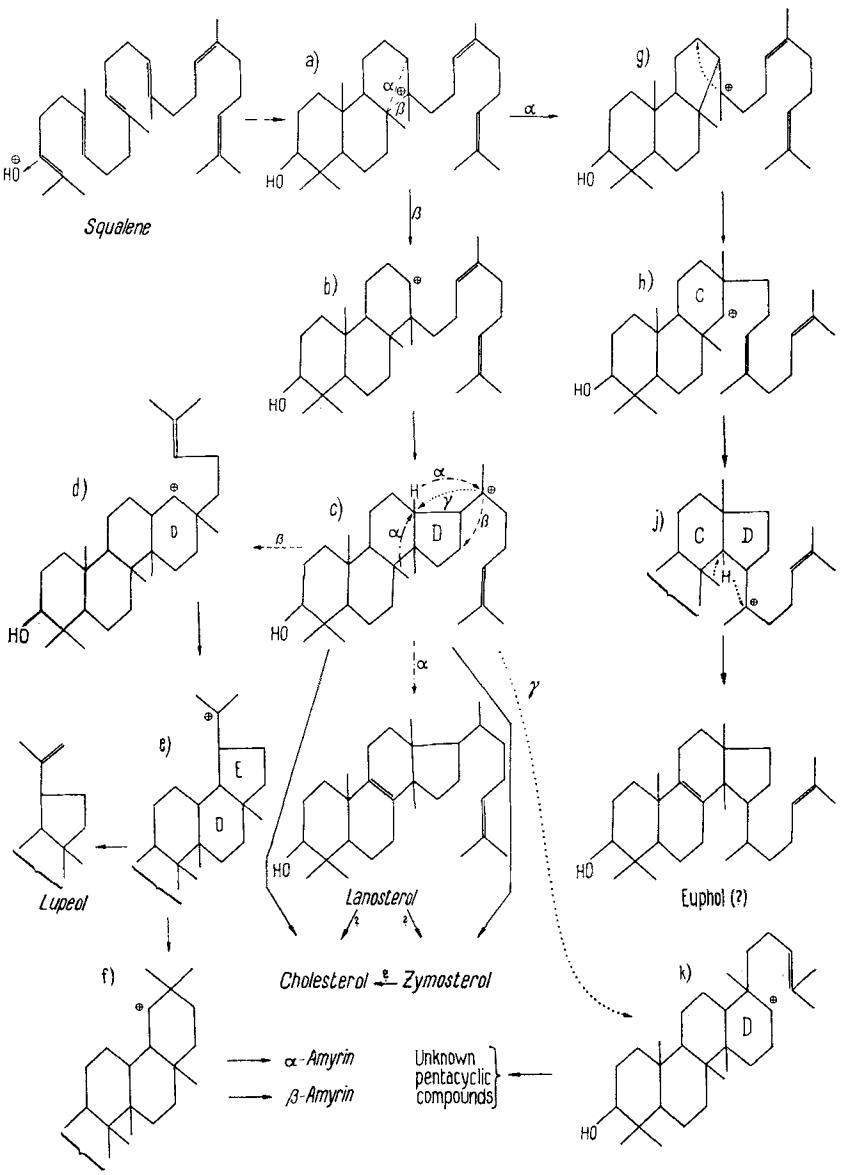
Though squalene most probably is the biological precursor of cholesterol, it should be emphasised that the only product obtained so far by acid-catalysed cyclisation of squalene is tetracyclosqualene[1] (XVI). It can be assumed that also ambrein is formed by cyclisation of squalene. In the formation of tetracyclosqualene, as well as in that of ambrein, cyclisation starts simultaneously at both ends of the squalene molecule (XVI).

An essential characteristic of the biological cyclisation of squalene to the steroids is the attack of a cation (e.g. OH^+) at one end only of squalene (XVII)[2], whereupon the cyclisation proceeds synchronously to completion[3]. In the case of *lanosterol, zymosterol,* and *cholesterol,* the cyclisation yields (by way of the intermediates XVII*a* and *b*) the cation XVII*c*, which is stabilised (reaction α in XVII*c*) through a 1–3 hydrogen shift[1] (from position 13 to position 20) and a double 1–2 methyl shift[2] (positions 8, 14, and 13).

The cation XVII*c* could also be stabilised by WAGNER-MEERWEIN rearrangement, and this by two different routes (routes β and γ in XVII*c*) each leading to different pentacylic systems. Both rearrangements result in the enlargement of ring D to a 6-membered ring (XVII*d* and *k*).

The cation XVII*d* leads to the formation of a 5-membered E-ring (XVII*e*), and hence to *lupeol.* A further WAGNER-MEERWEIN rearrangement of XVII*e* leads by way of the intermediate XVII*f* to *α-amyrin* and *β-amyrin.* This route from lupeol to β-amyrin via the intermediates XVII*e* and XVII*f* is nothing but a formulation of the well-known transformation of lupeol into compounds of the β-amyrin type, which, as we have seen, has been experimentally achieved (X).

[1] Or a double 1—2 shift. [2] Or a 1—3 shift.



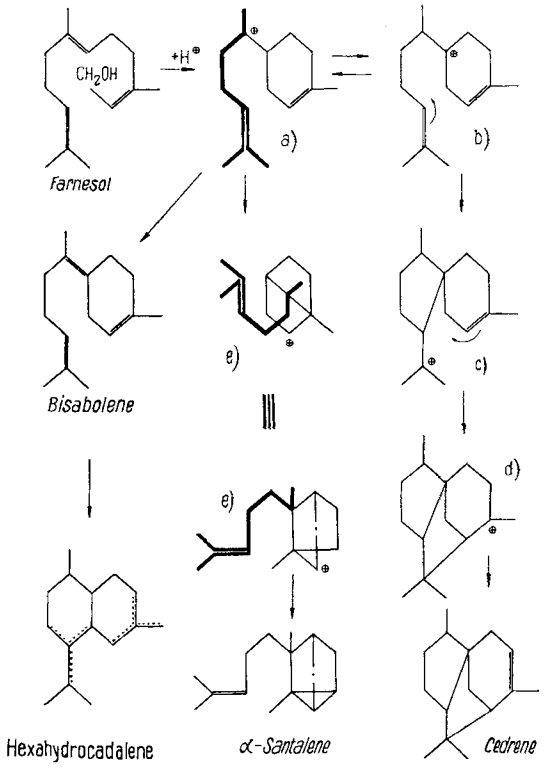
XVII.—Ionic mechanisms in the biogenesis of steroids and triterpenes from squalene.

[1] The position of the two double bonds in tetracyclosqualene, which has been proved by G. BÜCHI (private communication), explains why further cyclisation of tetracyclosqualene has so far been impossible.

[2] In Table XVII, for the sake of simplicity, the configuration of the methyl groups is not indicated.

[3] It is not known at what stage the methyl groups in positions 4 and 14 may be eliminated.

The cation XVII*k*, on the other hand, represents the precursor of pentacyclic compounds of structural types, which so far have not been observed in nature. It is worth noting that ring *E* in these hypothetical compounds would have structures similar to those of ring *E* in lupeol, α-amyrin, and β-amyrin.



XVIII.—Ionic mechanisms in the biogenesis of sesquiterpenes (6-membered ring intermediate).

A last variation of the squalene cyclisation should be mentioned. The limiting structure XVII*g* of the π-complex XVII*a* could undergo a WAGNER-MEERWEIN rearrangement (from 14 to 12) to an intermediate XVII*h*, with subsequent formation of the five-membered *D*-ring (XVII*j*). A 1–3 shift of a hydrogen atom (from 14 to 20) and a 1–2 shift of a methyl group (from 8 to 14) would lead to one of the formulae proposed for *euphol* (VII*b*). On the basis of this mechanism, formula VII*b* appears to be more likely than the one with the methyl group in 17 (VII*a*) and should therefore also be taken into consideration.

In an E.T.H. thesis[1] published in 1925, one may find the following statements:

"The *hypothesis* may be formulated that the *steroids* and the *triterpenes have, at least partially, a common origin*. For instance, one only has to insert a methyl group in each of the rings *A*, *B*, and *C* of cholesterol, in order to obtain a carbon skeleton corresponding to a triterpene. It would not be very profitable to discuss *the question whether steroids may originate from such triterpenes by elimination of three methyl groups*.[1]"

28 years later, in the light of BLOCH's experimental results and of the constitutional and configurational relations of steroids and triterpenes, the common origin of steroids and triterpenes is not only a matter of profitable discussion, but achieves the status of a working hypothesis for future chemical and biochemical work.

It should be emphasized that all of the biogenetic schemes discussed above are based on generally accepted reaction mechanisms. *Analogous biogenetic schemes*[2] *can also be proposed for the biosynthesis of sesquiterpenes, diterpenes, and monoterpenes.*

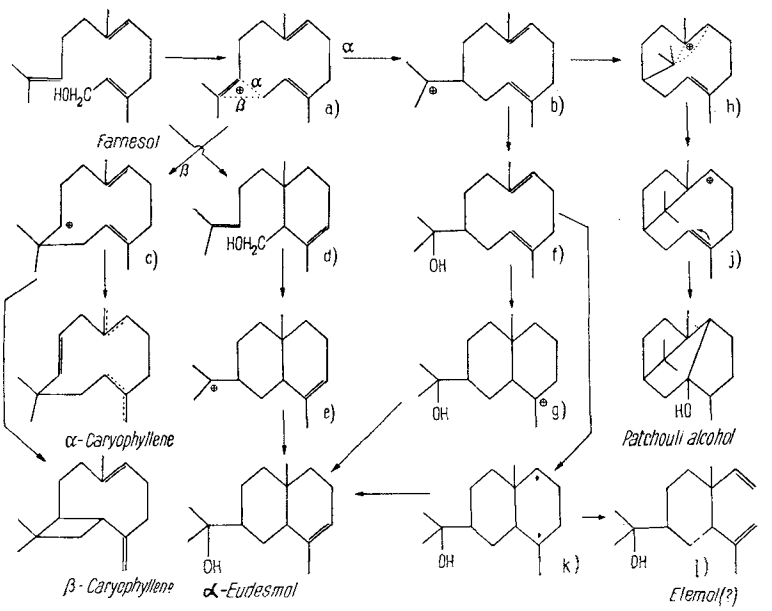
The biogenesis of *sesquiterpenes* possessing a carbon skeleton derived from farnesol may be assumed to follow a route starting from farnesol, farnesene or nerolidol.

One may distinguish two types of biogenetic cyclisation of farnesol. One is characterised by the formation of 6-membered ring intermediates and the other of 10- or 11-membered ring intermediates.

The *acid-catalysed cyclisation* of farnesol or nerolidol, which was carried out experimentally in Zurich in

[1] The cholesterol formula which had been proposed by WIELAND and WINDAUS when this Thesis was written was far from being established and no triterpene formula at all had as yet been published. *The above biogenetic hypothesis was advanced on principle, because it appeared more likely than the one deriving the steroids from fatty acids* (WIELAND).

[2] For the sake of greater clarity, the hypothetical intermediates in the acid-catalysed cyclisations are formulated as classical carbenium ions.



XIX.—Ionic and radical mechanisms in the biogenesis of sesquiterpenes (10- and 11-membered ring intermediates).

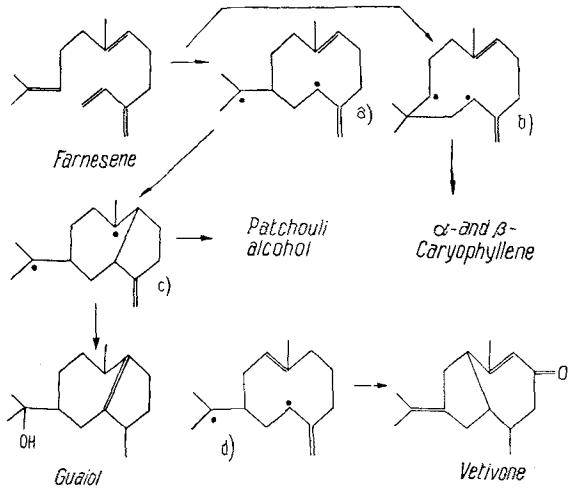
[1] E. A. RUDOLPH, Ph.-D. Thesis, E.T.H., Zürich, 1925, p. 98, 99.

1925, is an example of the first type. By way of the intermediate *6-membered ring* cation XVIII*a*, the reaction leads to *bisabolene* and further to a mixture of hexahydrocadalenes. The intermediate XVIII*a* could undergo a different cyclisation leading to the cation XVIII*e*, in analogy to the schematic transition from XXII*a* to the bornyl cation (XXII*b*). The cation XVIII*e* would be stabilised to α- or to *β-santalene*. The cation XVIII*b*, also derived by cyclisation of farnesol, leads to *cedrene* by way of the intermediates *c* and *d*.

The second type of farnesol cyclisation starts from the π-complex XIX*a* of the farnesyl cation, and may lead to a cation with a *10-membered ring* (*b*) or to a cation with an *11-membered ring* (*c*). The intermediate *c* yields *α-caryophyllene* (humulene) directly, or else, on formation of a 4-membered ring, *β-* or *γ-caryophyllene*.

The 10-membered ring intermediate XIX*b*, which is derived from the π-complex of the farnesyl cation, leads to *patchouli alcohol* by way of the π-complex XIX*h* and the bicyclic intermediate XIX*j*.

The biogenesis of *eudesmol* may be formulated along two different ionic routes. The one leads to α- or *β*-eudesmol by way of the already mentioned 10-membered ring intermediate XIX*b* and the intermediates *f* and *g*. The other route starts with a cyclogeraniol type cyclisation of farnesol to an intermediate XIX*d*, followed by an α-terpineol type cyclisation (XIX*e*) to α-eudesmol.



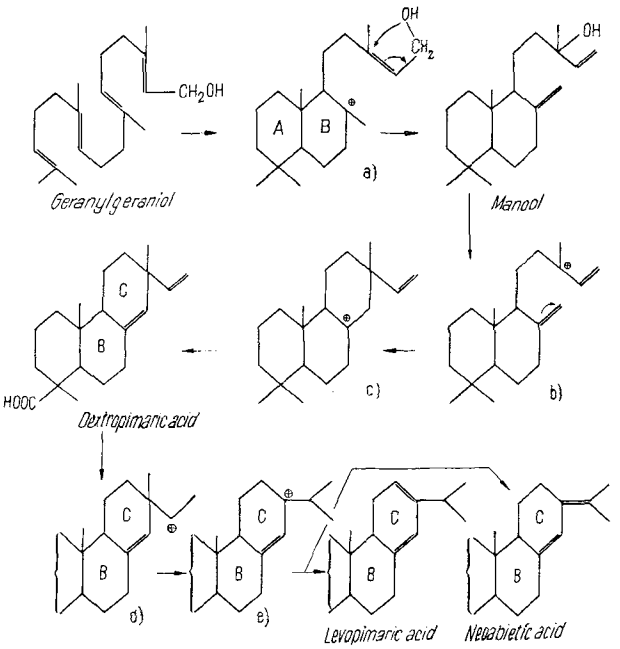
XX.—Radical mechanisms in the biogenesis of sesquiterpenes (10- and 11-membered ring intermediates).

So far only ionic cyclisations have been discussed. *Radical mechanisms* may, however, also be involved (XX). In fact, in certain cases the radical mechanism may be more appropriate than the ionic one, as for instance in the cyclisation of farnesene to guaiol, to vetivone, and also to the caryophyllenes. In the case of guaiol the intermediate would be the monocyclic biradical XX*a* which can cyclise to the bicyclic biradical *c* of the guaiol type. From here stabilisation may take place either directly to *guaiol* or by further cyclisation to patchouli alcohol (formula in Table XIX). The intermediate on the way from farnesene to the caryophyllenes (formulae in Table XIX) is the biradical XX*b*. A shift of the ring double bond of the biradical XX*a* would lead to a new 10-membered ring biradical (XX*d*), which may be stabilised by cyclisation, yielding *vetivone*.

Formula XIX*l*, recently advanced for *elemol* by ŠORM[1], on the basis of the isolation of methyldiisopropylbenzene on dehydrogenation, may be derived from the intermediate XIX*f* by cyclisation to the biradical XIX*k*, which could then undergo ring opening. This ring opening to XIX*l* corresponds closely to the one that occurs in the experimental conversion of α-pinene into ocimene (or *allo*-ocimene) and of *β*-pinene into myrcene by way of the biradicals XXIII*a* and XXIII*b*.

Another stabilisation route of the biradical XIX*k* would lead to α-eudesmol. This stabilisation is analogous to the conversion of the biradicals XXIII*a* and XXIII*b* into limonene, which has actually been obtained by pyrolysis of α- and *β*-pinene.

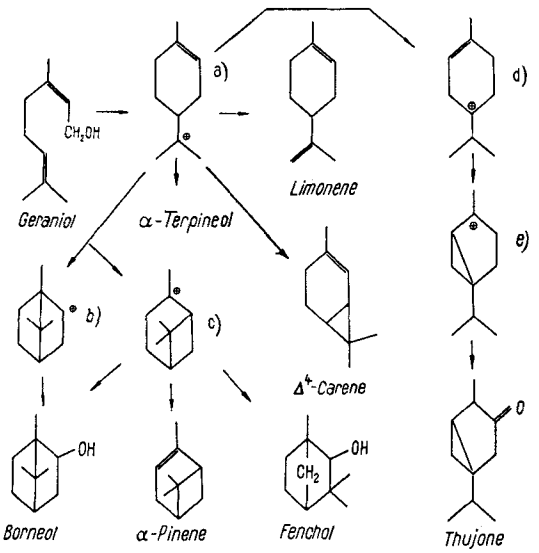
Farnesol thus occupies a key position not only because it possesses the carbon skeleton traceable in sesquiterpenes, but also because it is a possible biological precursor of the cyclic sesquiterpenes and, in the form of difarnesyl (*i.e.* squalene), also of the triterpenes and of the steroids.



XXI.—Ionic mechanisms in the biogenesis of diterpenes.

[1] F. ŠORM in a paper presented at the XIII[th] Internat. Congress of Pure and Applied Chemistry, Stockholm, 29[th] July, 1953.

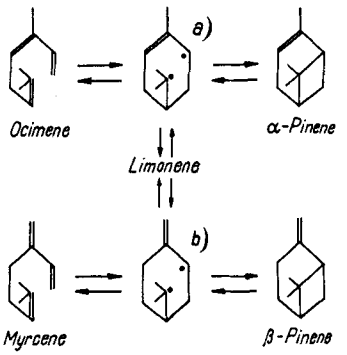
The *diterpenes* can easily be derived from geranylgeraniol[1], which so far has not been found in nature. Double cyclisation at one end of the molecule, accompanied by an allylic rearrangement at the other end (XXI*a*), would lead to *sclareol* and *manool*. Manool could in turn yield *dextropimaric acid* by cyclisation of the intermediate XXI*b* to XXI*c*. From dextropimaric acid, finally, one may derive *neoabietic acid* and *levopimaric acid*, by way of a WAGNER-MEERWEIN rearrangement of the intermediate XXI*d* to XXI*e*. As is well known, neoabietic acid and levopimaric acid readily yield *abietic acid*.



XXII.—Ionic mechanisms in the biogenesis of monoterpenes.

The cyclic *monoterpenes* can also be derived from an open chain precursor such as geraniol[2] by way of the common intermediate XXII*a*. In Table XXII the well-known formation of *limonene* from geraniol is formulated. The intermediate *a* can cyclise to XXII*b* or *c* and lead either to α- and *β-pinene*, or to *borneol*. From pinene, by way of XXII*c* bornyl and *fenchyl* derivatives can be obtained. By an intramolecular electrophylic substitution the intermediate XXII*a* can also lead to a 3-membered ring and consequently to *Δ⁴-carene*. *Thujone* and *sabinene* may be derived from the cation XXII*d*, an intermediate in the formation of α- and γ-terpinene from limonene, by way of the bicyclic cation *e*.

For the biogenesis of the monoterpenes it is also possible to propose radical mechanisms, as for example for the formation of limonene and α-pinene from ocimene, and of limonene and β-pinene from myrcene, by way of the intermediate biradicals XXIII*a* and *b*. The reactions in Table XXIII are nothing but the reversal of the experimentally achieved thermal ring openings of α-pinene to yield limonene and *allo*-ocimene (which is formed by isomerisation of the primary product ocimene), and of β-pinene to yield limonene and myrcene.



XXIII.—Radical mechanisms in the biogenesis of monoterpenes.

Isoprene rule

Only few of the cyclisations mentioned above have been carried out in the laboratory. These reactions can follow various routes, each leading to different natural compounds and, so far, most of the routes are, on steric grounds, not accessible *in vitro*. The course of a cyclisation is not only dependent on the reaction mechanism, but also to a high degree on the *constellation*[1] of the precursor and of the intermediate. It is an important function of the enzymes to bring about the required constellation and thus cause the cyclisation to follow one specific course.

An interesting example of the importance of the constellation in the biogenesis of terpenic compounds is given in the sesquiterpene group, where a great variety of carbon skeletons is encountered. The one carbon skeleton which has never been observed, is the bicyclofarnesol skeleton, so typical of all cyclic diterpenes and triterpenes. This appears to indicate that the biogenesis of steroids, diterpenes, and triterpenes differs in some fundamental detail from that of monoterpenes and sesquiterpenes.

Whether the hypothetical schemes which have been discussed have a real significance in the laboratory of nature, remains of course to be proved by experiment. BLOCH's merit is to have provided a firmer basis for such an experimental approach to the biogenesis of terpenic compounds as well as of the steroids.

Moreover, the *deduction of structures of natural terpenic compounds by accepted reaction mechanisms from the hypothesized simple precursors squalene, geranylgeraniol, farnesol, geraniol* (*"biogenetic isoprene rule"*) not only serves to outline possible biogenetic routes,

[1] Or from analogous compounds which can yield suitable ionic or radical intermediates, *e.g.* geranyllinalool, geranylmyrcene.

[2] Or from analogous compounds, *e.g.* linalool, myrcene.

[1] The word *"constellation"* (F. EBEL in K. FREUDENBERG, Stereochemie [F. Deuticke, Leipzig and Wien, 1933], p. 825) has the same meaning as *"conformation"* (*cf.* D. H. R. BARTON, Soc. *1953*, 1027).

but also represents a new helpful tool in the structural elucidation of terpenic compounds. This new tool limits the number of carbon skeletons which can be proposed on the basis of the original isoprene rule[1] (*"empirical isoprene rule"*) alone. It is obvious that any formula deduced in accordance with the "biogenetic" as well as with the "empirical" isoprene rule requires experimental proof.

The "empirical isoprene rule" was deduced formally from the structure of the natural terpenic compounds. From the "biogenetic isoprene rule", however, it would follow that the carbon skeleton of the biological end product is not necessarily identical to the carbon skeleton of the precursor. In other words the validity of the "empirical isoprene rule" depends on the mechanisms of formation of the natural compounds, and the failure of a terpene to obey this rule does not necessarily disprove its origin from isoprene units.

Nomenclature

The terms terpene, diterpene, triterpene are retained here in preference to the terms terpenoid, diterpenoid, triterpenoid, which are occasionally used in the modern literature. These terms have unnecessarily been introduced in analogy to the expression "steroid", which denotes a group of compounds having an irregularly varying number of carbon atoms. In the terpenic field the expression terpenoid should be reserved by analogy for compounds in which the number of carbon atoms varies irregularly[2], in contrast to the terpenes proper, where the number of carbon atoms is always a multiple of five.

The terms monoterpene, sesquiterpene, diterpene, triterpene are unambiguous and need not be replaced by nebulous synonyms ending in "oid". The term terpene, on the other hand, originally designated the monoterpenes alone. In order to avoid confusion the term terpene should be used only to designate the whole class of the terpenic compounds, and for the C_{10} group the term monoterpene should be used exclusively.

Zusammenfassung

Es wird eine Übersicht über die Kohlenstoffgerüste der Sesquiterpene, Diterpene und Triterpene gegeben. Die Isoprenregel zeigt in jeder dieser Gruppen besondere Eigenheiten. Auch die C_{30}-Steroide und ein Steroid mit 31 Kohlenstoffatomen, die mit den Triterpenen verwandt sind, werden besprochen. Die Zusammenhänge zwischen Diterpenen und Triterpenen in bezug auf ihre Konstitution und Konfiguration werden diskutiert, wobei auf die weitgehende Übereinstimmung ihrer Konfiguration mit der Konfiguration der Steroide hingewiesen wird. Die kürzlich durch die Experimentalarbeiten von BLOCH gewonnenen Kenntnisse über die biologische Entstehung des Cholesterins aus Essigsäure, unter sehr wahrscheinlicher Zwischenbildung des Triterpens Squalen, geben Anlass zur Erörterung hypothetischer Wege für die Biogenese der pentazyklischen Triterpene aus Squalen, der zyklischen Sesquiterpene aus Farnesol, der zyklischen Diterpene aus Geranyl-geraniol und schliesslich auch der zyklischen Monoterpene aus Geraniol, unter Beachtung der elektronischen Zyklisierungsmechanismen der organischen Chemie. Schliesslich wird die «biogenetische» Isoprenregel definiert und ihre Bedeutung diskutiert.

[1] *Cf.* page 357.

[2] *E.g. santene (C_9), irone (C_{14}), lupulone (C_{21}).*

Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

The Configuration of Digoxigenin

Although crystalline Digoxin (the tridigitoxoside of digoxigenin) was isolated in 1930[1] and has been in clinical use in the United Kingdom since 1933[2] the accepted proof of its structure is vitiated by a discrepancy between the optical rotation of the derived methyl 3:12-dihydroxyetianate, $[\alpha]_D$ + 39° (methanol)[3] and of authentic methyl 3α:12β-dihydroxyetianate, $[\alpha]_D$ + 52° (methanol)[1], with which it gave no melting point depression[2]. This matter was discussed with Professor REICHSTEIN and Dr. D. A. H. TAYLOR in 1952 and in the knowledge that the former was planning to prepare the four isomeric 3:12-dihydroxyetianates and the latter was repeating the degradation of digoxigenin, we studied partial acetylation of digoxigenin and the anhydrodigoxigenins.

[1] S. SMITH, J. Chem. Soc. *1930*, 508.

[2] Brit. Med. J. *295*, 864 (1933).

[3] M. STEIGER and T. REICHSTEIN, Helv. chim. Acta *21*, 828 (1938).

[1] V. WENNER and T. REICHSTEIN, Helv. chim. Acta *27*, 965 (1944).

[2] M. STEIGER and T. REICHSTEIN, Helv. chim. Acta *21*, 828 (1938). – H. L. MASON and W. M. HOEHN, J. Amer. Chem. Soc. *60*, 2824 (1938); *61*, 1614 (1939).

Wenn die Ansicht richtig ist, daß der heutige Zustand des Erdinnern das Ergebnis eines asymptotischen Vorgangs ist, so wird das Ergebnis mit dem von uns geschilderten (stoffliche Kontinuität im Erdinnern) im wesentlichen immer übereinstimmen müssen, mit dem Zusatz, daß in den Einzelheiten betreffend den Tiefgang der einzelnen Differenzierungsvorgänge und die *genaue* Verteilung der einzelnen Elemente noch Präzisierungen möglich sein werden.

Summary

According to considerations put forwards by W. KUHN and A. RITTMANN some years ago, it follows that the chemical composition of the interior of the earth must be rather homogeneous; the well-known discontinuity which occurs at 2900 km with respect to the propagation of longitudinal and transverse waves should not be due to a discontinuity of the material composition (not to an iron core). It is due to a continous decrease of the viscosity and thereby of the relaxation time; transverse waves of a period of e.g. 30 seconds will no more be propagated in a material whose relaxation time for tangential stress is below 30 seconds, while the longitudinal waves will suffer a decrease of the velocity at the same time.

A criticism put forward by A. EUCKEN consists in the argument that a material in which the time of relaxation for tangential stress becomes equal to the period of the vibration will exhibit a considerable absorption coefficient for longitudinal waves too. It is now shown that the distance on which the period of vibration and the relaxation time are approximately equal is small compared with the wave length of the seismic waves in question, from which it follows that the resulting absorption of the longitudinal waves too will only be small.

A further consideration shows that a mixture of 99 atomic % hydrogen and 1 atomic % of iron is most probably supercritical at a temperature of 5000^0 abs.

A survey of the solubilities in question shows further that the hydrogen present in a mixture of 90 % hydrogen and 10 % iron should on the strength of the absorption coefficient be completely absorbed by the iron at 5000^0 abs. and at a total pressure of $2 \cdot 10^6$ atmospheres.

The main argument why the assumption of an iron core inside the earth must be dismissed remains the fact that the present state must be the result of an asymptotic processus which at least in its final phase has occurred under conditions similar to the present conditions of temperature, pressure and viscosity; these latter conditions are far from permitting the processus of sedimentation etc. which would be required.

Die «Sprache» der Bienen und ihre Nutzanwendung in der Landwirtschaft[1]

Von KARL v. FRISCH, Graz

Man kann sich durch ein einfaches Experiment davon überzeugen, daß die Bewohner eines Bienenstockes imstande sind, einander Nachrichten zu geben. Ein Honigschälchen, in der Nähe des Stockes aufgestellt, bleibt oft stunden- und tagelang unbeachtet. Wird es aber von einer Biene entdeckt, so stellen sich nach kurzer Zeit auch andere ein, in steigender Zahl, und bald in Massen. Sie kommen aus demselben Heimatstock wie die Entdeckerin. Haben wir mehrere Schälchen rings um den Stock in dessen Nähe aufgestellt, und wurde *eines* von ihnen gefunden, so kommen Neulinge etwa gleichzeitig und in gleicher Zahl an sämtliche Schälchen. Die verständigten Bienen fliegen also nicht der Entdeckerin nach, sondern sie suchen selbständig nach allen Richtungen.

Was hier vorgeht, bleibt in einem gewöhnlichen Bienenkasten den Blicken entzogen. In einem Beobachtungsstock aber, dessen Waben nebeneinander angeordnet und durch Glasfenster in ihrer ganzen Ausdehnung überschaubar sind, sieht man folgendes: Die heimkehrende Sammlerin läuft in einen bestimmten Wabenbezirk und gibt zunächst etwas von dem eingetragenen Honig an die nächstsitzenden Bienen ab. Dann beginnt sie einen *Rundtanz*. Sie läuft in engen Kreisen abwechselnd rechts herum und links herum, wobei sie einen Schwanz anderer Bienen hinter sich herzieht, die ihr nachtrippeln und alle Wendungen mitmachen. Nach neuerlicher Futterabgabe wiederholt sich dasselbe Spiel an anderen Wabenstellen oft noch mehrere Male. Der Rundtanz bedeutet, daß in der Nähe des Stockes etwas zu holen ist. Bienen aus der Gefolgschaft der Tänzerin sind es, die hernach ausfliegen und nach allen Seiten suchen. Haben sie das Futter gefunden, so tanzen auch sie, und es steigert sich der Alarm im Bienenvolk.

Die naturgegebenen Trinkbecher der Bienen sind nicht Glasschälchen, sondern Blumen. Wir verändern das Experiment, indem wir in der Nähe des Stockes einige *Phlox*blüten anbringen und mit Zuckerwasser betropfen. Gezeichnete Bienen sammeln das Zuckerwasser auf diesen Blüten und tanzen im Stock. Am Futterplatz anfliegende Neulinge werden abgefangen, damit sie den Versuch nicht stören. An einem andern Ort der Umgebung stellen wir eine Schale mit Phlox und eine mit Zyklamen auf, ohne sie mit Zuckerwasser zu betropfen. Bald stellen sich auch hier alarmierte Neulinge ein und befliegen den *Phlox* in steigender Zahl. Sie setzen sich auf die Blüten und unter-

[1] Vortrag an der 126. Jahresversammlung der Schweizerischen naturforschenden Gesellschaft in Zürich am 8. September 1946.

suchen sie mit Ausdauer, obwohl ihnen der tief geborgene Nektar dieser Schmetterlingsblumen gar nicht zugänglich ist. Die Zyklamen werden nicht beachtet. Füttern wir aber nun die gezeichnete Bienenschar statt auf Phlox auf *Zyklamen*blüten, so wendet sich das Interesse der später ausschwärmenden Neulinge vom Phlox ab und den Zyklamen zu. Sie wissen, wonach sie suchen sollen!

Ich habe dieses Experiment mit vielerlei Blumenarten wiederholt und es ist immer gelungen, wenn an den Blüten ein Duft erkennbar war. Nur mit völlig duftlosen Blüten (Heidelbeere, wilder Wein, windblütige Gräser u. a.) geht es nicht. Demnach liegt das Verständigungsmittel offenbar im Duft der besuchten Blüten. Wenn dieser dem Körper der heimkehrenden Sammlerin noch erkennbar anhaftet, so wird auch klar, warum die Stockgenossen, die der Tänzerin nachtrippeln, deren Leib so eifrig mit ihren Riechwerkzeugen, den Fühlern, betasten. Sie merken sich den Duft, suchen draußen nach ihm und gelangen so an dieselbe Blütensorte, an der die Sammlerin gewesen war. Dieser Zusammenhang wird überzeugend deutlich, wenn man die Blumen wegläßt und ätherische Öle oder künstliche Riechstoffe anwendet. Wir füttern gezeichnete Bienen aus einem Glasschälchen auf einer Unterlage, die nach Pfefferminz duftet. Nach dem Einsetzen der Tänze befliegen die ausschwärmenden Neulinge alle Gegenstände, wie immer sie aussehen, wenn wir ihnen durch eine Spur von Pfefferminzöl dessen Geruch verliehen haben.

Es ist erstaunlich, daß diese Duftsprache der Bienen auch *bei sehr schwachem Blütenduft* nicht versagt. Der Versuch ist z. B. mit Schwalbenwurzenzian (*Gentiana asclepiadea* L.), mit Hahnenfuß (*Ranunculus acer* L.) oder Feuerbohnen (*Phaseolus multiflorus* Lmk. var. *coccineus* L.) gelungen, durchwegs Blumen mit so unauffälligem Duft, daß man sie gewöhnlich für geruchlos hält. Die Erklärung liegt zum Teil darin, daß Duftstoffe am Körper der Bienen länger haften als an anderen Gegenständen, wie Glas, Porzellan, verschiedenen Metallen, Watte, Papier, oder auch am Chitin anderer Insekten[1].

Es kommt aber noch etwas Weiteres hinzu: Der im Blütengrunde abgesonderte *Nektar* ist mit dem spezifischen Blütenduft geschwängert. Daher trägt die Sammlerin in ihrer Honigblase mit dem Nektar eine *Duftprobe* nach Hause, die sie beim Verfüttern des Tropfens den umgebenden Bienen zur Kenntnis bringt. Daß dies richtig ist, ergibt sich aus folgendem Versuch: Wir betropfen Phloxblüten mit Zuckerwasser, bis sich dieses nach 1–2 Stunden mit dem Phloxduft beladen hat. Dann lassen wir einige Bienen das phloxduftende Zuckerwasser aus einem engen Spalt saugen, so daß ihr Körper dem Duft *nicht* ausgesetzt ist (Fig. 1). Sie tanzen daheim und verfüttern das duftende Zuckerwasser. Die ausschwärmenden Neulinge suchen nach diesem Duft. Sie tun es mit gleicher, einwandfreier Deutlichkeit wie in einem Gegenversuch, bei

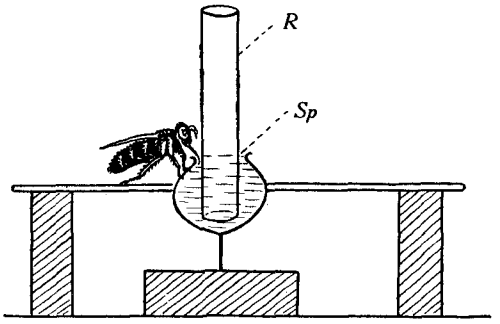


Fig. 1. Bienen saugen *nach Phlox duftendes Zuckerwasser* aus dem schmalen Spalt *Sp* zwischen dem Rande der Glaskugel und dem eingesetzten Glasrohr *R*. Der *Körper* der Bienen ist dem Duft *nicht* ausgesetzt. Das duftende Zuckerwasser wird mit einer Pipette durch die obere Öffnung des Glasrohrs nachgefüllt.

dem die sammelnden Bienen auf Blüten sitzen und aus dem engen Spalt *duftloses* Zuckerwasser aufsaugen (Fig. 2). Der äußerlich am Körper haftende und der innerlich in der Honigblase eingetragene Duft sind *beide* wirksam. Ich habe Bienen auf *Zyklamen*blüten sitzen lassen und ihnen gleichzeitig aus dem engen Spalt *phlox*duftendes Zuckerwasser geboten, also den äußerlich mit dem innerlich eingetragenen Duft gleichsam in Konkurrenz gesetzt. Am Beobachtungsplatz erhielt die Phloxschale doppelt so viele Anflüge als die

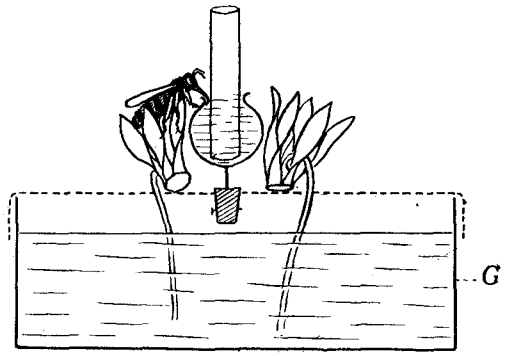


Fig. 2. Bienen saugen *duftloses Zuckerwasser* aus dem Spalt. Ihr *Körper* ist dem Duft von Zyklamenblüten ausgesetzt. *G* Glasschale mit Wasser, zum Einstecken der Blüten mit einem Gitter bedeckt.

Zyklamenschale. Im Gegenversuch saßen die Bienen auf Phloxblüten und tranken zyklamenduftendes Zukkerwasser; nun wurde die Zyklamenschale von Neulingen etwa zweimal so stark angeflogen als die danebenstehende Schale mit Phloxblüten. Demnach hat der von der Lösung absorbierte, im Magen eingetragene Blütenduft die größere Bedeutung. Er gewinnt den Wettbewerb entscheidend, wenn sich die Futterquelle in weiter Entfernung vom Bienenstock befindet. Ich habe den eben beschriebenen Versuch bei einem Abstand von 600 m zwischen Futterplatz und Bienenstock wiederholt. Bei weiter Flugstrecke

[1] Noch unveröffentlichte Untersuchungen von Frl. STEINHOFF.

wird der Körper stark gelüftet und der äußerlich anhaftende Duft verliert an Intensität. Nun richteten sich die ausschwärmenden Neulinge *ausschließlich* nach dem im Magen eingetragenen Blütenduft. Wir erkennen daraus die große biologische Bedeutung des vom Nektar absorbierten Blumenduftes, der von den Bienen in ihrer Honigblase wie in einem wohlverkorkten Fläschchen heimgetragen wird und dann im Stock zur Geltung kommt.

Der Alarm durch die tanzenden Bienen, in Verbindung mit dem Duftsignal, vermittelt den Stockgenossen in einfachster Weise die Kenntnis des Zieles, nach dem sie suchen sollen. Die Nachricht ist eindeutig, weil jede Pflanzenart ihren spezifischen Blütenduft besitzt und weil das Geruchsorgan der Bienen diese vielen Düfte auch zu unterscheiden vermag. Ich stellte einst einen Beobachtungsstock an der systematischen Abteilung des Münchner botanischen Gartens auf. Inmitten dieser Abteilung befand sich eine kleine Anpflanzung einer Immortellenart, die unter normalen Umständen nicht von Honigbienen beflogen wird (*Helichrysum lanatum*). Als ich gezeichnete Bienen an anderer Stelle auf einigen abgepflückten Immortellenblüten fütterte, kamen nach kurzer Zeit alarmierte Neulinge in ansehnlicher Zahl an jenes winzige Beet. Sie hatten das Ziel unter 700 anderen Pflanzenarten, die damals gleichzeitig auf dem engen Raum des Systemgartens in Blüte standen, richtig herausgefunden.

Die Tänze der Bienen erhalten ihren vollen biologischen Sinn erst durch den Umstand, daß sie *nur durch gute, ergiebige Futterquellen* ausgelöst werden. Bei einer Tracht, die ein großes Aufgebot nicht lohnt, wird nicht getanzt. Ein Schälchen mit Zuckerwasser gibt Anlaß zu immer wiederholten Tänzen, solange es gefüllt gehalten wird, stundenlang, vom Morgen bis zum Abend. Bietet man dieselbe Nahrung spärlich in zuckerwasserdurchfeuchtetem Fließpapier, so wird zwar weitergesammelt, aber nicht getanzt. Derselbe Gegensatz ist an natürlichen Blüten zu beobachten, wenn sie einmal reichlich, das andere Mal spärlich Nektar enthalten. Sind also bei einer neu erblühten Pflanzenart durch die Tänze der ersten Sammlerinnen so viele Bienen mobilisiert worden, daß sie den Honigsegen bewältigen und der Nektar in den Blüten spärlich wird, so unterbleiben die Tänze und es unterbleibt hiermit auch ein weiteres Anwachsen der Sammlerschar. Neben der *Menge* des Futters ist seine *Süßigkeit* von ausschlaggebender Bedeutung. Je süßer der gebotene Zuckersaft, desto lebhafter und anhaltender wird getanzt. Bei abnehmender Süßigkeit werden die Tänze matt und unterbleiben schließlich ganz, auch wenn die Sammeltätigkeit noch fortgesetzt wird. Das bedeutet, daß die Blüten mit dem meisten und süßesten Nektar die stärksten Tänze auslösen, daher am besten beflogen und am sichersten befruchtet werden – gewiß für die Blüten ein mächtiger Faktor zur Steigerung der wunderbaren Fähigkeit, in ihren Kelchen hochkonzentrierten Zuckersaft zur Abscheidung zu bringen.

Wenn die Blumendüfte, wie prägnante Ausdrücke einer Wortsprache, zur Verständigung über das Ziel der Sammelflüge dienen, so gesellt sich dazu als weiteres «Wort» der Bienensprache noch ein körpereigener Lockduft, der mit Nachdruck die Stockgenossen dahin ruft, wo ihre Kameraden bereits erfolgreich tätig sind. Die Honigbiene besitzt am Hinterleib ein *Duftorgan*, wie solche von weiblichen Insekten vielfach benützt werden, um die Männchen anzulocken. Bei der Bienenarbeiterin hat es soziale Bedeutung gewonnen. Beim «Sterzeln» vor dem Flugloch zeigen sie den noch unerfahrenen Kameraden als lebende Duftmarken den Weg zum Eingang des Stockes, und beim Anflug an eine Trachtquelle locken sie die in der Nähe herumsuchenden Neulinge durch das Ausstülpen der Duftdrüse an den richtigen Ort (Fig. 3). Man kann bei Bienen, die an einem Glasschälchen Zuckerwasser sammeln, das Duftorgan dadurch ausschalten, daß man die Hinterleibsringe, zwischen denen das Organ bei seiner Betätigung hervortritt, mit Schellack aneinanderklebt. Der Zustrom an Neulingen wird dadurch etwa auf den zehnten Teil vermindert. So mächtig ist die lockende Kraft dieser winzigen Drüse. Es erscheint durchaus logisch, daß die Biene nur dann von ihr Gebrauch macht, wenn sie auf der Wabe getanzt hat. Wenn bei schlechter Tracht die Tänze unterlassen werden, dann unterbleibt auch das Ausstülpen der Duftdrüse beim neuerlichen Anfliegen des Weideplatzes; dann sind ja auch keine neu angeworbenen Helferinnen unterwegs, für die der Wink von Nutzen wäre.

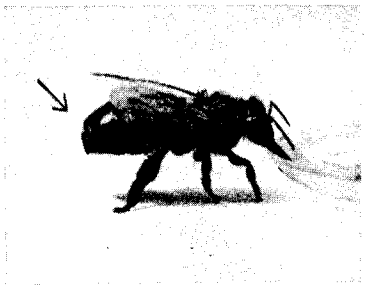


Fig. 3. Biene am Futterschälchen. Der Pfeil weist auf die ausgestülpte Duftfalte hin.

Bei meinen alten Versuchen über die Sprache der Bienen bot ich das Futter stets in unmittelbarer Nähe des Beobachtungsstockes, um die Vorgänge auf den Waben und den Futterplatz gleichzeitig im Auge behalten zu können. Daß sich die ausschwärmenden Neulinge in der Nähe des Stockes rasch und in großer Zahl, an entfernteren Plätzen später und viel spärlicher einstellten, erschien nicht auffällig. Vor zwei Jahren legte ich zum erstenmal einen Futterplatz einige hundert Meter vom Bienenstock entfernt an und er-

lebte die große Überraschung, daß nun die Neulinge in dieser Entfernung sehr bald und zahlreich, in der Nähe des Stockes aber viel spärlicher suchten. Es schien so, als könnten die Tänzerinnen auch sagen, wie weit man fliegen muß, um die Futterstelle zu finden. Ich richtete es nun so ein, daß Bienen eines Beobachtungsstockes in dessen Nähe, an einem 12 m

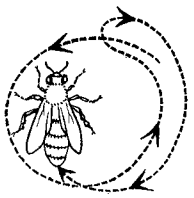


Fig. 4. Laufkurve der Biene beim *Rundtanz*.

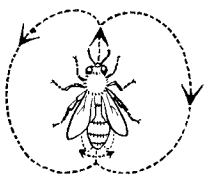


Fig. 5. Laufkurve der Biene beim *Schwänzeltanz*.

entfernten Futterschälchen, andere Bienen aus demselben Volk an einem fernen Schälchen in etwa 300 m Abstand sammelten. Die einen waren durch einen blauen, die anderen durch einen roten Tupfen auf dem Hinterleib auffallend gekennzeichnet. Als ich nach diesen Vorbereitungen an beiden Plätzen gleichzeitig Futter bot und den Beobachtungsstock öffnete, traute ich kaum meinen Augen. Alle Nahsammler machten *Rundtänze* (Fig. 4), alle Fernsammler führten *Schwänzeltänze* (Fig. 5) auf. Beim Schwänzeltanz läuft die Biene einen Halbkreis, dann geradlinig zum Ausgangspunkt zurück, einen Halbkreis nach der anderen Seite, wieder geradlinig zurück, usw. Beim Geradelauf erfolgt jedesmal ein Schwänzeln mit dem Hinterleib. Der Schwänzeltanz erregt genau so wie der Rundtanz das lebhafte Interesse der umgebenden Bienen. Ich verlegte den nahen Futterplatz stufenweise in größere Entfernung. Bei einem Abstande von 50–100 m gingen die Rundtänze in Schwänzeltänze über. Ich brachte gleichzeitig das Futterschälchen der Fernsammler stufenweise immer näher an den Heimatstock. Zwischen 100 und 50 m wurden die Schwänzeltänze durch Rundtänze abgelöst. In vielen weiteren Versuchsreihen hat sich seither dieses Verhalten ausnahmslos bestätigt. Rundtanz und Schwänzeltanz sind zwei verschiedene Ausdrücke der Bienensprache, die auf nahe gelegene und ferne Futterquellen hinweisen und, wie sich zeigen läßt, von den Stockgenossen in diesem Sinne verstanden werden[1].

Die Bienen geben einander genaue Kunde von der Art der entdeckten Blütensorte, sie berichten über die Üppigkeit der Tracht, sie rufen beim Anflug an die Futterquelle die anderen, noch Unerfahrenen, zu sich heran. Sollte sich wirklich eine so differenzierte Sprache auf die Mitteilung «näher» oder «weiter als 100 m vom Stock» beschränken, wo sich doch die Sammelflüge eines Volkes bis zu Entfernungen von 2–3 km ausdehnen? Diese Überlegung gab Anlaß zu vergleichenden Beobachtungen über den Schwänzeltanz bei stufenweiser Verlagerung des Futterplatzes bis an die Grenzen des Flugbereiches. Es zeigte sich, daß mit zunehmender Entfernung der Rhythmus des Tanzes in gesetzmäßiger Weise geändert wird. Bei einem Abstand der Futterquelle von 100 m vollzieht sich der Tanz in hastigen Wendungen; die kurzen, rasch hingeworfenen Schwänzelläufe wiederholen sich etwa zehnmal in einer Viertelminute. Je größer die Entfernung des Weideplatzes ist, desto geringer wird die Zahl der Wendungen beim Schwänzeltanz, um bei einem Abstand von 3 km auf fast nur zwei in einer Viertelminute abzusinken (Fig. 6). Die tanzenden Bienen drehen sich langsamer, während gleichzeitig der geradlinige Schwänzellauf verlängert wird und an Nachdrücklichkeit gewinnt. So kann man mit der Uhr in der Hand auf etwa 100 m genau die Entfernung angeben, in der die Tänzerin gesammelt hat.

Die ausfliegenden Neulinge haben aber nicht nur eine gewisse Kenntnis von der *Entfernung* der aufzusuchenden Trachtquelle, sondern auch von der *Richtung*, in der sie gelegen ist. Ein Beispiel mag dies anschaulich

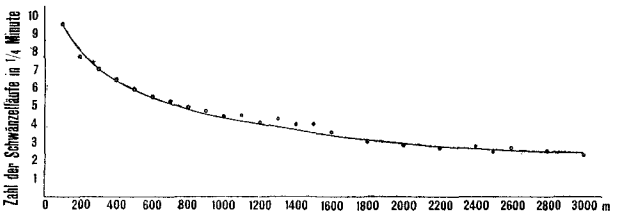


Fig. 6. Der Rhythmus des Schwänzeltanzes als Indikator für die Entfernung des Futterplatzes. Auf der Abszisse ist die Entfernung des Futterplatzes vom Bienenstock, auf der Ordinate die Zahl der Wendungen beim Tanz (Zahl der Schwänzelläufe) pro ¼ Minute aufgetragen. (Mittelwerte aus den bisher vorliegenden Beobachtungen.)

machen. Bei *B* (Fig. 7) befand sich ein Bienenstock. Bei *F*, 300 m in nördlicher Richtung entfernt, wurden 9 gezeichnete Bienen dieses Stockes auf einer Unterlage, die nach Thymianöl duftete, 1 Stunde lang mit Zuckerwasser gefüttert. Alle Neulinge, die sich zugesellten, wurden abgefangen. An verschiedenen Plätzen der Umgebung in gleicher wie auch in entgegengesetzter Richtung lagen kleine Kartonplatten im Grase, ohne Futter, aber mit Thymianduft. Bei jeder saß ein Beobachter und zählte die anschwärmenden,

[1] Ich habe früher den Schwänzeltanz als den Tanz der *Pollen*sammler aufgefaßt. Der Irrtum war dadurch entstanden, daß ich Zuckerwasser und Nektar stets in der Nähe des Stockes geboten hatte, während ich die leicht kenntlichen Pollensammler bei ihrer natürlichen Sammeltätigkeit an weiter abgelegenen Trachtquellen zu beobachten pflegte. Auch Pollensammler machen Rundtänze, wenn ihr Weideplatz näher als 50 bis 100 m, und Schwänzeltanze nur dann, wenn er weiter als 50 bis 100 m vom Stock abliegt.

von den Tänzerinnen hinausgeschickten Bienen. An den entfernten Plätzen, die angenähert in der Richtung der Futterstelle lagen, kamen während jener Stunde je über hundert, während eine gleichartige Duftplatte in unmittelbarer Nähe des Stockes wie auch solche,

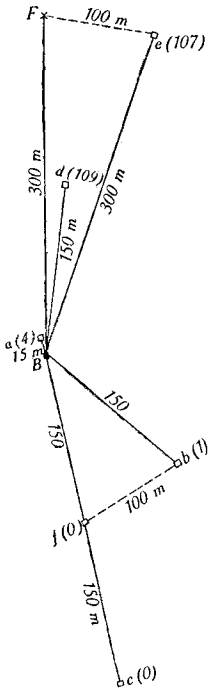


Fig. 7. Versuch über die Richtungsweisung. *B* Bienenstock, *F* Futterplatz in 300 m Abstand vom Bienenstock; die Unterlage des Futterschälchens war mit Thymianöl versehen. *a–f* Duftplatten mit Thymianöl, ohne Futter, in verschiedener Entfernung und Richtung; in Klammer beigefügt: die Zahl der angeflogenen Bienen.

die in großer Entfernung, aber entgegengesetzter Richtung aufgelegt waren, nur vereinzelte oder überhaupt keine Besuche erhielten. Als der Futterplatz in einem anderen Versuch im Süden lag, wandte sich der Strom der Neulinge den südlichen Duftplatten zu und die nördlichen blieben unbeachtet.

Bei aufmerksamer Betrachtung der Schwänzeltänze im Beobachtungsstock lüftete sich der Schleier, der fürs erste über diesen Tatbestand gebreitet war. Bienen, die von verschiedenen Seiten mit Bürde beladen heimkehren, laufen beim Schwänzeltanz die geradlinige Strecke, in welcher das Schwänzeln erfolgt, in verschiedener Richtung. Bei allen Tänzerinnen von derselben Sammelstelle ist der Schwänzellauf gleichgerichtet. Er ändert aber seine Richtung mit der fortschreitenden Tagesstunde. Er ändert die Richtung um denselben Winkel, den die Sonne inzwischen durchmessen hat, aber mit entgegengesetzter Drehung. Hieraus war klar, daß die Tänzerinnen bei ihrer Richtungsweisung irgendwie auf den Sonnenstand Bezug nehmen. Ich suchte nun dem Sinn ihrer Tanzweise auf die Spur zu kommen. Auch einfache Dinge können, solange man sie nicht weiß, dem Verständnis Schwierigkeiten machen. Diese werden geringer, wenn man möglichst übersichtliche Verhältnisse schafft. Ich legte darum in aneinander schließenden Versuchsreihen den Futterplatz genau südlich, dann östlich, nördlich und westlich vom Stock an und beobachtete die Tänze zu verschiedenen Tagesstunden unter genauer Beachtung des Sonnenstandes. Dabei offenbarte sich rasch eine sehr eigenartige Seite im Lexikon der Bienensprache: Befindet sich zur Zeit der Beobachtung der Futterplatz, vom Stock aus gesehen, in derselben Richtung wie die Sonne, so legen die Tänzerinnen auf der Wabe die geradlinige Tanzstrecke, den Schwänzellauf, genau nach oben (Fig. 8 *a*). Liegt der Futterplatz links vom Sonnenstande, so richten sie auch den Schwänzellauf nach links, und zwar um jenen Winkel, um den sich die Stockgenossen nach links von der Sonne halten müssen, um an den Weideplatz zu kommen (Fig. 8 *b*). Liegt der Futterplatz rechts vom Sonnenstande, so wird um den entsprechenden Winkelbetrag nach rechts von der Richtung nach oben getanzt (Fig. 8 *c*). Und liegt der Futterplatz genau entgegengesetzt zum Sonnenstande, so geht der Schwänzellauf auf der Wabe senkrecht nach unten (Fig. 8 *d*). Diese Richtungsweisung nach dem Sonnenstande versagt auch bei bedecktem Himmel nicht. Sie versagt auch dann nicht, wenn man ein Bienenvolk bei bedecktem Himmel in eine ihm unbekannte Gegend versetzt und den Versuch durchführt, bevor ein Sonnenstrahl durch eine Lücke der Wolkendecke hervorbrechen konnte. Daraus geht zwingend hervor, daß die Bienen den jeweiligen Sonnenstand auch zu erkennen

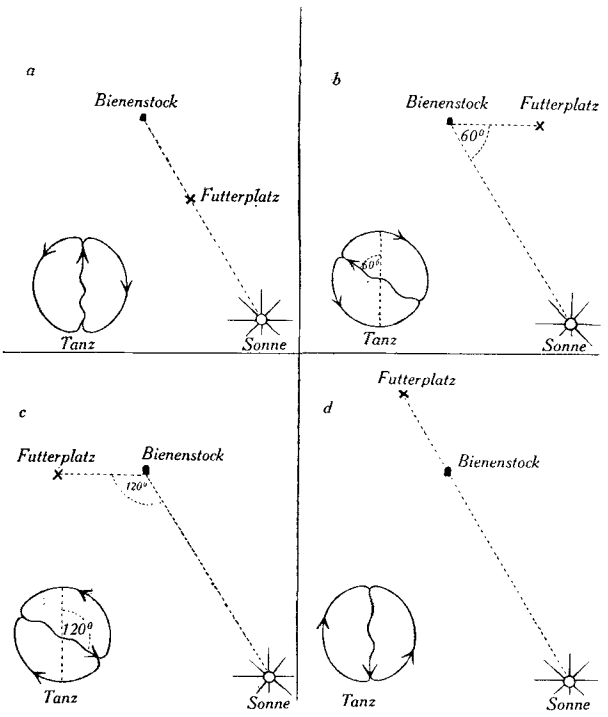


Fig. 8. Vier Beispiele für die Richtungsweisung. Die Sonne steht südöstlich vom Stock. Die Skizze links unten in jeder Abbildung zeigt schematisch, wie bei der angegebenen Lage des Futterplatzes der Schwänzeltanz verläuft.

vermögen, wenn er unseren Blicken durch Wolken verhüllt ist. Vielleicht liegt dieses Rätsels Lösung in einem feinen Empfindungsvermögen für die Richtung der Wärmestrahlen, die ja durch eine Wolke hindurchgehen, ohne nach allen Seiten zerstreut zu werden. Gewißheit darüber können erst künftige Versuche bringen.

Was ich von der Entfernungs- und Richtungsweisung gesagt habe, mag Ihnen phantastisch scheinen. Ich hätte es nicht vorgetragen, wenn ich mir nicht bewußt wäre, vor meinem Bienenstock jeden Zweifler von der Wahrheit der Sache überzeugen zu können.

Vielleicht denken Sie, daß die Bienen meines Beobachtungsvolkes sozusagen schon gelehrte Bienen sind, die sich nicht normal verhalten. Ich habe mich an herausgehobenen Waben eines gewöhnlichen *Zander*kastens überzeugt, daß seine Bewohner ebenso tanzen und ebenso die Richtung weisen. Mit einer solchen emporgehobenen Wabe in der Hand war ich versucht, dieselbe *horizontal* zu legen. Was würden dann die Tänzerinnen machen!? Es geschah etwas völlig Unerwartetes. Die Richtungsweisung nach dem Sonnenstande, auf der senkrecht hängenden Wabe die allein mögliche Art, wird auf horizontal liegender Tanzfläche abgelöst von einer unmittelbaren Angabe der Richtung zur Futterstelle: Die geradlinige Laufstrecke des Schwänzeltanzes weist direkt nach der Himmelsrichtung, in der sich der Futterplatz befindet. Bei Drehung der herausgehobenen Wabe in der horizontalen Ebene läßt sich die Tänzerin den Tanzboden unter den Füßen wegdrehen und hält die Richtung ein, wie eine Kompaßnadel. Ich habe den ganzen Beobachtungsstock horizontal gelegt und stundenlang den Tänzerinnen zugeschaut. Sie ändern nun die Tanzrichtung nicht mehr mit dem wandernden Tagesgestirn, sondern halten bei ihrem Schwänzellauf von früh bis abends die Himmelsrichtung ein, in der ihr Weideplatz zu finden ist. Man muß sich gegenwärtig halten, daß ein Tanz auf horizontalem Boden keine unbiologische Zumutung bedeutet. Bei vorlagernden Völkern kann man Tänze heimkehrender Sammlerinnen nicht selten auf der horizontalen Anflugfläche vor dem Flugloch sehen, und auch im Stock selbst, bei Waben, die nach unten nicht ganz ausgebaut sind, habe ich solche auf der inneren, horizontalen Fläche des Wabenrähmchens mehrfach beobachtet.

Da wir nun wissen, wie sich die Bienen über Entfernung und Richtung einer gegebenen Örtlichkeit verständigen, wird auch eine jedem Imker geläufige Tatsache begreiflich, die bisher in ein undurchdringliches Dunkel gehüllt schien: daß die Kundschafter eines Bienenschwarms, die *Spurbienen*, die eine geeignete Wohnung aufgespürt haben, die Schwarmtraube veranlassen, sich aufzumachen und geraden Weges ihrer künftigen Behausung zuzufliegen, auch wenn diese in kilometerweiter Entfernung liegt. Man hat auf der Oberfläche einer Schwarmtraube tanzende Bienen beobachtet. Ich glaube, daß solche Tänze nicht nur eine Aufforderung zum Aufbruch bedeuten, sondern daß sie ebenso, wie die Tänze der heimkehrenden Sammlerinnen, klare Angaben über die Entfernung und Richtung des Ziels zum Inhalt haben.

Lange, bevor mir die zuletzt geschilderten Feinheiten der «Sprache» der Bienen bekannt waren, habe ich vorgeschlagen, ihr Nachrichtenwesen auszunützen und sie durch künstlich ausgelöste Tänze mit entsprechender Duftparole rasch und in großer Zahl an bestimmte Blüten hinzulenken, wo solches dem Praktiker erwünscht ist. Ursprünglich zur Steigerung der Honigernten beim Wanderbetrieb gedacht, ist dieser Vorschlag dann zuerst zur Verbesserung der Bestäubung und zur Steigerung der Samenernten bei *Rotklee* von russischen Bienenforschern nach 1930 für die Praxis ausgearbeitet und bald mit Nutzen in ihre Landwirtschaft eingeführt worden. Ich selbst habe mich in den letzten Jahren mit Unterstützung der deutschen Imkerschaft und einer großen Zahl von Mitarbeitern dieser praktischen Auswertung zugewandt. Ohne hier auf technische Einzelheiten einzugehen, in denen wir, wie ich glaube, die russischen Versuche erfolgreich weiterentwickelt haben, möchte ich über Ziel und Weg der neuen Methode und über die erreichten Ergebnisse nur das Folgende sagen:

Rotkleeblüten setzen keine Samen an, wenn sie nicht von Insekten besucht und befruchtet werden. Langrüsselige Hummeln, die natürlichen Bestäuber dieser Blüten, sind zu spärlich, um an ausgedehnten Kulturen die Befruchtungsarbeit zu bewältigen. Bienen pflegen andere Blumen zu bevorzugen, weil ihrem kürzeren Rüssel der Nektar in den tiefen Kronröhren des Rotklees nur teilweise zugänglich ist. Die Samenerträge sind daher vielfach sehr unbefriedigend, die Beschaffung des nötigen Saatgutes für diese wichtige Futterpflanze ist in Frage gestellt.

Man kann einen ausreichenden Beflug der Blüten durch Bienen bewirken, wenn man etwa vier Völker je Hektar in der Nähe der Felder zur Aufstellung bringt und sie durch täglich wiederholte Fütterung mit kleinen Mengen Zuckerwasser unter Beigabe von Rotkleeblüten auf den Klee hinlenkt. Die gefütterten Bienen tanzen im Stock, sie duften nach Rotklee und machen dadurch bei ihren Kameraden Propaganda für den Besuch der Rotkleeblüten. Feldversuche großen Maßstabes sollten zeigen, wieweit sich auf diese Weise der Beflug und der Samenertrag steigern läßt. Es wurden für jeden Versuch zwei Felder gewählt, die in ihrer Größe und Lage, in Bodenbeschaffenheit, Düngung und Saatgut einander nach Möglichkeit entsprachen. An jedem wurde die gleiche Zahl von Bienenvölkern aufgestellt. Der Abstand beider Felder war so groß gewählt, daß jede Bienengruppe nur *ihr* Rot-

kleefeld beflog und das andere ihrem Flugbereich entzogen war. Am *Versuchsfeld* wurde die Duftlenkung durchgeführt. Am *Kontrollfeld* erhielten die Bienen zu den gleichen Zeiten genau gleiche Mengen Zuckerlösung, aber ohne Beigabe von Rotkleeblüten. Aus dem Vergleich beider Felder war somit die Wirkung der *Duftlenkung* erkennbar.

In 12 solchen Feldversuchen wurde das duftbelenkte Rotkleefeld ohne Ausnahme deutlich stärker beflogen, im Durchschnitt drei- bis viermal so stark als das Kontrollfeld. Der Samenertrag lag in neun zahlenmäßig kontrollierten Versuchen auf den belenkten Feldern im Durchschnitt um rund 40% höher als auf den zugehörigen Kontrollfeldern[1].

Dem Anwendungsbereich der Duftlenkung auf dem Gebiete der Landwirtschaft sind aber die Grenzen weiter gesteckt. Auch der Besuch von Blüten, die von den Bienen schon ohne künstlichen Anreiz gern beflogen werden, kann durch das Duftlenkungsverfahren einen gewaltigen zusätzlichen Antrieb erhalten. Die Sammlerinnen beginnen ihre Arbeit früher am Tage, sie setzen sie länger fort, sind eifriger am Werke und fliegen auch bei verhältnismäßig ungünstiger Witterung. In sechs Versuchen an *Raps* wurden die belenkten Felder stets stärker beflogen als die Kontrollfelder. Der Samenertrag war in vier verwertbaren Versuchen um 12–33% erhöht. In drei Versuchen am nahe verwandten *Rübsen*, der stärker als Raps auf Fremdbestäubung angewiesen ist, wurde auf den belenkten Feldern eine Steigerung des Samenertrags um 27%, 44% und 51% erzielt. Auch bei *Buchweizen* scheint ein Erfolg in Aussicht zu stehen, doch bleiben hier, wie bei anderen landwirtschaftlichen Nutzpflanzen, erst weitere Versuche abzuwarten.

Wenn wir die Bienen auf Rotklee lenken und sie hierdurch von anderen, lohnenderen Trachtquellen abziehen, so kann es dem *Imker* zum Nachteil gereichen. Im Durchschnitt war allerdings bei unseren Versuchen der Verlust durch den gesteigerten Sammeleifer ausgeglichen, so daß der Bienenwirt nicht zu Schaden kam. Bei guten Trachtpflanzen kann eine sachgemäß durchgeführte Duftlenkung für den Imker von großem Vorteil sein. Im Vergleich mit einer entsprechenden Zahl duftlos gefütterter Kontrollvölker lieferten 71 auf *Schwedenklee* oder *Weißklee* gelenkte Völker durchschnittlich einen Mehrertrag von rund 25–50%, 77 auf *Raps* und *Rübsen* gelenkte Völker durchschnittlich Mehrerträge von rund 20–40%, 39 auf *Heidekraut* gelenkte Völker solche von 13–34%, 9 auf *Kohldisteln* gelenkte Völker Mehrerträge von 35–95% usw. Bei richtiger Anwendung wird sich das Verfahren für den Imker um so mehr lohnen, als ja der aufgewandte Zucker nicht verlorengeht, sondern der Entwicklung und dem Ertrag der Völker zugute kommt, also in die Tasche des Imkers zurückkehrt.

Das neue Verfahren in die Praxis einzuführen wäre eine dankenswerte Aufgabe für die bienenkundlichen Versuchsanstalten. Dann werden, wie es schon so oft geschah, die Früchte einer theoretischen Arbeit der Praxis zugute kommen. Sosehr ich dies hoffe und erwarte, so gestatten Sie mir doch das Geständnis: die reinste Freude bleibt die der Erkenntnis, frei vom irdischen Hauch einer nutzbringenden Auswertung.

[1] Bei einem Zuckerverbrauch von 3,5 kg pro Volk für eine fünfwöchige Lenkung erfordert 1 Hektar Feldfläche 14 kg Zucker, oder, bei einem Preis von 1,10 Fr., einen Kostenaufwand von 15,40 Fr. Bei einem Durchschnittsertrag von 100 kg pro Hektar würde ein Mehrertrag von 40 kg (bei einem Preis von 6,60 Fr. für 1 kg Rotkleesamen) einen Mehrgewinn von rund 250 Fr. pro Hektar bedeuten.

Summary

If honey-bees find a feeding place, after return they report the discovery by dancing. The species of flowers from which they are coming is indicated by means of the flower-scent adhering to their bodies, and also by the scent of nectar brought into the hive within the honey-stomach. By a long flight the scent adhering to the outer surface is diminished. But the scent within the honey-stomach is still the same. Therefore the scent of nectar (that is the specific flower-scent absorbed by nectar) is especially important if the feeding place is far away from the hive.

Bees dance only in case there is plenty of food. Then the informed bees fly out and look for the flowers having the scent indicated by the dancing bees. In this way the number of visiting bees increases, and the nectar becomes scarce. Then honey collecting is still continued, but there is no more dancing in the bee-hive and the number of bees does not increase, so that there always is the correct relation between the amount of nectar and the number of collecting bees.

If the feeding place is at a distance of some hundred meters there are many bees seeking for food at that distance but only a few seeking near the hive. By using an observation-hive the matter could be cleared up. Bees collecting at a feeding place nearer than 50 to 100 m make round-dances (Fig. 4, p. 400). Bees coming from a feeding place more distant make tail-wagging dances (Fig. 5, p. 400). The tail-wagging dance not only indicates that the food has been found at a distance of more than 50–100 m, but it also gives a very exact knowledge of the distance by the number of turnings (Fig. 6, p. 400). Moreover the way to the feeding place is indicated by the direction of the straight run. Running upwards means that the feeding place is situated in the same direction as the sun. Running downwards means that the opposite direction has to be taken for finding the feeding place. Running to the left in a certain angle to the direction upwards indicates that in the same angle left of the sun the food can be found (Fig. 8, p. 401).

Our knowledge about the "language" of bees can be used for leading bees to certain flowers *for which it is desirable to be more visited*. In this way the pollination and therefore the crop of red clover and other plants has been improved (on average about 40%) and the honey-production has been increased.

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Communications provisoires - Vorläufige Mitteilungen
Comunicazioni preliminari - Preliminary reports

Latin squares of the sixth order

The properties of a square array of n letters, each repeated n times, and such that each letter occurs once in each row and once in each column of the n^2 cells, were first discussed by EULER[1] (1782); since then the enumeration and the investigation of other properties of these *Latin Squares* have interested many mathematicians. Some aspects of the problem assumed practical importance about twenty years ago, when Latin square arrangements began to be used extensively in the design of agricultural and other scientific experiments.

One problem to which EULER gave particular attention was that of finding a Græco-Latin square, or as he termed it *un quarré magique complet*, namely two superposed squares, one in Roman and one in Greek letters, with the additional restriction that each of the n^2 pairs of one Roman and one Greek letter should occur once and once only. On evidence that was scarcely adequate, he concluded that for 6×6 squares no such arrangement existed; his conclusion was finally confirmed by FISHER and YATES[2] (1934) after a systematic enumeration of Latin squares of this order.

A Græco-Latin square of order n may be looked upon as a (1^n) partition of an $n\times n$ Latin square, since it entails the subdivision of the n^2 cells into n sets such that each contains one cell from each row, one from each column, and one of each letter; to each of these sets of n cells, usually termed *directrices* and possessing the property of being orthogonal with rows, columns, and Roman letters, a different Greek letter is then assigned. Certain agricultural problems recently caused the writer to investigate the existence of less complete partitions of a 6×6 Latin square, and in particular of (3^2) and (2^3) partitions, these requiring the subdivision of the 36 cells into either two sets of eighteen or three sets of twelve such that each set contains three or two from each row, column, and letter. He demonstrated the existence of (2^3) partitions for every 6×6 square and of (3^2) partitions for most squares (FINNEY[3], 1945).

This study suggested the further problem of discovering other orthogonal partitions of the 6×6 squares and of enumerating them. FISHER and YATES have classified the squares into twelve adjugate sets, such that any orthogonal property of the kind under discussion is common to all squares of the same set; consequently only a detailed examination of one square from each set was necessary. The procedure followed was essentially the same as that used by EULER in his search for Græco-Latin squares, namely the exhaustive ascertainment of directrices followed by the listing of sets of directrices having no cells in common. EULER found one square to have a set of four directrices giving a $(1^4, 2)$ partition, and from this can be formed, by combination of parts, every other type of partition except the (1^6); the existence of $(1^4, 2)$ partitions has been demonstrated for four of the twelve adjugate sets.

As an illustration of the results obtained, the properties of one square will be discussed in some detail. The square chosen is:

A	B	C	D	E	F
B	A	F	E	D	C
C	D	A	B	F	E
D	F	E	A	C	B
E	C	B	F	A	D
F	E	D	C	B	A

which is a representative of an adjugate set (FISHER and YATES' XV, XVI) having a remarkably high degree of symmetry in its orthogonal partitions. There are 24 directrices, and each cell of the square lies on four of these; for example, the first cell of the first row has passing through it the directrices AFBCDE, AEFBCD, ADEFBC, ACDEFB, the six letters being taken from the six rows of the square in the order specified. Sets of four directrices leading to $(1^4, 2)$ partitions can be chosen in thirty different ways, and these use every directrix five times. A typical one of these partitions, the nearest approach possible to a 6×6 Græco-Latin square, is:

$A\alpha$	$B\beta$	$C\gamma$	$D\delta$	$E\varepsilon$	$F\varepsilon$
$B\varepsilon$	$A\varepsilon$	$F\alpha$	$E\gamma$	$D\beta$	$C\delta$
$C\beta$	$D\gamma$	$A\delta$	$B\alpha$	$F\varepsilon$	$E\varepsilon$
$D\varepsilon$	$F\delta$	$E\beta$	$A\varepsilon$	$C\alpha$	$B\gamma$
$E\delta$	$C\varepsilon$	$B\varepsilon$	$F\beta$	$A\gamma$	$D\alpha$
$F\gamma$	$E\alpha$	$D\varepsilon$	$C\varepsilon$	$B\delta$	$A\beta$

[1] L. EULER, "Recherches sur une nouvelle espèce de quarrés magiques", Verhandelingen uitgegeven door het Zeeuwsch Genootschap der Wetenschappen te Vlissingen *9*, 85–239 (1782).

[2] R. A. FISHER and F. YATES, "The 6×6 Latin squares", Proc. Cambridge Philos. Soc. *30*, 492–507 (1934).

[3] D. J. FINNEY, "Some orthogonal properties of the 4×4 and 6×6 Latin squares", Ann. Eugenics *12*, 213–219 (1945).

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Predacious Fungi and Nematodes

By C. L. DUDDINGTON*

The existence of predacious fungi in the soil and elsewhere has been known since the classic paper of ZOPF (1888)[1] described the capture of nematodes by *Arthrobotrys oligospora*, *Dactylella ellipsospora* and others. Even before that time, the characteristic networks formed by the reticulate Hyphomycetes had been observed and recorded, though the function of these peculiar structures was not even guessed at. It was not for nearly half a century after the appearance of ZOPF's paper, however, that it was realized that the predacious fungi are not occasional biological curiosities, but common organisms that form an important part of the micro-flora of the soil, rotting vegetation, dung and other habitats where free-living nematodes abound. Even now we should probably know little about them but for the work of DRECHSLER[2–4] in America, whose observations on the predacious fungi during the last quarter of a century will go down in history as one of the classic researches in mycology.

The predacious fungi that attack nematodes fall into two categories: those that capture their prey alive with the aid of various ingenious trapping devices, and those that are internally parasitic in nematodes, effecting entry into their host usually by means of sticky spores that adhere to the animal's body on contact and, when they germinate, intrude a germ tube through the integument of the host that gives rise to an endozoic mycelium.

Most of the nematode-trapping predacious fungi belong to the Moniliales (Hyphomycetes), an Order of the Fungi Imperfecti. They have a mycelium of colourless, septate hyphae on which the nematode traps are borne. The traps are of two main kinds: adhesive traps, by which the prey is captured and held by means of a sticky secretion, and mechanical traps where no adhesive substance is needed.

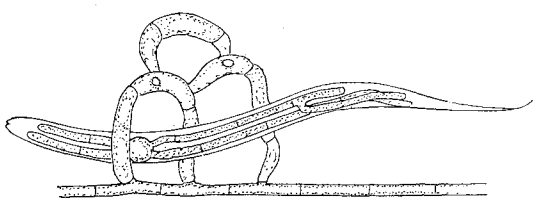

Fig. 1. *Arthrobotrys oligospora*. Diagram of a network with a captured nematode. Note infection bulb and trophic hyphae in the carcass of the captive.

The best known of the adhesive traps is the sticky network, such as is found in *Arthrobotrys oligospora*, the commonest of all the predacious fungi. The structure and functioning of the traps has been described in detail by DRECHSLER[2]. The networks are formed by short lateral branches from the mycelium that curl round and join up to form loops (Figure 1) by anastomosis either with the parent mycelium or with other similar branches. The loops tend to be orientated roughly at right angles to one another, rather like the semicircular canals of the mammalian ear, so that a three-dimensional reticulum is formed.

The surface of the networks only is sticky: there is no sticky material produced by the rest of the mycelium. If a nematode happens to come into contact with any part of a network it is held firmly, and it is possible to see that a viscous adhesive fluid has been secreted by the cells of the network. The action of the sticky substance is highly efficient: a nematode seldom, if ever, escapes once it has been fairly held.

Immediately after capture the captive struggles violently, pulling the mycelium of the fungus this way and that, but without avail. In due course its struggles become less and less, until within two hours at the most it is quiescent and, apparently, moribund. The cause of death is uncertain: it may be that a toxin is produced by the fungus, but there is no evidence of this.

Once the captured nematode has become moribund, invasion of its body by the fungus begins. This process has been described in detail by SHEPHERD[5]. A swelling appears on the fungal network at the point where the nematode is held, and from this a minute outgrowth penetrates the integument of the animal and forms, inside its body, a globular infection bulb from which

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[1] W. ZOPF, Nova Acta Leop.-Carol. *52*, 314 (1888).
[2] C. DRECHSLER, Mycologia *29*, 447 (1937).
[3] C. DRECHSLER, Phytopathology *31*, 773 (1941).
[4] C. DRECHSLER, Mycologia *42*, 1 (1950).
[5] A. M. SHEPHERD, Nature, Lond. *175*, 475 (1955).

trophic hyphae grow out and fill the carcass, consuming its contents as they do so. In about twenty-four hours only the integument of the nematode is left, still attached to the network and filled with trophic hyphae. Finally, the contents of the trophic hyphae themselves are passed back to the parent mycelium.

Other variations of the sticky trap are found amongst the predacious Hyphomycetes. In *Dactylella cionopaga* (DRECHSLER)[4] nematodes are captured by short lateral branches of the mycelium that are sticky and function in the same way as the sticky networks of *Arthrobotrys oligospora*. Each branch usually consists of one, two or three cells (Figure 2), though sometimes they proliferate and form simple networks: the complex three-dimensional networks shown by the reticulate species are, however, never formed.

In *Dactylella ellipsospora* (DRECHSLER[2]) the eelworm traps consist of small subspherical knobs attached to the mycelium by short, two-celled stalks. Here the knobs are sticky, and nematodes are captured by adhesion (Figure 3). The sequence of events following capture is the same with the sticky branches and stalked knobs as with the networks: when the captive is dead or moribund its integument is penetrated by a slender process that gives rise to an infection bulb from which trophic hyphae arise and consume the body-contents of the nematode.

The mechanical traps, which do not depend on the production of a sticky secretion for their operation, are of two kinds: non-constricting rings and constricting rings, the latter being both commoner and more effective. The best-known fungus with non-constricting rings is *Dactylaria candida* (DRECHSLER[2]). The rings are formed by slender lateral branches that curl round and join up with themselves, forming three-celled rings attached to the mycelium by usually three-celled stalks (Figure 4). The internal diameter of a ring is such that, if a wandering nematode accidentally pushes its anterior end into the opening, it gets wedged in trying to force its way through. Capture is followed by the intrusion of trophic hyphae into the body of the captive, and the absorption of its body-contents, in the usual way.

The action of the non-constricting ring is entirely passive: there is no secretion of sticky material as far as we know, and the nematode is captured and held simply because it has, by its own misguided efforts, jammed its body into the ring. The constricting ring, on the other hand, captures nematodes by positive action. It works on the principle of a rabbit snare, or the lassoo of an American cowboy. A nematode that thrusts its anterior end into a constricting ring is gripped by a sudden inflation of the ring cells, and held as in a garotte.

Predacious fungi with constricting rings are very common: *Dactylaria gracilis* (DUDDINGTON[6]) is an example. In appearance, the constricting ring is very

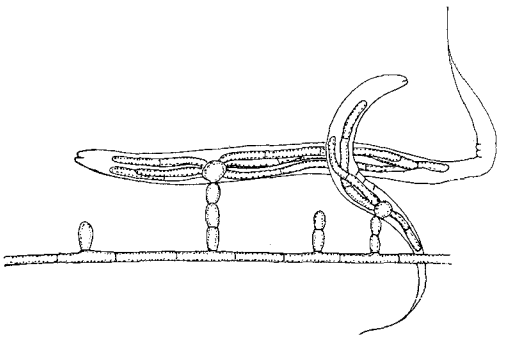


Fig. 2. *Dactylella cionopaga*. Diagram of part of the mycelium, with adhesive branches. Two nematodes have been captured.

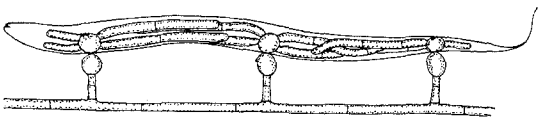


Fig. 3. *Dactylella ellipsospora*. Diagram of part of the mycelium, showing three adhesive knobs and a captured nematode.

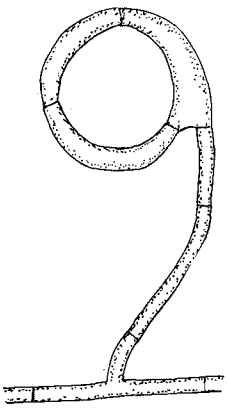


Fig. 4. A non-constricting ring of *Dactylaria candida*.

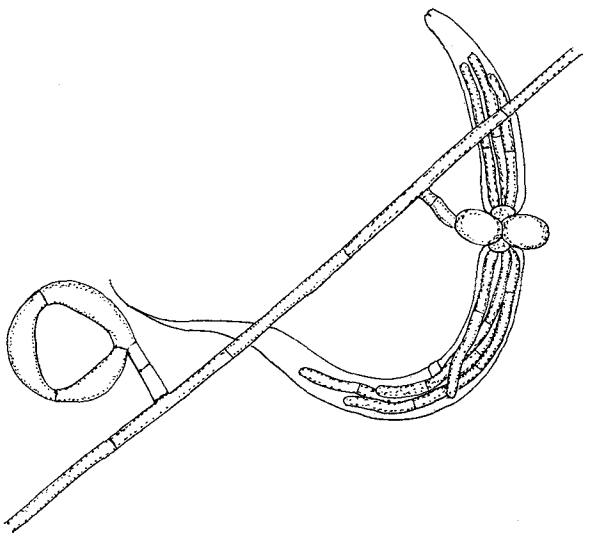


Fig. 5. *Dactylaria gracilis*. Diagram of part of the mycelium with one ring in its 'open' state and another, in side view, that has captured a nematode.

[6] C. L. DUDDINGTON, Trans. Brit. mycol. Soc. *34*, 194 (1951).

like the non-constricting ring, and it is formed in much the same way, by a short lateral branch of the mycelium curling round to form a three-celled ring on a stalk (Figure 5). The constricting ring is, however, somewhat stouter in construction than the non-constricting ring, and its stalk is shorter, consisting normally of only two cells.

The three cells that make up a constricting ring are sensitive to touch on their inner sides. When a nematode introduces its body into a ring, the contact stimulus as it rubs against the ring cells is sufficient to trigger off the mechanism. The ring cells suddenly inflate inwards, greatly reducing the size of the opening of the ring and gripping the unfortunate nematode so tightly that its body is deeply constricted by the pressure of the inflated ring cells. The action of the cells is very quick: after a lag phase of a few seconds, the actual swelling takes only about one tenth of a second. The captured nematode struggles violently for a time after the ring has closed on it, but its movements become progressively weaker. When it has ceased to struggle, trophic hyphae grow from the ring cells into its body and consume its contents.

All the predacious fungi so far described are members of the Moniliales (Hyphomycetes), and they all appear to be closely related to one another. Another small group of nematode-capturing fungi is found in the Zoopagales, an Order of Phycomycetes showing strong taxonomic relationships with the Mucorales. The Zoopagales have non-septate hyphae, reproduce asexually by means of conidia borne singly or in groups on erect aerial fertile hyphae, and in many cases show sexual reproduction of a typically zygomycetous type. Most of the Zoopagales are predacious on amoebae and other Protozoa, but a number of species capture nematodes. No specialized organs of capture are formed: the prey is captured by adhesion, the mycelium apparently being sticky all over its surface. A typical example is *Stylopage grandis* (DUDDINGTON[7]). Here the mycelium consists of a fairly stout, non-septate hyphae that branch occasionally. Nematodes coming into contact with the hyphae are held by a sticky secretion, and capture is followed by the intrusion of trophic hyphae into the body of the victim, as in the Hyphomycetes that capture nematodes with sticky traps (Figure 6).

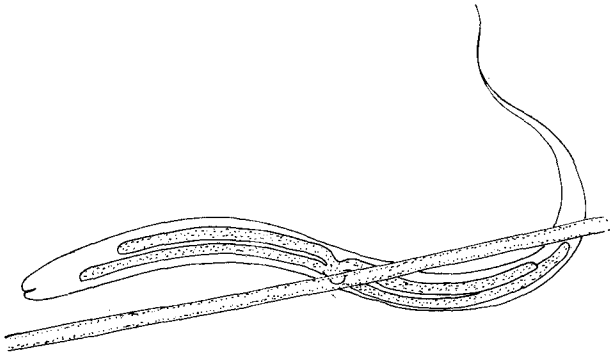


Fig. 6. Diagram of a nematode captured by the adhesive mycelium of *Stylopage grandis*.

The nematode-trapping Hyphomycetes are not dependent on nematodes as a source of food, for when isolated into pure culture they will grow freely on most of the normal media used for cultivating fungi. Grown without nematodes, they do not usually form their characteristic traps, but, if nematodes are added to the cultures, traps are quickly formed and nematodes are captured. It is not even necessary to add living nematodes to the cultures, for most of the nematode-trapping Hyphomycetes that have been tested will form traps if given sterile filtered water in which nematodes have lived. It seems clear that trap formation can be initiated by a chemical stimulus, in this case a substance, the nature of which is at present unknown, that has diffused out of nematodes into the water surrounding them.

Nematode-trapping Hyphomycetes will sometimes form traps spontaneously in pure culture, without the addition of any stimulating substance: the fungi with constricting rings are especially prone to do this. The reason for this behaviour is as yet unknown, but recent work (FEDER, EVERARD and DUDDINGTON[8]) suggests that these fungi are heterocaryotic with regard to the capacity for spontaneous ring formation.

Fungi with constricting rings will form rings in pure culture in response to the presence of a wide variety of different substances (ROUBAUD and DESCHIENS[9], LAMY[10]). The substances that gave positive reactions included blood serum of the horse and other animals, and aqueous extracts of various animal organs. Plant extracts were not effective.

The network-forming Hyphomycetes are less prone to develop their traps in pure culture, though they sometimes do so for no apparent reason. They will, however, produce networks fairly readily if treated with sterile filtered water in which nematodes have lived (COMANDON and DE FONBRUNE[11,12]). PRAMER and STOLL[13] obtained and partially purified a substance from nematode cultures containing an active principle that stimulated network formation in *Arthrobotrys* spp., and to this hypothetical stimulant they have given the name 'nemin'.

The physiology of the operation of the constricting ring traps of the predacious Hyphomycetes has cap-

[7] C. L. DUDDINGTON, Mycologia *47*, 245 (1955).
[8] W. S. FEDER, C. O. R. EVERARD, and C. L. DUDDINGTON, Science *131*, 922 (1960).
[9] E. ROUBAUD and R. DESCHIENS, C.R. Acad. Sci. Paris *209*, 77 (1939).
[10] L. LAMY, C. R. Soc. Biol. Paris *137*, 337 (1943).
[11] J. COMANDON and P. DE FONBRUNE, C. R. Soc. Biol. Paris *129*, 619 (1938).
[12] J. COMANDON and P. DE FONBRUNE, C.R. Acad. Sci. Paris *207*, 304 (1939).
[13] D. PRAMER and N. R. STOLL, Science *129*, 966 (1959).

tured the attention of a number of workers. COUCH[14] failed to induce closure of the rings of *Dactylella bembicodes* by passing a fine micromanipulator needle into them, an observation that is contradicted by COMANDON and DE FONBRUNE[11], who showed conclusively that the rings would close in response to a mechanical stimulus such as stroking the inner edges of the ring cells with a fine needle. The French workers also showed that the outer edges of the ring cells are insensitive to tactile stimuli. COUCH[14] showed that the rings could be closed by the application of moderate heat, either by dropping warm water on to them or by holding a hot scalpel near them; this observation was confirmed by MULLER[15].

The most striking feature of the action of the constricting rings is their great speed in closing. When suitably stimulated the ring appears to undergo a lag phase of several seconds, after which the ring cells suddenly inflate inwards, the time needed for the actual inflation being no more than one tenth of a second. When fully inflated, the ring cells occupy about three times their former volume – a very considerable increase. No completely satisfactory explanation of this sudden and rapid inflation has yet been put forward, but some observations by MULLER[15] on the osmotic relationships of the ring cells are interesting. MULLER found that, after inflation, the osmotic potential of the cells was approximately the same as it was before inflation took place, in spite of the fact that the volume of the cells had increased threefold. This suggests that osmotically active material must be rapidly produced in the cells either during or immediately after inflation, and he favours the view that the swelling is due to a relaxation of turgor pressure in the cells brought about by a change in the elasticity of the inner cell wall, followed by the production of osmotically active material within the cell to counterbalance the intake of water. This theory is plausible, but needs confirmation, especially in view of the fact that MULLER, in his experiments, slowed down the rate of closure of the rings one hundredfold by treatment with sugar solution.

The predacious fungi that are internally parasitic in nematodes form a group distinct from the nematode-trappers. Here the mycelium of the fungus is entirely endozoic, only the fertile hyphae that bear the spores emerging from the body of the host. Some of these fungi are extremely common: *Harposporium anguillulae* (LOHDE[16], KARLING[17]), for instance, is to be found in fertile soils over a wide area in this country and abroad.

A typical example of a hyphomycete internally parasitic in nematodes is seen in *Acrostalagmus obovatus* (DRECHSLER[3]), a species that is very common in soil. The small spores of the fungus adhere to the integuments of nematodes that come into contact with them. On germination, the spore puts out a germ tube that penetrates the integument of the nematode and gives rise to an extensive mycelium of branched, septate hyphae within its body. As the mycelium of the parasite develops the body-contents of the host are consumed, until finally the carcass of the nematode is filled with hyphae. As this stage approaches, the reproductive phase of the fungus begins. Branches from the internal mycelium penetrate the integument of the host in an outward direction, and grow into procumbent fertile hyphae, on which numerous flask-shaped phialides are formed. Each phialide produces up to twenty small, obovate spores, which cohere in a bunch round the neck of the phialide (Figure 7). The spores are readily picked up by passing nematodes, and in this way the parasite is dispersed.

In *Acrostalagmus obovatus*, as in other Hyphomycetes, sexual reproduction is unknown.

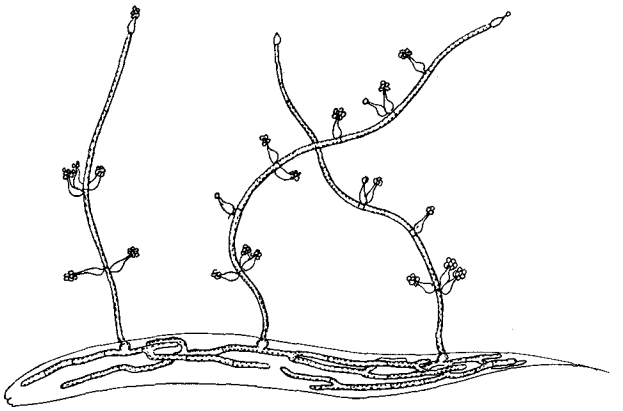


Fig. 7. *Acrostalagmus obovatus*. Body of an infected nematode, with internal mycelium and three emergent fertile hyphae bearing phialides and spores.

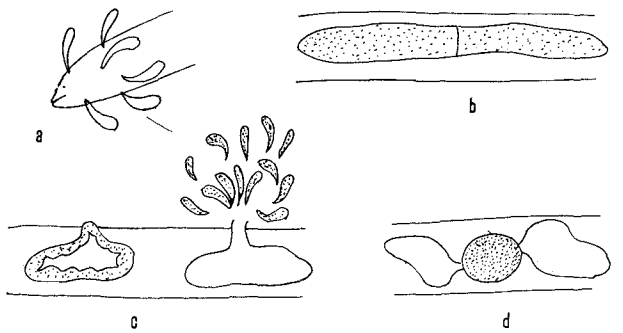


Fig. 8. *Protascus subuliformis*. a, Spores sticking to the anterior end of a nematode. b, Young thallus in the body of a nematode. c, a young sporangium, and an old sporangium that has discharged its spores. d, a developing zygospore.

[14] J. N. COUCH, J. Elisha Mitchell Sci. Soc. *53*, 301 (1937).
[15] H. G. MULLER, Trans. Brit. mycol. Soc. *41*, 341 (1958).
[16] G. LOHDE, *Einige neue parasitische Pilze*. Tageblatt der 47. Versammlung deutscher Naturforscher und Ärzte in Breslau (1874), p. 203.
[17] J. S. KARLING, Mycologia *30*, 512 (1938).

A small but interesting group of fungi that are endozoic in nematodes is found in the Lagenidiales, a primitive Order of Phycomycetes most of which are parasitic in fresh-water algae. *Protascus subuliformis* DANGEARD[18], KARLING[19]) is a not uncommon example. Here the spores are club-shaped and slightly curved (Figure 8), and they adhere to the bodies of nematodes by their pointed ends, which appear to be sticky. When a spore germinates, the germ tube penetrates the integument of the nematode and forms a naked protoplast within its body. This grows at the expense of the body-contents of the nematode, forming an elongated thallus. Multiple infection from a number of spores is common.

As the thallus of the fungus grows it forms a wide, irregular filament, which eventually becomes septate. Finally, the thallus fragments at the septa. Each segment then becomes a sporangium in which the cytoplasm divides up to form a large number of spores: an exit tube grows out through the integument of the host, and the spores are discharged to the exterior. Sometimes the fungus undergoes sexual reproduction, adjacent segments functioning as male and female gametangia and conjugating with one another, the contents of the male gametangium passing through a conjugation tube into the female gametangium. Conjugation is followed by the formation of a thick-walled zygospore.

Protascus subuliformis is unusual in that its asexual spores are non-motile, whereas in most of the Lagenidiales they are motile zoöspores. It seems likely that this is connected with the type of host: nematodes are motile and gregarious, and it is reasonable to suppose that, in attacking such prey, motile spores would carry no advantage, and might well be inferior to sticky aplanospores. It is worthy of note that most of the Lagenidiales that parasitize nematodes have non-motile spores.

Various other species of lower fungi are internally parasitic in nematodes: descriptions of these can be found in the literature (DUDDINGTON[20], SPARROW[21]).

Unlike the nematode-trapping Hyphomycetes, the endozoic predacious fungi appear to be obligate parasites: there are no fully-confirmed accounts of their isolation into pure culture. The same applies to nematode-trapping species of Zoopagales such as *Stylopage grandis*.

Until recently, the occurrence of nematode-attacking predacious fungi in the soil had not been closely studied, though it has been known that they are abundantly represented. LINFORD and OLIVEIRA[22] found no less than seventeen different species in soil from Hawaiian pineapple fields, and a survey of predacious fungi from British arable soils (DUDDINGTON[23]) produced eighty-two records from forty-nine samples, at least twenty different species being represented. The predacious fungi are, in fact, an important fraction of the natural biome of the soil. They are probably absent from extreme mineral soils where there is a marked lack of organic matter, such as the soil of Wareham Heath, Dorset (England), and in my experience the acid peats are also inhospitable to them, though they contain plenty of free-living nematodes.

The state of activity of the nematode-trapping fungi in the soil has long been a matter of speculation. Most of these fungi can live and reproduce freely without nematodes if supplies of nourishment are sufficient; they are facultative saprophytes, and may even be facultative predators, depending on a diet of nematodes only when food is limited. Since the abundance of predacious fungi in the soil may be a biotic factor of some importance in the lives of soil nematodes, this is a matter on which information is badly needed.

Shortly before the second world war, LINFORD et al. in Hawaii, working on the biological control of the pineapple root-knot eelworm, showed that the efficacy of predacious fungi in keeping down the soil population of root-knot eelworm was greatly enhanced by the addition to the soil of easily-decomposable organic matter such as chopped green pineapple tops (LINFORD[24], LINFORD and YAPP[25, 26], LINFORD, YAPP and OLIVEIRA[27]). This effect of organic matter on predacious fungi has been confirmed by more recent work in England (DUDDINGTON[28], DUDDINGTON and DUTHOIT[29], DUDDINGTON, DUTHOIT, and EVERARD[30]). LINFORD advanced the theory that the stimulating effect of organic matter on soil predacious fungi was an indirect one, postulating that the presence of the organic matter produced a great increase in the numbers of free-living nematodes, and that the rise in the nematode population then reacted on the fungi. For many years this view was generally accepted, but recent work by COOKE[31] indicates that it is no longer tenable.

COOKE investigated the effect of organic matter on the activity of predacious fungi in the soil by an ingenious agar disk technique. COOKE buried disks of

[18] P. A. DANGEARD, C. R. Acad. Sci. Paris *136*, 627 (1903).
[19] J. S. KARLING, *Simple Holocarpic Biflagellate Phycomycetes* (New York 1942).
[20] C. L. DUDDINGTON, Biol. Rev. *31*, 152 (1956).
[21] F. K. SPARROW, *Aquatic Phycomycetes*, 2nd ed. (University of Michigan Press, Michigan 1962).
[22] M. D. LINFORD and J. M. OLIVEIRA, Phytopathology *28*, 14 (1938).
[23] C. L. DUDDINGTON, Nature, Lond. *173*, 500 (1954).
[24] M. B. LINFORD, Science *85*, 123 (1937).
[25] M. B. LINFORD and F. YAPP, Phytopathology *28*, 14 (1938).
[26] M. B. LINFORD and F. YAPP, Phytopathology *29*, 596 (1939).
[27] M. B. LINFORD, F. YAPP, and J. M. OLIVEIRA, Soil Sci. *45*, 45, 127 (1938).
[28] C. L. DUDDINGTON, *The Friendly Fungi* (Faber and Faber, London; The Macmillan Company, New York 1957).
[29] C. L. DUDDINGTON and C. M. G. DUTHOIT, Plant Pathology *9*, 7 (1960).
[30] C. L. DUDDINGTON, C. M. G. DUTHOIT, and C. O. R. EVERARD, Plant Pathology *10*, 108 (1961).
[31] R. C. COOKE, Nature, Lond. *191*, 1411 (1961).

agar in soil contained in large petri dishes under carefully controlled conditions, and enriched the soil with various quantities of chopped cabbage leaf. The agar disks were removed each week and replaced with fresh ones: in this way COOKE was able to study the behaviour of predacious fungi in his soil samples over a period of time.

The results obtained by COOKE were most interesting. He found that the addition of chopped cabbage leaf to the soil produces a dramatic increase in the population of free-living nematodes. He also found, on studying the fungal flora of his agar disks, that the activity of predacious fungi, as indicated by the number of traps formed, increased to a notable extent. On comparing the effects of organic amendment of the soil on the nematode population and on the activity of predacious fungi, however, a discrepancy appeared. On the addition of the chopped cabbage leaf to the soil, the figures for nematode population and fungal activity rose together to a maximum. The activity of the fungi then rapidly fell away, while the increase in nematode population was maintained for several weeks, declining only slowly. At a time when the activity of the predacious fungi had dropped back to its former value before organic amendment, the eelworm population was still very high—higher, in fact, than it was when predacious activity was nearing its peak. It did not look as if the increase in predacious activity was due solely to the increase in nematode population, for if that had been the case the fungal activity should have remained high as long as the increase in nematode population was maintained.

COOKE then tried sucrose as a soil amendment instead of chopped cabbage leaves, and here his results were even more striking. He found that the addition of sucrose produced a sharp rise in the activity of predacious fungi, without a corresponding increase in the nematode population. In this case the increase in predacious activity could not possibly be attributed to increased numbers of nematodes.

In view of COOKE's results, it is impossible to retain LINFORD's hypothesis that the stimulating effect of organic matter on the activity of soil predacious fungi is simply a function of the rise in nematode population that follows the addition of easily-decomposable organic matter to the soil. We must look for some other explanation. It looks as if the organic amendment of the soil has some direct action on the predacious fungi, but what that action may be we cannot even guess. Further work is badly needed to elucidate this point.

LINFORD and COOKE agree on one very interesting point. Both found that there was an optimum amount of organic soil amendment for the activity of predacious fungi. If this optimum was exceeded, the activity of the fungi fell off. The reason for this has not yet been explained.

In view of the fact that predacious fungi are abundant in soil, it is not surprising that a number of workers have seen in them a possible means for the biological control of nematodes that attack crops. The first work in this field was carried out by LINFORD et al.[22,24–27], working on the pineapple root-knot eelworm in Hawaii. They found that direct inoculation of the soil with cultures of five different predacious Hyphomycetes was ineffective, but that incorporation of chopped green pineapple tops into the soil produced a useful degree of control, which they attributed to the increased activity of soil predacious fungi. This work was given up shortly before the outbreak of World War II, and was not resumed.

During the war, a great deal of work on the use of predacious fungi for the control of nematodes, both in plants and animals, was carried out in France. This work has been summarized by DOLLFUS[32]. Most of the French work was concentrated on methods of growing the fungi in bulk, and on demonstrating that they could effectively attack pathogenic nematodes and that they were harmless to crop plants and domestic animals. Only two experiments on nematode control were carried out, both of which were inconclusive owing to their small scale.

Experiments on the biological control of nematodes by predacious fungi in England began in 1951, and are still in progress. Initially, the work was mainly concerned with the control of the potato root eelworm (*Heterodera rostochiensis*), and a preliminary series of pot experiments confirmed LINFORD's observation that the activity of the fungi was much affected by the presence of organic matter in the soil. The results of experiments in the field were inconclusive, though indications that the fungi might be of potential value were obtained (DUDDINGTON[28]). Later trials with cereal root eelworm (*Heterodera avenae*) in oats again confirmed LINFORD's experiments on the effect of organic matter, and demonstrated that the use of predacious fungi in conjunction with organic soil amendment produced a marked reduction in the number of *Heterodera* larvae invading the roots of oat seedlings (DUDDINGTON and DUTHOIT[29], DUDDINGTON, DUTHOIT, and EVERARD[30]).

In a recent series of trials, HAMS and WILKIN[33] used cultures of predacious fungi grown in deep culture. In pot experiments against potato root eelworm, pea root eelworm and cereal root eelworm, the incidence of nematode attack on the plants was reduced, but later experiments on a field scale were inconclusive. The design of the field trials, however, and the method of assessing the results, were not beyond criticism.

[32] R. P. DOLLFUS, *Parasites (animaux et végétaux) des helminthes* (Lechevalier, Paris 1946).

[33] A. F. HAMS and G. D. WILKIN, Ann. appl. Biol. *49*, 515 (1961).

In recent years, a great deal of work has been done in Russia on the biological control of nematodes by predacious fungi, and some interesting and encouraging results have been reported. The work has been monographed by SOPRUNOV[34]. In a more recent paper, SOPRUNOV and TENDETNIK[35] state that they have found *Arthrobotrys oligospora* and *A. dolioformis* the most useful fungi for eelworm control, and recommend chopped maize and oatmeal as the best culture substrates. After growth and sporulation of the fungi the cultures are dried and powdered, and the authors claim that a powder with up to 2000000 spores/g is effective and can be produced at a cost of about 4.50 roubles/kg. The value of the Russian work is difficult to assess at present, but, if their results are substantiated, they appear to be well ahead of the rest of the world in the field of biological control of nematodes.

In the United States, interest in the use of predacious fungi for the control of soil nematodes has recently been aroused, and a number of very able workers have entered the field. It is as yet too soon to evaluate what is going on, but if the vigorous American attack on the problem continues it is unlikely that the Russians will hold their lead for long.

Zusammenfassung. Die zahlreichen, sich von Nematoden ernährenden Pilzarten zerfallen in zwei Gruppen. Die eine Gruppe fängt die Würmer mit klebrigen, netz- oder knopfförmigen Mycelien oder mit Zellringen, in denen sich die Nematoden verfangen oder durch plötzliches Quellen des Ringes aktiv festgehalten werden. Die zweite Gruppe dringt von am Wurm haftenden Sporen in seinen Körper ein.

Die Pilzmycelien besitzen keine Fangeinrichtungen, wenn sie ohne Nematoden gezüchtet werden. Zugabe von sterilem Wasser, in dem sich früher Nematoden aufgehalten hatten, bringt die Wurmfallen zur Entwicklung. Auch zahlreiche andere Substanzen, wie Pferdeserum oder wässrige Auszüge verschiedener tierischer Organe wirkt bei den Formen mit quellbaren Fangringen als Bildungsreiz. Es wurde ausserdem festgestellt, dass gewisse organische Stoffe ihre Aktivität im Nematodenfang beträchtlich erhöhen.

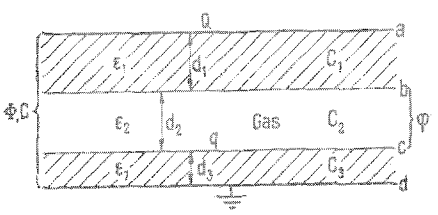
Nematodenfangende Pilze sind häufige Bodenbewohner und ihre Bedeutung als Nematodenvertilger ist erwiesen. Es sind Versuche im Gang, sie für die biologische Kontrolle von Feldwürmern auszunutzen, die als Erreger von Pflanzenkrankheiten schädlich sind.

[34] F. F. SOPRUNOV, *Predacious Fungi—Hyphomycetes and their Application in the Fight against Pathogenic Nematodes* (Ashkabad 1958).
[35] F. F. SOPRUNOV and YU. YA. TENDETNIK, Trud. gel'mint. Lab. *10*, 192 (1960).

Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

Intermittierende elektrische Entladungen im Gasraum zwischen zwei Dielektrika

Wird in einem dielektrischen System, das einen Gasraum einschliesst, z.B. einem zusammengesetzten, unendlich ausgedehnten Plattenkondensator, wie ihn die Abbildung im Schema zeigt, ein von $+E$ bis $-E$



alternierendes Potential Φ angelegt, so tritt im mittleren Gasraum (Dielektrizitätskonstante ε_2, Kapazität/cm² C_2) jeweilen eine Entladung ein, wenn das dort herrschende Potentialgefälle Φ den Wert der Zündspannung $\Phi = A$ erreicht hat, und die Entladung hört auf, wenn $\Phi = B$, gleich der Löschspannung geworden ist. Durch die Entladung, die in Form einer stillen elektrischen Entladung abläuft, solange kein Durchschlag durch das Dielektrikum erfolgt, entstehen an den Dielektrikumsflächen b und c elektrische Ladungen, die das Absinken des Potentials Φ von A auf B bedingen, was eine Nachlieferung von Elektrizität durch die Stromquelle auf die Belegungen a und d des Kondensators erfordert zur Aufrechterhaltung des angelegten Potentials Φ. Dass diese Nachlieferung von Elektrizität, auch wenn die Entladung im Gasraum sehr rasch abläuft, ohne vorübergehendes Absinken von Φ vor sich gehe, wird angesichts der geringen Menge der transportierten Elektrizität als sehr wahrscheinlich erachtet, denn der im Hochspannungsstromkreis gemessene Strom, der den aus den Gasentladungen resultierenden Leitungsstrom enthält, hat eine Stärke in der Grössenordnung von nur 10^{-2} bis 10^{-3} mA pro cm² Kondensatorfläche. Tatsächlich haben SPRETER und BRINER[1] in Versuchen mit stiller elektrischer Ent-

[1] V. SPRETER und E. BRINER, Helv. chim. Acta *32*, 2527 (1949).

sein, ohne besondere Schwierigkeiten die einschlägigen Originalarbeiten zu lesen und zu verstehen – dies insbesondere deshalb, weil die Nomenklatur erfreulicherweise alle Extravaganzen vermeidet und dem heutigen mehr oder weniger angenommenen Gebrauche folgt –. Darüber hinaus dürfte es dem Leser aber auch möglich sein, unter Beiziehung des Buches selbst thermodynamische Probleme der Chemie in Angriff zu nehmen.

F. GRÜN

Comparative Animal Physiology

By C. L. PROSSER

888 pp. (W. B. Saunders Company, Philadelphia and London, 1950) (63/-)

A modern comprehensive book on comparative animal physiology is certainly very welcome as it fills a gap in the physiological literature. As it is pointed out in the preface, the book is meant for the advanced student as well as for the investigator, as a source of information and references. Without doubt this aim has been entirely accomplished. While being concise and easy to read, the most recent aspects and developments of the problems are outlined and discussed. It has therefore the added merit of being a stimulating book.

The book is the result of the cooperation of five physiologists, each one dealing with a group of separate subjects: Water; Inorganic ions; Protein specificity; Nutrition; Feeding and digestion; Nitrogen excretion; Respiratory functions of body fluids; Temperature; Circulation of body fluids; Muscle and electric organs; Amoeboid movements; Nervous system (C. L. PROSSER); Respiration and metabolism (D. W. BISHOP); Photoreception; Chemoreception; Phonoreception (T. L. JAHN and W. J. WULFF); Mechano- and equilibrium reception (W. J. WULFF and C. L. PROSSER); Cilia; Trichocysts and Nematocysts; Bioluminescence; Chromatophores and colour change; Endocrine mechanisms (F. A. BROWN, Jr.).

Considering all these good qualities, it seems desirable that some inexactitudes (e. g. to include the frog *Discoglossus* among the Prochordates, p. 290 and Table 48, and to consider Echinochrome as an iron-containing respiratory pigment, p. 292) should be corrected in the next edition.

A. MONROY

Informations - Informationen - Informazioni - Notes

EXPLICATIONES

Zur Klimaänderung der Gegenwart

Über die in den letzten Jahrzehnten, insbesondere seit 1920, sich bemerkbar machende Erwärmung des Klimas besteht eine umfangreiche Literatur. Eine Zusammenstellung der klimatischen Faktoren nebst Literaturverzeichnis gibt uns LYSGAARD[1]; weitere Angaben, insbesondere auf botanischem Gebiete, ebenfalls nebst Bibliographie, stellte der Verfasser dieser Mitteilung zusammen[2]. Anschließend an den VII. Botanischen Kongreß in Stockholm im Juli 1950 nahm Verfasser an der von Dr. ARNBORG geführten Exkursion durch das Nadelwaldgebiet in Schweden teil, die einen Querschnitt von Gästrikland nördlich Stockholm bis Abisko im Lappland bot. Obwohl Verfasser schon mehrfach Lappland bereist hatte, zuerst vor dem ersten Weltkriege, also vor Eintritt der sichtbaren Klimaänderung, fuhr er erneut dahin, um vor allem den Einfluß der Klimaänderung auf die Wälder an der polaren Waldgrenze zu beobachten. Dies gelang ihm im Muddus-Nationalpark unweit von Gällivara in Lappland und bei Abisko. Die hier gemachten Beobachtungen bestätigten vollauf die von finnischen Forschern im früheren Gebiet von Petsamo und von russischen Forschern gemachten Untersuchungen (siehe die Arbeiten des Verfassers).

Schon RENVALL[3] hatte 1912 darauf hingewiesen, daß die Kiefer an der polaren Waldgrenze nur einmal im Laufe von 100 Jahren reife Samen hervorbringt, daß aber weiter südlich die Samenjahre häufiger auftreten. NEKRASOWA[1] zeigte, daß auf der Halbinsel Kola die Fichte alle 6–7 Jahre Samen reift, je Hektar jedoch nur 100–150 000 Samen den Boden erreichen. Dies ist jedenfalls eine Folge des unweit der polaren Waldgrenze herrschenden, für die Verjüngung der Bäume ungünstigen Klimas. Wird dieses wärmer, so müssen die Samenjahre häufiger auftreten, dies bestätigen auch Beobachtungen von HUSTICH, AARIO und anderen im nördlichen Finnland und im nördlichen Norwegen. Beobachtungen in dieser Richtung haben vor solchen an der alpinen Waldgrenze den Vorzug, daß die polare Waldgrenze vom Menschen bedeutend weniger beeinflußt ist – es kommen eigentlich nur die Nomaden mit ihren Rentieren in Betracht – als die letztere, die für die Zwecke des Sennereibetriebes stark herabgedrückt ist.

Da die polare Waldgrenze als ein Produkt der bisher herrschenden klimatischen Bedingungen angesehen werden kann und daher äußerst labil ist, so genügen kleine Schwankungen des Klimas, um eine Verschiebung in der einen oder anderen Richtung hin hervorzurufen.

Allerdings haben wir es im schwedischen Lappland, bzw. in der Gegend von Gällivara und Abisko nicht mit der eigentlichen polaren Waldgrenze zu tun, die ja weiter nördlich verläuft, sondern mit einer alpinen Waldgrenze, liegt doch Abisko in mehr als 400 m Höhe, doch ist hier im Norden kein wesentlicher Unterschied zwischen polarer und alpiner Waldgrenze zu beobachten, und weiter nördlich fallen beide zusammen. Der Grad der menschlichen Beeinflussung ist hier aber ein und derselbe; eine Depression der Waldgrenze für die Zwecke des Sennereibetriebes und der Viehwirtschaft, wie wir sie in den Alpen beobachten, kommt in Lappland nicht in Frage.

[1] L. LYSGAARD, Folia Geographica Danica, V. København, H. Hagerup (1949).

[2] C. REGEL, Österr. Bot. Z. *96*, 369 (1949); Ber. Geobot. Forsch.-Inst. Rübel, Zürich 1949, 11 (1950).

[3] R. RENVALL, *Die periodischen Erscheinungen der Reproduktion der Kiefer an der polaren Waldgrenze* (Helsingfors 1912). (Diss.).

[1] T. P. NEKRASOWA, Reprodukcija jeli na Kolskom Sewere. Botan. J. XXXIII, Nr. 2, 239. (Moskwa-Leningrad 1948).

Die Nadelwälder des südlichen Teiles der Nadelwaldzone, wie zum Beispiel bei Stockholm, enthalten Bäume aller Altersklassen. Neben alten Kiefern sieht man solche mittleren Alters, kleine und kleinste Bäume, so daß der Wald von den vielen Bäumen dicht ist. Im Gegensatz hierzu stehen die Nadelwälder Lapplands, in denen man nur ausgewachsene Bäume sieht und dann den Jungwuchs am Boden, während die dazwischen wachsenden Altersklassen fehlen. Jedenfalls fällt der nordische Nadelwald dadurch auf, daß man zwischen seinen Stämmen eine weite Sicht hat, denn diese wird nicht durch die Bäume verschiedensten Alters verdeckt. Diese eigentümliche Erscheinung ist eine Folge der erwähnten Samenjahre. Wird das Klima wärmer und treten infolgedessen die Samenjahre häufiger auf, so muß der nordische Wald allmählich den Aufbau eines südlicheren Waldes mit Bäumen verschiedenster Altersklassen aufweisen. Können wir diesen Vorgang in Lappland beobachten?

Es kam uns vor allem darauf an, festzustellen, inwieweit die Samenjahre der Kiefer bei Gällivara und bei Abisko häufiger auftreten als früher. Im Muddus-Nationalpark bei Gällivara wurde auf trockenen Böden mehrfach reicher Jungwuchs der Kiefer verschiedener Jahresklassen beobachtet. Folgende Feststellungen wurden gemacht:

a) Wald aus Birken und Kiefern *(Pinus silvestris)* mit Heidekraut (*Calluna vulgaris*) und Krähenbeere (*Empetrum*) in der Feldschicht. Der Jungwuchs der Kiefer besteht hier aus den verschiedenen Altersklassen und ist 1–1,5 m hoch.

b) Ebensolcher Wald mit Kiefern-Jungwuchs aller Altersklassen, darunter auch einige junge Fichten.

c) Ebensolcher Wald, alte Kiefern von 350 Jahren, mit Brandspuren von vor 175 Jahren, darunter stellenweise dichter Jungwuchs aller Jahresklassen, 6–10 Kiefern auf Flächen von 4 m². Samenjahre beim Jungwuchs alle 6–7 Jahre vorhanden. Ein besonders reiches Samenjahr war das Jahr 1946.

d) Kiefernwald auf trockenem Boden auf Anhöhe. Hier wurden bis zu 430 Jahre alte Kiefern festgestellt, die nächste Generation war 280 Jahre alt, dann wieder 125 Jahre alt, dazu kamen einige 110 Jahre alte Fichten und einige 50 Jahre alten Kiefern. Schließlich gab es Jungwuchs mehrerer Jahresklassen der letzten Jahre.

e) Kiefernwald mit dichtem Unterwuchs aus Schwarzbeeren (*Vaccinium Myrtillus*). Der Jungwuchs der Kiefer ist reichlich und umfaßt verschiedene Jahrgänge.

Auffallend ist ferner, daß sich dieser Jungwuchs nur auf die Kiefer bezieht, nicht aber auf die Fichte, die keinen oder aber einen nur spärlichen Jungwuchs aufweist, im Gegensatz zur Halbinsel Kola, wo Verfasser, allerdings noch vor Eintritt der Klimaänderung, stellenweise einen dichten Jungwuchs der Fichte beobachten konnte[1]. Die Erklärung liegt darin, daß das jetzt wärmere und zugleich trockenere Klima vor allem der Kiefer zugute kommt, zuletzt aber der Fichte, deren Optimum in einem kälteren und feuchteren Klima liegt, wie es bis vor kurzem herrschte. Dies ist auch von anderen Forschern beobachtet worden.

Abisko liegt schon in der Stufe des Birkenwaldes, der in den Gebirgen des Nordens die Grenze gegen die alpine Stufe bildet. Nur einige wenige Kiefergruppen wachsen inmitten des Birkenwaldes unweit der Touristenstation. Hier wurden folgende Aufzeichnungen gemacht, die die stärkere Verjüngung des Baumes in den letzten Dezennien charakterisieren.

a) Alte Kiefer im Birkenwalde, stark mit Zapfen besetzt, die aus zwei nacheinanderfolgenden Jahren stammen.

b) Heide mit Krähenbeeren (*Empetrum nigrum*) bewachsen, dazwischen zerstreut einige Kiefern wachsend, 15 Jahre alt, 10–12 Jahre alt und 8 Jahre alt.

c) Kieferngruppe im lichten Birkenwald. Kiefern 100–150 Jahre alt, einzelne auch bis zu 200 Jahre alt, darunter jüngere Kiefern, 45, 20–25, 15 und 4 Jahre alt. Stellenweise sind fünf Generationen innerhalb der letzten 15 Jahre zu beobachten.

Auch die Birke zeigt reichen Jungwuchs, sowohl bei Abisko als auch weiter an der oberen Waldgrenze bei Björkliden und bei Riksgränsen. Alle von Bäumen entblößten Flächen sind dicht mit jungen Birken bestanden; der Birkenwald erobert das Gebiet zurück, auf dem er vernichtet war, während dies früher Jahrzehnte dauerte und eine solche schnelle Rückeroberung nicht zu beobachten war. Dasselbe berichten auch finnische Forscher von der Fischerhalbinsel im früheren Gebiete von Petsamo.

Aus vorliegenden Beobachtungen, die diejenigen finnischer und norwegischer Forscher in anderen Gegenden des schwedischen, finnischen und norwegischen Lapplandes bestätigen, läßt sich ersehen, daß die Kiefer im Muddus-Nationalpark bei Gällivara und bei Abisko in den letzten Jahren häufig Samen getragen hat und daher Jungwuchs verschiedener Jahresklassen vorhanden ist, während ein solcher aus früheren Jahren nicht vorhanden ist und die Samenjahre nur selten vorkamen. In Abisko wurden junge Kiefern beobachtet, deren Jahresunterschiede nur 4–10 Jahre betrugen, woraus man auf ein häufiges Eintreten der Samenjahre hier in den letzten Dezennien schließen kann. Diese Beobachtungen bestätigen die Beobachtungen zahlreicher anderer Forscher und lassen sich in die Reihe übriger Beweise für eine Erwärmung des Klimas in den letzten Dezennien einreihen. Eine jede Klimaänderung bewirkt eine Verschiebung der den eurasiatischen Kontinent durchziehenden Landschaftszonen, der Tundra, des Nadelwaldes, des Laubwaldes, der Steppe, der Wüste nach Norden oder nach Süden hin. Wird das Klima, wie es jetzt der Fall ist, wärmer, so rückt die Zone des Nadelwaldes in die Tundra vor, indem die Samenjahre an der Waldgrenze häufiger auftreten als früher. Doch da diese Verschiebung der Zonen nicht nur die Verjüngung der Wälder beeinflußt, sondern alle Merkmale der betreffenden Zone, sowohl Pflanzen- und Tierwelt als auch die leblosen, wie zum Beispiel die Gletscher, so muß eine Untersuchung der Klimaänderung sich auf alle diese Merkmale zusammen erstrecken. Eine Zusammenstellung dieser Merkmale durch den Verfasser ist in Vorbereitung.

Was aber die Waldgrenze in Schwedisch-Lappland und das häufige Auftreten der Samenjahre anbelangt, so wäre deren eingehendere Untersuchung an Hand von Dauerprobeflächen, sowohl im Nationalpark von Muddus als auch in Abisko, wo eine Biologische Station besteht, wünschenswert.

C. Regel

Zürich, den 7. August 1951.

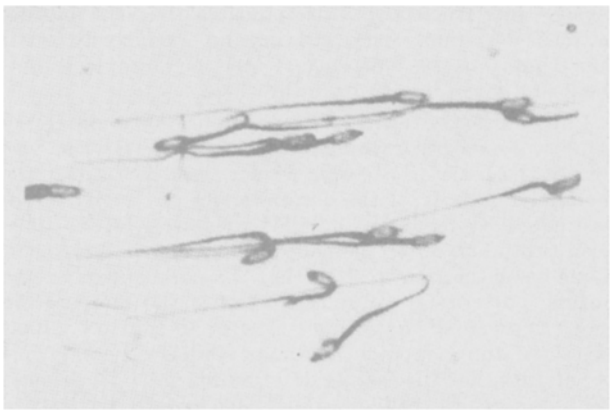
[1] C. Regel: *Die Vegetationsverhältnisse der Halbinsel Kola*, Repert. Spec. nov. regni vegetabilis. Beiheft LXXXI (Verlag Prof. Dr. Fr. Fedde, Dahlem 1941).

Résumé

L'auteur fait part de ses observations faites pendant l'excursion dans la région des forêts de la Suède septentrionale à la suite du Congrès international de botanique à Stockholm en 1950. Sous l'influence du climat devenu plus chaud les forêts à Muddus reverdissent et ont la tendence d'envahir la toundra, un fait observé par des savants finnois, norvégiens et russes.

Chiroptera) revealed two distinct types. One with a pointed anterior end and the other with a blunt club-shaped head end (Figure). According to the shape of the anterior tip, the acrosome is was pointed with a broad base or lies flat, closely attached to the nuclei in either case. The nuclei of the former type were conical, whereas in the latter they were more rectangular. On the posterior aspects of the nuclei in both the types, the 'post nuclear regions' or the 'post nuclear caps' were well pronounced. A centriole region was clearly marked in close association with the 'post nuclear cap'. The flagellum in each case seemed to develop from the centriole region, passing through the 'middle piece' and coming out as a vibratile tail. Both the sperm types were actively motile.

Uterine sections from the freshly copulated females showed the presence of both the sperm types in their lumen. Whether both are functional in fertilizing the ovum is not clear to us.



Dimorphic sperms of *Rhinopoma kinneari* as seen in the fixed smears. (1420×)

Equal numbers of both types of living sperms were measured from ten different adult individuals. The mean size was found to be 50.8 ± 12.9 μ for pointed head sperms and 52.8 ± 12.0 μ for blunt headed sperms. These size differences appear to have no statistical significance. The head lengths were also measured. There was no appreciable difference between the two types. Further the ratio of the two types was not constant in the individuals examined by us.

The outstanding differences between the two types of sperms are, (1) in their shape, and (2) in their overall lengths. There appears to be no difference in the head lengths but there is a difference in the lengths of the flagella (tail). Whether the dimorphism is due to the differences in the chromosomal complement, is not apparent in the size of the sperm heads as has been seen in the human sperms[1,2], but in their morphology[3].

Résumé. Les spermatozoïdes de *Rhinopoma kinneari* sont dimorphes et diffèrent les uns des autres dans leur forme et leur longeur totale, mais la longueur de leur tête reste constante. Ils peuvent être du type X et Y comme ceux des autres mammifères.

R. S. Mathur and T. C. A. Kumar

Department of Zoology, University of Rajasthan, Jodhpur (India), May 8, 1962.

[4] We wish to record our thanks to Prof. L. S. Ramaswami for helpful criticism and to Dr. P. N. Srivastava for helping us in statistical calculations.

A New Puffing Pattern Induced by Temperature Shock and DNP in Drosophila

The different puffing patterns of the polytene chromosomes of Diptera show organ-specificity, developmental stage-specificity and sometimes zone-specificity[1-4]. These patterns can be explained in terms of variations of chromosome activity. It is known that puffs are due to the uncoiling of some characteristic bands[5] similar to those which have been shown to correspond to certain Mendelian loci[6,7].

It has also been shown recently[8-11] that puffs are sites of synthetic activity and that their major product is RNA. For these reasons the different puffing patterns can now be more precisely interpreted in terms of activity of genes probably due to different metabolic situations occurring in the various organs and developmental stages investigated.

Some recent investigations show that it is possible to induce directed variations in the puffing patterns. This was accomplished by Kroeger[12] by transplanting salivary gland nuclei of *D. busckii* into egg cytoplasm of *D. melanogaster*, and by Clever and Karlson by means of injections of ecdysone in *Chironomus* larvae[13].

The purpose of this paper is to report our results on the effect of temperature on the puffing patterns of the salivary glands chromosomes of *Drosophila busckii*. It will clearly appear that temperature shocks may induce well defined variations in the puffing patterns and that the variation always interests the same bands and involves specific metabolic activities.

These observations are limited to the 2L chromosomes, since the main variations are found in this region. In the

	2L 8	2L 14	2L 15	2L 20
Normal at 25°C	+	—	—	—
After 30 min at 30°C	—	+	+	+

[1] W. Beermann, Chromosoma *5*, 139 (1952).
[2] R. Mechelke, Chromosoma *5*, 511 (1953).
[3] M. E. Breuer and C. Pavan, Chromosoma *7*, 371 (1955).
[4] W. Beermann, *Developmental Cytology* (Ed. Rudnik, Ronal Press, New York 1959).
[5] W. Beermann and G. F. Bahr, Exp. Cell Res. *6*, 195 (1954).
[6] O. Mackensen, J. Hered. *26*, 136 (1935).
[7] H. Slizynska, Genetics *23*, 291 (1938).
[8] D. J. Gross, Nature *180*, 440 (1957).
[9] G. Pelling, Nature *184*, 655 (1959).
[10] J. L. Sirlin, Exp. Cell Res. *19*, 177 (1960).
[11] F. Ritossa, Atti Assoc. Gen. Ital. *7*, 147 (1962).
[12] H. Kroeger, Chromosoma *11*, 129 (1960).
[13] U. Clever and P. Karlson, Exp. Cell Res. *20*, 623 (1960).

Table the puffing pattern is given of third instar larvae about 15 h before pupation of *D. buschii* grown at 25°C. In Figure 1 the regions 2L 14 and 2L 15 of the same larvae are shown[14].

If the larvae are subjected to a temperature shock (30°C or more) for about 30 min, a clear cut change in the puffing pattern is observed. The principal variations are shown in the Table and in Figure 2 and consist in the appearance of puffs in regions 2L 14, 2L 15 and 2L 20 and in the regression of the normal puff in 2L 8. The induction of a new puffing pattern by temperature shock has also been obtained in *D. melanogaster* and will be reported elsewhere. If the larvae that have undergone a temperature shock are transferred back to 25°C, after about 1 h the induced puffs recede while the puff in 2L 8 reappears again. When the same larvae are subjected once more to the temperature shock, the typical induced puffing pattern again makes its appearence.

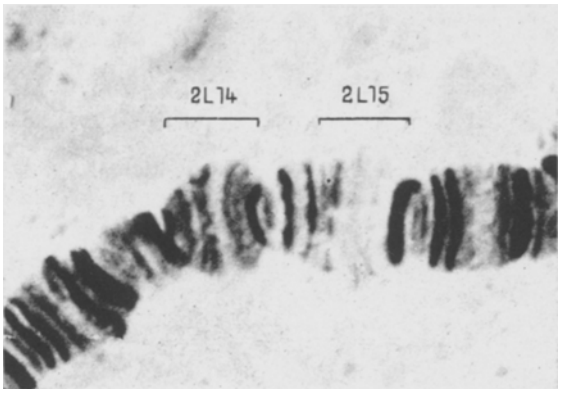


Fig. 1. The 2L 14 and 15 regions of salivary gland chromosome of *D. buschii* larvae reared at 25°C about 15 h before pupation.

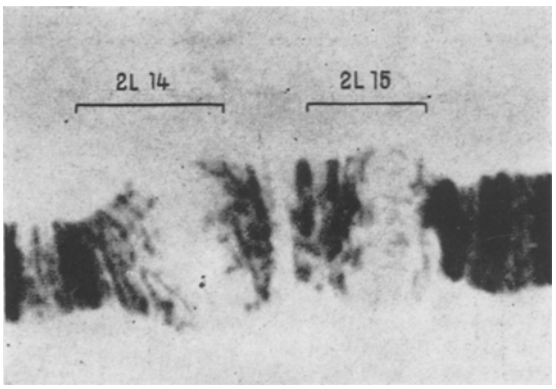


Fig. 2. The same regions as in Figure 1 after a thermal shock of 30 min at 30°C. Larvae near to pupation.

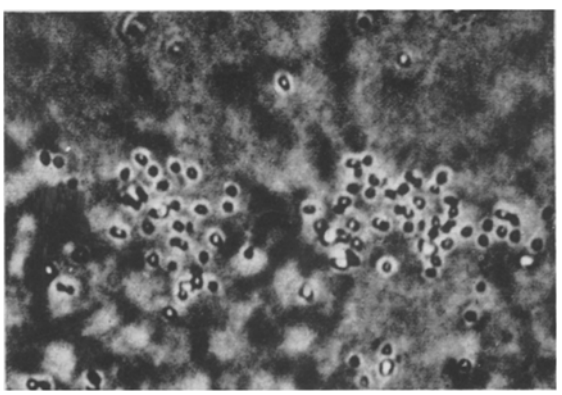


Fig. 3. The induced puffs after 10 min of incubation in 2.5 mm³ of a saline solution (0.17 mC/ml) of tritiated cytidine (Schwarz, s.a. 1 C/m*M*). 4 days exposition. Stripping films Kodak AR. 10.

It is also possible to induce the new puffs in larvae which have reached a stage very close to pupation, where puff 2L 8 has normally receded. Also in this case the induction has 100% efficiency.

When larvae grown at 19°C are brought to 25°C, some effects are sometimes noticeable, but with very low intensity and efficiency. This indicates that the puffing pattern variations may not depend exclusively on a 5–6°C jump in the temperature but on the rapid attainment of a given temperature threshold.

I have recently reported[11] a procedure for maintaining salivary glands of Drosophila metabolically active *in vitro* for about 2 h under oil drops. Under these conditions, the metabolic activity of the chromosomes can easily be investigated by means of labelled precursors which have been proved to enter the cell in few seconds. This technique has been used to show that the same effects on the puffing patterns are obtained whether the temperature shock is given to the whole larvae or to salivary glands extracted and incubated in Ringer solution. It may thus be inferred that the factors determining the phenomenon are limited to the salivary gland cells and do not involve organ interaction. The induced structural modifications can be shown to correspond to actual changes in the synthetic activity of the chromosomes bands concerned.

Tritiated cytidine was administered to salivary glands incubated *in vitro* and heated in order to induce the new puffing pattern. The presence of rather large quantities of this tracer in the puffs is apparent already after 3–4 min and it reaches high values after 10 min (Figure 3). Radioactivity is removed by RNAse[16]. This proves, as it was previously shown for the normal spontaneous puffs, that, also in the temperature-induced puffs (2L 14, 2L 15, 2L 20), a rather high rate of RNA synthesis occurs. On the other hand, this activity is found to be absent at the site of the original puff which has regressed (2L 8). Since most of the information so far available supports the idea that puffs are 'active genes', our experiments could be interpreted in the sense that function of genes (or at least of some genes) is reversible and dependent upon environmental conditions.

In an attempt to elucidate the possible mechanism of action of temperature, according to SZENT-GYÖRGYI's hypothesis[17], the effects of 2–4 dinitrophenol (DNP) and salicilate have been studied. For this experiment, salivary glands were incubated under oil in Ringer solution containing $10^{-3}M$ DNP or $10^{-2}M$ sodium salicilate for 30 min at 25°C. It was found that these substances can

[14] These positions are approximate; they have been inferred from the Sirotina's schematic maps[15]. The nomenclature is KRIVJCENKO's
[15] M. I. SIROTINA, Mem. Gent., Acad. Sci. Urk. SSR. *2*, 61 (1938).
[16] Other evidences on the synthetic activity of puffs will be published elsewhere.
[17] A. SZENT-GYÖRGYI, *Bioenergetics* (Academic Press, New York 1957).

mimic the temperature effect for the induction of the new puffing pattern. Since a good deal of information is available on the chemical mechanisms by which these substances act, we may now study the metabolic variations that can induce the formation of these puffs[18].

Riassunto. Si è notato che shocks di temperatura possono indurre una variazione di «puffing pattern» in ghiandole salivari di Drosophila. Tali «puffing» sono perfettamente reversibili e rappresentano zone di intensa sintesi di RNA. Si è notato che DNP e Na salicilato portano a simili variazioni di «puffing pattern».

F. RITOSSA

Laboratorio Internazionale di Genetica e Biofisica, Napoli (Italy), July 6, 1962.

[18] The author wishes to thank Dr. I. E. RASMUSSEN and Prof. G. MAGNI of the Genetics Institute of the University of Pavia for encouragement and critical discussion during the course of this work.

Über Korrelationen zwischen der Hemmung des aktiven Na-Transportes bei Erythrocyten und der positiv-inotropen Wirkung von Digitaliskörpern nach Phenylbutazonschädigung

SCHATZMANN[1], KAHN[2,3], SOLOMON et al.[4], GLYNN[5], MACHOVÁ[6] und REPKE et al.[7] zeigten, dass Digitaliskörper in niedrigen Konzentrationen den aktiven Ionentransport kältegeschädigter menschlicher Erythrocyten hemmen und dass diese Fähigkeit sich mit einigen Struktur-Wirkungsbeziehungen, mit den Toxizitätswerten und der Hemmung der für den Ionentransport verantwortlichen ATP-ase korrelieren lässt. Unbeantwortet blieb bisher die Frage, ob auch therapeutische Konzentrationen von Digitaliskörpern, die am isolierten Herzen einen positiv inotropen Effekt hervorrufen, den aktiven Kationentransport hemmen. In früheren Untersuchungen[8–14] haben wir gezeigt, dass zwischen einer Reihe von Geninen und Glykosiden strukturabhängige qualitative Wirkungsunterschiede in dem Sinne bestehen, dass Konzentrationen, die am ungeschädigten isolierten Herzen einen äquieffektiven positiv inotropen Effekt hervorrufen, eine experimentell ausgelöste akute «Herzinsuffizienz» unterschiedlich stark kompensieren können. Als vorläufige Arbeitshypothese wurde die Vermutung ausgesprochen[11], dass eine verschieden starke Hemmwirkung auf den aktiven Ionentransport und damit auf die von SKOU nachgewiesene ATP-ase für diese spezifischen Wirkungsdifferenzen verantwortlich sein könnte. In den vorliegenden Versuchen sollte diese Arbeitshypothese überprüft werden.

Methodisch hielten wir uns an die Literaturangaben von SCHATZMANN, KAHN et al.[1–3]. Nach 4–5tägiger Aufbewahrung des Blutes bei 4° C wurde nach Reinkubation bei 37° C die Abnahme des akkumulierten Natriums flammenphotometrisch gemessen. Die Hemmwirkung auf den Na-Austausch wurde mit 15 Geninen[15], deren positiv inotroper Effekt am ungeschädigten und geschädigten isolierten Meerschweinchenvorhofpräparat festgestellt worden war[11–13], bestimmt. Die sich ergebenden Dosiswirkungskurven wurden statistisch nach der Kovarianzanalyse (BONNIER und TEDIN[16]) auf Parallelität und gemittelten mittleren Abstand überprüft. Für die in den pharmakologischen Versuchen am isolierten Vorhofpräparat gefundenen äquieffektiven Geninkonzentrationen wurde von den Dosiswirkungskurven die prozentuale Hemmwirkung auf den Na-Austausch abgelesen und eine Korrelation zwischen dieser Hemmungsstärke und der Stärke des positiv inotropen Effektes der Genine nach Chinin- und Phenylbutazonschädigung aufgestellt.

Die Strukturformeln der untersuchten 15 Genine sind in Tabelle I gegenübergestellt.

Ergebnisse. Entgegen den Befunden von KAHN[2,3] und MACHOVÁ[6], die für die Hemmung des aktiven *Kalium*-transportes bei allen untersuchten Digitaliskörpern parallel verlaufende Dosiswirkungskurven feststellten, fanden wir für die Hemmung des *Natrium*-Austausches signifikante strukturabhängige Unterschiede einiger Regressionskoeffizienten. Die Dosiswirkungskurven der beiden 3,14-Dihydroxygenine (Digitoxigenin und Bufalin) verlaufen flacher als die Kurven der 3,14,12- und 3,14,16-Trihydroxygenine ($P = 0{,}02$). Die Substitution einer Aldehyd- statt der Methylgruppe in C_{10} (Strophanthidin: Periplogenin) vermindert ebenfalls den Anstiegswinkel. Der Einfluss des Laktonringes ist unterschiedlich: bei den 3,14,16-Trihydroxygeninen besitzen die drei Bufadienolide (*Bufotalin*, *Desacetylbufotalin* und *Cinobufagin*) höhere Regressionskoeffizienten als die beiden Cardenolide (Gitoxigenin und Oleandrigenin). Bei den 3,5,14-Trihydroxygeninen ist das umgekehrte Verhalten festzustellen (Figur 1). Setzt man ähnlich wie bei MACHOVÁ die ED_{50}-Werte der Dosiswirkungskurven zu den Toxizitätswerten bei der Katze in Beziehung, so ist der Korrelationskoeffizient hoch signifikant ($P < 0{,}001$).

In Tabelle II sind die Hemmung des aktiven Na-Austausches und die Kompensation nach Chinin- und Phenylbutazonschädigung durch die am ungeschädigten Herzen äquieffektiven Konzentrationen der Genine gegenübergestellt.

[1] H. J. SCHATZMANN, Helv. physiol. Acta *11*, 346 (1953).
[2] J. B. KAHN Jr. und G. H. ACHESON, J. Pharmacol. *115*, 305 (1955).
[3] J. B. KAHN Jr., J. Pharmacol. *121*, 234 (1957).
[4] A. K. SOLOMON, T. J. GILL und G. L. GOLD, J. gen. Physiol. *40*, 327 (1956), zit. nach [6].
[5] J. M. GLYNN, J. Physiol. *136*, 148 (1957).
[6] J. MACHOVÁ, Exper. *16*, 553 (1960).
[7] K. REPKE und H. J. PORTIUS, Arch. exp. Path. Pharmak. *241*, 535 (1961).
[8] W. FÖRSTER, Exper. *17*, 220 (1961).
[9] W. FÖRSTER, Exper. *17*, 308 (1961).
[10] W. FÖRSTER, Acta biol. med. germ. Suppl. *1*, 165 (1961).
[11] W. FÖRSTER, Habilitationsschrift, Magdeburg (1961).
[12] W. FÖRSTER, Acta biol. med. germ., im Druck (1962).
[13] W. FÖRSTER, Acta biol. med. germ., im Druck (1962).
[14] W. SZIEGOLEIT und W. FÖRSTER, Acta biol. med. germ. *7*, 614 (1961).
[15] Die verwendeten Genine wurden uns freundlicherweise zur Verfügung gestellt von Herrn Prof. K. MEYER, Basel (Desacetylbufotalin, Cinobufagin, Bufotalin, Telocinobufagin, Bufalin, Periplogenin und Oleandrigenin), Herrn Prof. TAMM, Basel (12β-Hydroxybufalin), Herrn Prof. WOLLENBERGER, Berlin (Ouabagenin), Herrn Prof. THIEN, AWD Dresden (Digoxigenin), Herrn Dr. DÜBLER, Deutsche Hoffmann-La Roche AG, Grenzach Baden, (Gitoxigenin und Digitoxigenin). Strophanthidin und Strophanthidol stammten von der Arco-Chemie, Berlin.
[16] G. BONNIER und O. TEDIN, *Biologisk variationsanalys* (Stockholm 1940).

de nos travaux, la détermination des molécules marquées à l'azote 15 s'effectue après leur séparation par des techniques chromatographiques. La principale difficulté de cette application est la contamination par l'azote extérieur et l'azote présent aux différents niveaux biologiques. Cette technique n'atteint pas la sensibilité des molécules marquées au carbone 14 mais permet par contre l'utilisation d'isotopes stables chez l'homme sans problème éthique.

Crédit FNRS, n° 3.726.72

Cyclosporin A: A New Antilymphocytic Agent

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Medizinisch-biologische und pharmazeutisch-chemische Forschung der Sandoz AG, CH–4002 Basel

The metabolite cyclosporin A derived from 2 fungus species represents a novel antilymphocytic agent. It is a small molecular weight ring-peptide. Cyclosporin A suppresses humoral as well as cellular immunity in several animal models. It also inhibits specifically in vitro proliferation of murine and human blood lymphocytes. This compound is furthermore highly effective in the experimentally induced polyarthritis in rats, but not in acute inflammation. It is orally active and its effects are comparable to those obtained with appropriate reference compounds. In contrast to other immunosuppressive agents and to cytostatic drugs, the weak side effects on the haemopoietic tissues suggest that its action may be directed mainly towards the immunocompetent lymphocytes. It is speculated that cyclosporin A interferes at an early stage of mitogenic stimulation of the lymphoid cells.

The Role of Parietal Cells for the Gastrotoxicity of Salicylates

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Basing on the clinical evidence which implicates parietal cells in the development of gastric side effects of salicylates we performed the following studies: (a) Morphological studies: Electron microscopical evaluation of gastric mucosa of rats revealed early and preferential damage of parietal cells occurring already minutes after oral administration of acetylsalicylic acid. (b) Biochemical studies: Measurement of radioactivity in samples of rat gastric mucosa 1 min after administration of C^{14} labelled acetylsalicylic acid revealed about 4 times higher concentrations in parietal cells containing areas as compared to samples devoid of parietal cells. – From these and other results it is concluded that parietal cells which are unprotected by mucus may serve as an entrance for (non-ionized) acetylsalicylic acid. They trap these molecules, get destroyed and open a gateway to further tissue damage.

The Influence of Intracerebral Cyclic AMP on Drinking Responses to Angiotensin (AT)

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Institut de pharmacologie de l'Université de Lausanne, CH–1011 Lausanne

Enhancement of drinking by intrahypothalamic or systemic beta-adrenergic agents and the second messenger role of cAMP at other beta-adrenergic effector sites suggested an investigation of the role of cAMP. – When injected unilaterally into the preoptic brain area of water-satiated rats neither cAMP (non-acylated; 0.004 to 4.0 µg), nor the phosphodiesterase inhibitor theophylline (0.002 to 2.0 µg), nor both agents combined elicited any drinking response. When injected 15 min after 2.5 mU of partially purified hog renin, cAMP (4 µg) significantly increased the drinking response from 5.1 ± 0.9 to 7.4 ± 1.1 ml/3 h (n = 17); similarly water intake after 125 ng AT II-amide rose from 2.3 ± 0.5 ml to 4.2 ± 1.0 ml/3 h (n = 19). Neither systemic (i.p.) nor intracerebral theophylline (2 µg; THE) influenced drinking to AT II. Paradoxically, intracerebral cAMP plus THE injected 2 min before 125 µg AT II depressed the drinking response from 4.0 ± 0.9 to 1.0 ± 0.4 ml/3 h (n = 16). I.p. THE (75 mg/kg) injected 30 min before access to water increased drinking in rats kept thirsty for 21 h from 16.3 ± 0.6 to 18.1 ± 1.0 ml/3 h.

Supported by SNSF, grant 3.389.74

Effect of *p*-Nitrophenyl Diazonium Fluoroborate on Cholinergic Mechanisms

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Pharmakologisches Institut der Universität, Gloriastrasse 32, CH–8006 Zürich

Electrophysiological experiments were done to investigate the effect of *p*-nitrophenyl diazonium fluoroborate (*p*-NPD) on the motor endplate of the frog. When added to the bath *p*-NPD stabilizes the postsynaptic membrane. Longtime iontophoretic ejections of *p*-NPD produce a biphasic effect: initially a potentiation of the depolarization due to ACh and subsequently an inhibition. When the AChE is inactivated previously only the inhibition is demonstrable. Ionophoretic ejections of *p*-NPD on cholinergic neurons in the hippocampal cortex of the cat produce also a biphasic effect on ACh-induced activity. – The association constant of *p*-NPD to purified AChE and to membrane fragments of electroplax was determined biochemically. Its affinity to the AChE is about 20 times greater than to the ACh-receptor. – The biphasic effect seems to depend on the capacity of *p*-NPD to combine with both the AChE and the ACh-receptor. The AChE is first inhibited thereby potentiating the ACh-response. The ACh-receptor is then inhibited too thereby blocking the postsynaptic excitability.

Differential Effects of Neuroleptic Drugs on Hyperactivity and Stereotyped Behaviour Induced by D-Amphetamine in the Rat

A. Delini-Stula
Ciba-Geigy Limited, CH– 4002 Basle, K-125.12.20, PH 2.426

Low and high doses of D-amphetamine induce two distinct types of behavioural excitation: hyperactivity and stereotypies. These are thought to be mediated by

sondern um kleine Anteile aller Globulinfraktionen. Die Ergebnisse führen zu dem Schluss, dass bei zunehmender Absorption von Kationen nur diejenigen Proteine im Sediment bleiben, denen anionische Gruppen verbleiben, die also nach Auflösung im alkalischen Barbituratpuffer am schnellsten zur Anode wandern. Das würde bedeuten, dass bei einem gegebenen pH nur ein Teil der Globuline mit Kationen abgesättigt wird, während ein anderer anionische Gruppen behält, sei es, dass diese im Innern des geknäuelten Moleküls liegen, sei es, dass jedes Proteinmolekül gleichviel Kationen absorbiert, ohne Rücksicht auf die Zahl seiner negativen Gruppen. Letztere Auffassung steht zunächst im Widerspruch zum Massenwirkungsgesetz.

Bei der Hitzedenaturierung wird elektrophoretisch wie chromatographisch ein Ladungsausgleich beobachtet. Daraus resultiert auch die einheitliche Fällbarkeit. Die Gesamtzahl der ionisierten Gruppen wird durch die Denaturierung nicht geändert, wie der unveränderte Säureverbrauch beweist[1].

K. Simon

Medizinische Universitäts-Poliklinik, München, den 4. Dezember 1953[2].

Summary

Diluted serum was treated with diluted acid. Between pH 6·8 and 6·5 a part of the globulines is precipitated whose composition changes in favour of the more rapidly migrating fractions with rising pH. Sera denaturated by heat are flocculated quantitatively and behave like one monodisperse protein also in electrophoresis and chromatography. This leads to the conclusion, other than after denaturation, that negative loads of native proteins possess different strengths of absorption toward H^+.

[1] K. SIMON, Exper. *8*, 55 (1952).
[2] Gegenwärtige Adresse: München-Solln, Whistlerweg 21.

PRO EXPERIMENTIS

Ein neuer Weg zur Gewinnung von Zellen- und Gewebebestandteilen

Zellen- und Gewebebestandteile lassen sich nach Gefriertrocknung in «nicht wässrigem» Milieu (BEHRENS, 1929) oder nach Homogenisieren in «wässrigem» Milieu (BENSLEY, 1934; DOUNCE, 1943) gewinnen. Bei beiden Methoden wird der ursprüngliche Zustand der histologischen Gebilde verändert. Der Wert der Trennungen wird dadurch wesentlich gemindert. Es wurde versucht, ein besseres Trennverfahren zu finden. Folgender Weg erwies sich als gangbar:

Die zu trennenden Gewebe werden in einem indifferenten Öl (Paraffinöl) emulgiert. Die histologischen Strukturen verteilen sich dabei, wie die mikroskopische Untersuchung zeigt, auf die verschiedenen Teilchen der dispersen Phase. Es gelingt nun, gleichartige Teilchen abzutrennen und damit bestimmte Zellen- und Gewebebestandteile zu gewinnen. Die Trennung kann nach der Grösse durch dosiertes Zentrifugieren der Emulsion oder nach dem spezifischen Gewicht in geeigneten Tetrachlorkohlenstoff-Petroläther-Mischungen erfolgen. Der Austausch des Öls durch diese Mischungen bietet keinerlei Schwierigkeiten. Bis jetzt wurden mit der neuen Methode Zellkerne aus Erythrozyten von Scyllium, aus dem Hepato-Pankreas des Octopus und der Leber der Maus isoliert.

Da die Zellkerne nur mit Paraffinöl, Tetrachlorkohlenstoff und Petroläther in Berührung gekommen sind, kann angenommen werden, dass sie nur geringfügige Veränderungen erfahren haben. Lediglich Fette und freie Lipoide dürften teilweise extrahiert worden sein.

M. BEHRENS

Physiologisch-chemisches Institut der Medizinischen Akademie der Justus-Liebig-Hochschule, Giessen, und Zoologische Station, Neapel, 25. Oktober 1954.

Summary

A new method to obtain cells and tissue ingredients almost unalterated is described. The tissue is emulsified in an indifferent oil in which process the different histological structures are separated into the different droplets. Separating similar droplets, it is possible to gain single cells and tissue components.

PRO EXPERIMENTIS

A new Method for Measuring Membrane Potentials with External Electrodes

The true value of membrane potentials of biological core conductors can only be measured with external electrodes if the flow of injury current between a normal and an injured or depolarised part of membrane can be prevented by

(i) compensating the potential drop which is at the origin of current flow[1], or

(ii) increasing the longitudinal resistance of the outer medium.

Current flow in the core and in the outer medium in fact reduces the true value of the membrane potential measured with external electrodes by the short-circuiting factor

$$\frac{r_1}{r_1+r_2}$$

where r_1 and r_2 are longitudinal resistances of the external and internal medium per unit length respectively.

An increase of r_1 to much higher values than r_2 will therefore tend to increase the short circuiting factor to unity. The potential measured with external electrodes will thus approximate the true membrane potential of the uninjured membrane. These conditions can be realised by the following procedure:

A bundle of myelinated nerve fibers of a frog is introduced into a hole of slightly greater diameter through which an isotonic sucrose solution of at least 2×10^6 Ω cm specific resistance flows at constant rate. The inflow of sucrose is made in the middle of the hole and the outflowing sucrose is spilled away by RINGER or test solutions flowing through vertical channels at both ends of

[1] A. F. HUXLEY and R. STÄMPFLI, J. Physiol. *112*, 476 (1951).

it (Fig. 1). The nerve fibers are bent upwards into the two channels, thus being in contact with the inflowing solutions. The resistance of the liquid in the vertical channels is very small. Each of them can be considered as equipotential. There is a sharp increase of longitudinal

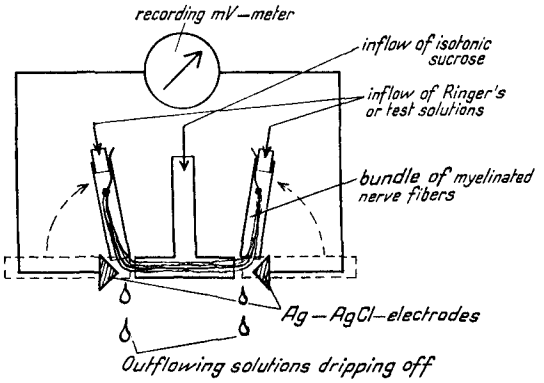

Fig. 1.—Arrangement of polyethylene tubes for recording membrane potentials. The nerve fibers are pulled across three tubes. The middle one is circulated with isotonic sucrose solution of high specific resistance, the outer ones with RINGER's solution on one side and RINGER's solution or test solutions on the other side. After introduction of the nerve fibers both tubes are moved into vertical position in order to have a sharp change of resistance at both ends of the middle section by spilling the sucrose solution away.

resistance at the entry of the horizontal hole. The potential difference between chlorided silver electrodes at the lower end of the channels, with RINGER's solution on one side and an isotonic KCl solution on the other, should therefore be equal to the resting potential of the fibers. Values of about 70 mV are obtained, in good agreement with the compensating method[1]. The advantages of the new method are obvious: Bundles of nerve or muscle fibers, freed from connective tissue, can be used instead of single fibers. No particular skill is necessary and the arrangement is simple. The potential changes can easily be recorded and the time course of the action of ions or drugs affecting the membrane potential can be compared quantitatively (Fig. 2). Although it is not yet possible to measure action potentials by this procedure, these advantages appear to justify publication. The main difficulty is to make a sucrose solution of high enough specific resistance. This can be done by using distilled water from an ion exchanger column for

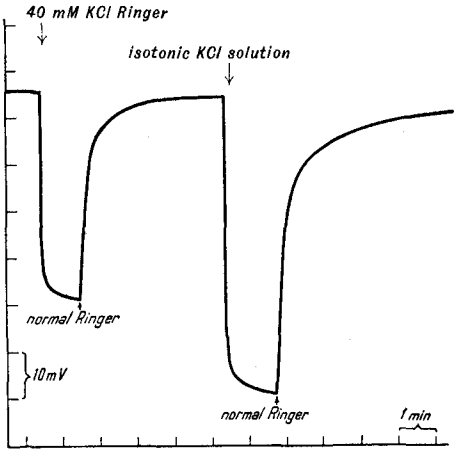

Fig. 2.—Example of the depressing action of K-rich solutions. 40 mM K-Ringer (left) depresses the membrane potential of a bundle of myelinated frog nerve fibers by at least 46 mV (equilibrium was not yet obtained when RINGER's was given again) and isotonic KCl-solution brings it down by at least 64 mV (same remark as above) x-axis = time in minutes, y-axis = millivolts. Note sharp decrease in potential at application of K-rich solutions and slower recovery after change to RINGER's. Complete recovery is regularly observed at small K-concentrations and frequently with isotonic or hypertonic K-solutions.

dilution or by circulating the diluted sucrose through an ion exchanger.

R. STÄMPFLI[1]

Physiological Institutes of the University of Berne, Switzerland, and of the Saar-University, Homburg, Saarland, June 6, 1954.

Zusammenfassung

Beschreibung einer neuen Methode, die es gestattet, mit Aussenelektroden die wahre Grösse der Membranpotentialschwankungen, die durch den Einfluss von hyper- oder hypopolarisierenden Substanzen oder durch die Erregung in markhaltigen Nervenfasern erzeugt werden, zu registrieren.

[1] A. F. HUXLEY and R. STÄMPFLI, J. Physiol. *112*, 476 (1951).

[1] Present address: Physiological Institute of the University of Saarland, Homburg, Saar.