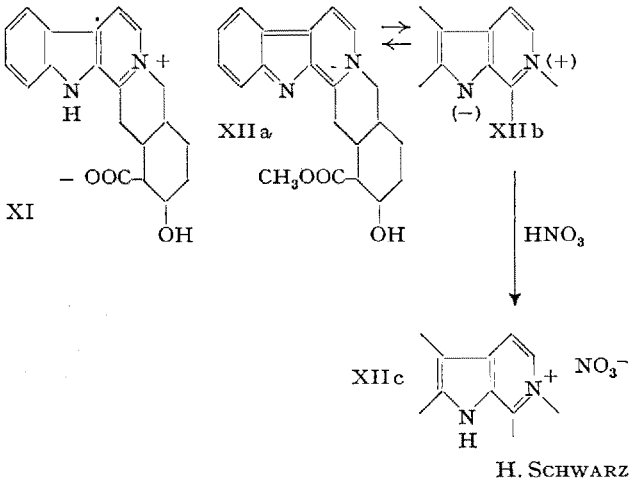
Absorptionsspektren und dem ganzen chemischen Verhalten dieser Verbindungen besser entsprechen:



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Organisch-chemische Anstalt der Universität Basel, den 24. Juni 1950.

Summary

From the study of absorption spectra of tetradehydroyohimbic acid, tetradehydroyohimbine, and its salts a new constitution is proposed for these substances.

Contribution à l'étude de l'héparine

Dans un travail qui vient de paraître, JORPES, BERGSTRÖM et MUTT[1] concluent que l'héparine renferme des groupes sulfamides du type CH—NH—SO_3H. Etant donné que de notre côté, et par des voies différentes, nous sommes arrivés à la même conclusion, nous pensons utile de faire part de nos résultats.

L'héparine Roche qui nous a servi pour nos essais renferme au maximum 10% de groupes aminés libres. D'autre part, conformément à JORPES et à WOLFROM[2], nous avons trouvé que la faible quantité de groupes acétyles qu'elle contient ne peut expliquer le blocage complet du reste des groupes aminés. Il faut donc admettre un autre mode de liaison de l'azote dans l'héparine.

L'hypothèse d'une liaison NH-glucosidique, telle que l'avait envisagée WOLFROM, doit être écartée. En effet, l'héparine forme dans les conditions de la réaction de VAN SLYKE (acide acétique + nitrite de soude) une nitrosamine. Ce corps que l'on peut isoler est biologiquement inactif; il se décompose lentement dans le milieu réactionnel avec libération d'azote. S'il s'agissait d'un nitroso-composé issu d'une liaison NH-glucosidique, sa décomposition provoquerait nécessairement une rupture de la chaîne, entraînant une baisse de la viscosité. Or, ce n'est pas le cas.

D'autre part le groupe aminé n'est pas non plus lié au groupe carboxyle de l'acide glucuronique, celui-ci étant libre comme l'indique les courbes de titration. Enfin, on ne trouve à côté de l'acide acétique et de l'acide sulfurique aucun autre acide qui pourrait être rendu responsable du blocage du groupe aminé.

[1] E. J. JORPES, H. BOSTRÖM et V. MUTT, J. Biol. Chem. *183*, 607 (1950).

[2] M. L. WOLFROM et W. H. MCNEELY, J. Amer. Chem. Soc. *67*, 748 (1945).

La seule possibilité qui reste est celle d'une liaison NH—SO_3H. Par hydrolyse acide de l'héparine (HCl N/25 et 100° C), on constate la scission d'environ 1,5 groupes sulfates par groupe aminé libéré. Les groupes NH—SO_3H de l'héparine seraient donc plus rapidement scindés que ses groupes C—O—SO_3H, dont le nombre dépasse nettement celui des précédents. Or, ce fait semble en contradiction avec la stabilité à l'hydrolyse des sulfamates simples décrits par TRAUBE[1]. En effet, nous avons pu confirmer que les sulfamates du type RNH—SO_3H, (CH_3NH—SO_3H, p. ex.) sont plus stables que les esters O-sulfuriques correspondants (p.ex. CH_3O—SO_3H). Cependant, la synthèse de la glucosamine-N-sulfate nous a permis de comparer sa vitesse d'hydrolyse à celle du glucose-6-sulfate. Or, dans ce cas comme dans celui de l'héparine, c'est la liaison N-sulfate, qui est la plus rapidement hydrolysée. La labilité de la liaison-N-sulfate de l'héparine n'a donc rien d'exceptionnel.

Nous devons donc admettre la présence de groupes CH—NH—SO_3H dans l'héparine; ceux-ci semblent de plus être essentiels à l'activité biologique du polysaccharide, car leur transformation en groupes C—N(NO)SO_3H entraîne instantanément la perte totale de l'activité.

KURT H. MEYER et D. E. SCHWARTZ

Laboratoires de chimie organique et inorganique de l'Université de Genève, le 8 juin 1950.

Summary

Evidence has been obtained for the presence in heparin of glucosamine-N-sulfate groups (—NH—SO_3H) by the elimination of other alternatives and by transformation of heparin into an inactive nitroso compound.

[1] W. TRAUBE, Ber. Dtsch. Chem. Ges. *52*, 1274 (1919).

Mutation in the Enzymatic Equipment of *Escherichia coli* and *Proteus* OX 19 Directed by Desoxyribonucleic Acid Isolated from Bacteria of the same and of different Species.

GRIFFITH[1] discovered the possibility of transforming *in vivo* one type of pneumococcus into another type by using heat-killed bacteria of the other type of pneumococcus. This phenomenon was confirmed by DAWSON and SIA[2], who were able to reproduce it *in vitro*, and by ALLOWAY[3].

The transforming principle has been identified by AVERY, MACLEOD and MACCARTY[4] with bacterial desoxyribonucleic acid. Identical results were recently obtained by H. E. TAYLOR[5].

BOIVIN *et al.*[6] have isolated from cells of *Bacterium coli* a desoxyribonucleic acid which induced mutations of the phase and of the enzymatic equipment in another strain of *B. coli*. Further experiments along the same line were performed with *S. paradysenteriæ* (WEIL and BINDER[7]) and with *B. anthracis* (MANNINGER and

[1] F. GRIFFITH, J. Hyg. *27*, 113 (1928).

[2] M. H. DAWSON and R. H. P. SIA, J. Exp. Med. *54*, 681 (1931).

[3] J. L. ALLOWAY, J. Exp. Med. *55*, 91 (1932); ib. *57*, 265 (1933).

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

[5] H. E. TAYLOR, J. Exp. Med. *89*, 399 (1949).

[6] A. BOIVIN, Cold Spring Harbor Symp. on Quant. Biol. *12*, 7 (1948).

[7] A. J. WEIL and A. J. BINDER, Proc. Soc. Exp. Biol. Med. *66*, 349 (1947).

Oxidation of some carbohydrates studied by the bacterial strains (+ oxidation, — no oxidation)
(The results are based on at least 4 experiments each)

Bactreial strains	Arabinose	*l*(+)-Xylose	Glucose	Galactose	Mannose	Fructose	Maltose	Sucrose	Lactose	Raffinose	Dextrin	Soluble starch	Inulin	Mannitol	Inositol	Salicyne
Coli I	+	+	+	+	+	−	+	−	+	+	−	−	−	+	+	+
Coli UQ	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Coli SG	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Coli 10	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	−
Proteus OX 19	+	−	+	+	+	−	+	+	−	+	+	−	+	+	+	+
Proteus OX 2	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Salmonella typhi murium	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Salmonella newport	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Staphylococcus pyogenes Oxford	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+
B. subtilis	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+
B. pyocyaneum	−	+	+	+	+	+	+	+	+	+	−	−	+	−	−	−
B. prodigiosus	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+

NÓGRÁDI[1]). No mutations were, on the contrary, obtained in *B. anthracis* by TOMCSIK[2].

In 1934 KAUFFMANN[3] discovered that many Colon Bacilli isolated from faecal matter of normal subjects and of patients with gastroenteric diseases possessed some antigenic fractions of *Salmonellæ*. This fact was confirmed by GIOVANARDI *et al.*[4] and by BUONOMINI *et al.*[5]. ZIRONI[6] has described the phenomenon of paraagglutination: a bacterial strain, living in the presence of another biologically related one, imposes on to the latter its own antigenic structure.

Experiments on mutations in the enzymatic equipment of some bacterial strains are described in this paper.

The bacteria studied are: 4 strains of *B. coli*, which are designated as *Coli* I, *Coli* UQ, *Coli* SG, and *Coli* IO, isolated from patients with urinary diseases; 2 strains of *Proteus* (*Proteus* OX 19 and *Proteus* OX 2); 2 *Salmonellæ* (*S. typhi murium* and *S. newport*; a strain of *Staphylococcus pyogenes* var. Oxford; a strain of *B. subtilis*; a strain of *B. prodigiosus* and of *B. pyocyaneum*.

The enzymatic equipment of these strains has been investigated, especially with respect to oxidation of carbohydrates. The oxidation of the sugars by the bacterial cells was studied using WARBURG's manometric technique. The sugars were added in buffered 0·2 M solutions to the bacterial suspensions (p_H 7·4). The following carbohydrates were used: arabinose, *l*(+)-xylose, glucose, galactose, mannose, fructose, maltose, sucrose, lactose, raffinose, dextrin, soluble starch, inulin, mannitol, inositol, salicyne. After every determination, microscopic and serological purity controls were performed. The results are summarized in the Table.

From all these strains of microorganims the nucleoprotein fraction was isolated, using the method of VENDRELY[7] (autolysis at 37° C for two days, of the chloroform-killed bacterial cells suspended in saline in presence of sodium citrate, which inhibits the activity of desoxyribonuclease [MACCARTY[1]], removal of the bacterial bodies by centrifuging at high speed, precipitation of the nucleoprotein fraction with acetic acid until complete removal of the bacterial glucydolipidic complex, serologically tested).

With the nucleoprotein fraction isolated from microorganisms which are able to oxidize one sugar, I treated another bacterial strain unable to oxidize it. Mutations in the enzymatic equipment of two strains, *Coli* I and *Proteus* OX 19, were so obtained, while the other strains did not show any modification.

(A) *Coli* I: this strain was treated with the nucleoprotein fraction from *Coli* UQ, *Coli* SG, and *Coli* IO.

After 13 passages with the nucleoprotein fraction from *Coli* UQ and after 16 passages with *Coli* SG, *Coli* I oxidized sucrose, while the untreated original strain remained unable to oxidize it. No mutation was obtained with the nucleoprotein fraction from *Coli* IO after 39 passages.

The transforming principle of *Coli* UQ and *Coli* SG resisted triptic and peptic digestion as well as the action of ribonuclease (isolated from pancreatine following the method of DUBOS and THOMPSON[2], but did not resist desoxyribonuclease (isolated from fresh beef pancreas according to FISCHER, BÖTTGER, and LEHMANN-ECHTERNACHT[3]. This fact strongly suggests that the inducing principle is related to desoxyribonucleic acid.

No mutations were obtained in *Coli* UQ, *Coli* SG, and *Coli* IO after treatment with the nucleoprotein fraction isolated from the other strains of Colon Bacilli. *Coli* I was also treated with the nucleoproteic fraction of the other microorganisms of different species. After 13 passages with the nucleoprotein isolated from *Proteus* OX 2, after 8 passages with the nucleoprotein from *S. typhi murium* and 13 with the one from *S. newport*, *Coli* I was able to oxidize sucrose, while the original untreated strain remained unaltered. No mutation was observed with the nucleoprotein fraction from *Proteus* OX 19, *Staphylococcus pyogenes* Oxford, *B. subtilis*, *B.*

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[3] F. KAUFFMANN, Z. Hyg. *114*, 97 (1933).
[4] A. GIOVANARDI, Zbl. Bakt., I Orig. *141*, 341 (1938).
[5] G. BUONOMINI and G. D'ALESSANDRO, Boll. Soc. It. Microbiologia *19*, 129 (1941); Boll. Soc. It. Biol. Sper. *20*, 245 (1945).
[6] A. ZIRONI, Atti Soc. Lomb. Sci. Med. Biol. *6*, 25 (1938).
[7] R. VENDRELY, Exper. *3*, 196 (1946).

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[2] R. J. DUBOS and R. H. S. THOMPSON, J. Biol. Chem. *124*, 501 (1938).
[3] F. G. FISCHER, I. BÖTTGER, and H. LEHMANN-ECHTERNACHT, Z. Physiol. Chem. *271*, 246 (1941).

pyocyaneum, *B. prodigiosus*. *Coli* UQ, *Coli* SG, and *Coli* IO remained unchanged after treatment with the nucleoproteins of all the other strains.

(B) *Proteus* OX 19: this strain, which does not attack lactose, fructose, and xylose, was treated with the nucleoprotein fraction isolated from *Proteus* OX 2. *Proteus* OX 2 cannot oxidize lactose, but can easily oxidize xylose and fructose. After 6 passages with the nucleoprotein from *Proteus* OX 2, *Proteus* OX 19 has developed the enzymes necessary for the oxidation of fructose and xylose, but did not attack lactose.

Treatment of *Proteus* OX 19 with the nucleoprotein fraction of bacteria of different species has shown that this strain also can mutate with the nucleic acids from some microorganisms of different, but biologically related, species. I have observed mutation in the oxidation of all three substrates mentioned above after treatment with the nucleoprotein from *Coli* I (13th passage). I have also observed mutation in the oxidation of lactose and fructose by the nucleoprotein fraction isolated from *Coli* SG. No mutation was observed with the nucleoprotein from *Coli* IO and *Coli* UQ. After treatment with the nucleoprotein of *S. typhi murium* and *S. newport*, which oxidize xylose and fructose, but cannot attack lactose, *Proteus* OX 19 has attacked xylose and fructose, but not lactose.

The mutation has failed with the nucleoprotein fraction of *Staphylococcus* Oxford, *B. subtilis*, *B. pyocyaneum*, and *B. prodigiosus*.

The transforming principle is resistant to tryptic and peptic digestion, and to ribonuclease, but is completely destroyed by desoxyribonuclease. These experiments confirm BOIVIN's results. Partial mutation in the enzymatic equipment has been observed in *Coli* I and in *Proteus* OX 19 also after treatment with desoxyribonucleic acid isolated from bacteria of different, but biologically related species, e. g. other Enterobacteria.

The experiments reported here give experimental support to KAUFFMANN's observation who isolated from fæcal matter of normal subjects and of patients with gastroenteric diseases strains of *B. coli* containing some antigenic fractions of *Salmonellæ*. Explanation is given also to the paraagglutination phenomenon described by ZIRONI. It follows that the presence of common antigenic fractions in bacteria is likely to be of mutative origin.

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Department of General Pathology, University of Siena, November 14, 1949.

Résumé

L'auteur a obtenu des mutations de l'équipement enzymatique (oxydation de la saccharose) d'une souche de *E. coli* traitée avec la fraction nucléoprotéique extraite de deux souches diverses du Colibacille attaquant ce sucre. Une souche de *Proteus* OX 19 également a muté par l'oxydation des deux sucres, xylose et fructose, sous l'influence d'un principe de nature nucléoprotéique extrait d'une souche de *Proteus* OX 2 attaquant ces substances. D'autres mutations ont pu être obtenues par l'oxydation des carbohydrates dans les mêmes souches de Colibacille et de *Proteus*, à la suite du traitement avec la fraction nucléoprotéique extraite de quelques souches de bactéries intéstinales appartenant à des espèces différentes (*Coli*, *Proteus*, *Salmonella*), mais biologiquement parentes. L'auteur n'a pas obtenu de mutations chez le Colibacille et le *Proteus* en se servant de nucléoprotéines extraites de souches biologiquement éloignées. Le principe actif peut être identifié avec l'acide désoxyribonucléique, car il reste inaltéré après la digestion tryptique et la digestion peptique et résiste à la ribonucléase, tandis qu'il est complètement inactivé par la désoxyribonucléase.

Experiments with Chromosomes Isolated from Intermitotic Nuclei

By manipulating his preparation of *chromosin*—a desoxyribosenucleoprotein complex derived from the cell nucleus—from liver, kidney, and other tissues, MIRSKY[1] found certain bodies which he explained as isolated chromosomes. The *chromatin threads* isolated from the intermitotic (resting) nucleus vary little in size and organization. Usually they show distinct doubleness, a specific pattern with satellites, heterochromatic and euchromatic sections, and often one finds a repeated occurrence of a precise type of chromosome. As with chromosomes, the threads can be uncoiled by agents such as KCN. Some bodies considered as nucleoli can be seen in a smear, stained with FEULGEN's reagent and light green, still attached to a chromosome or as separate particles. Likewise the *chemical* composition of the isolated chromatin threads has already been described by MIRSKY[2]. Because CLAUDE and POTTER[3] isolated chromatin threads which they suggested as represented chromosomes, I regarded it as desirable to examine MIRSKY's bodies by means of polarized light and in regard to their microdissectional behaviour.

In addition to cultures of muscle explants[4] grown *in vitro* after aseptic extraction from *Calliphora erythrocephala* MEIG., I explanted similarly the *follicle epithelium* of Drosophila spec., and then I fragmented the cultures *in vitro*, suspended in a large volume of saline, by placing them in a colloid mill. By such *treatment*, all the cells and nearly all the nuclei are broken, and by centrifuging a bright-coloured precipitate under a dark-coloured supernatant is obtained. In order to reduce autolytic changes, the procedures have to be carried out as rapidly as possible, and must be performed in a cold room kept at 1° C. Rise of temperature, for the same reason, has to be prevented. After suspension in a saline, the precipitate is placed in the colloid mill once more for 1–2 minutes. After continued washing and centrifuging the precipitate consists, at last, of chromosome threads isolated from intermitotic nuclei of the explant.

Microdissectional stretchings between microneedles have been made as usual. The *measurements of birefringence* (path difference or retardation, to be divided by the length of the path travelled by light) have been performed with a BRACE compensator (rotating λ/30-mica compensator) and the half-shadow wedge according to J. MACÉ DE LÉPINAY[5]. Measurements with this method are known to be extraordinarily sensitive[6].

The *isolated threads* present, to say the least, a model of a chromosome, being an aggregate of polypeptide protamine molecules in association with nucleic acid. Often they possess two arms separated by a (primary) constriction which cannot be stained by FEULGEN's reagent. In addition to that, one often sees small secondary constrictions. *Between crossed nicols* one observes, in general, no reliable indication of birefringence. But if the bodies are treated with ethyl

[1] A.E. MIRSKY and A.W. POLLISTER, J. Gen. Physiol. *30*, 117 (1946), with H. RIS, *ib.* *31*, 1 (1947).

[2] A.E. MIRSKY and H. RIS, J. Gen. Physiol. *31*, 7 (1947).

[3] A. CLAUDE and J.S. POTTER, J. Exp. Med. *77*, 345 (1943).

[4] H.H. PFEIFFER, Z. Naturforsch. *4b*, 307 (1949).

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[6] H.H. PFEIFFER, Optik (Stuttgart) *5*, 217 (1949).