byproducts was obtained by treating a solution of noradrenaline hydrochloride (0.5 g) in water (150 ml) with potassium iodate (0.8 g); stirring the reaction mixture for $1^1/_2$ h; adjusting the pH to 2 (with hydrochloric acid) and finally allowing the solution to stand at 4° overnight. Direct acetylation of 2-iodonoradrenochrome (II: R = H) with acetic anhydride and dry pyridine gave 3,5,6-triacetoxy-2-iodoindole (III: R = H) (m.p. 207–208°) (Found: C, 40.32; H, 2.92; N, 3.14; I, 30.53. $C_{14}H_{12}O_6NI$ requires C, 40.30; H, 2.89; N, 3.36; I, 30.43%) which on deiodination with zinc and acetic acid gave 3,5,6-triacetoxyindole (IV: R = H) (m. p. 123–124°) (Found: C, 57.79; H, 4.53; N, 4.81. $C_{14}H_{13}O_6N$ requires C, 57.73; H, 4.50; N, 4.82%). These two compounds had previously been described as 3,5,6-triacetoxy-N-acetyl-2-iodoindole (III: $R = COCH_3$) and 3,5,6-triacetoxy-N-acetylindole (IV: $R = COCH_3$) respectively[7], however identification was based on carbon and hydrogen analysis alone, which in these cases does not give a sufficiently clear cut differentiation between the tri- and tetra-acetyl derivatives. In neither case does the infrared spectrum show any amide carbonyl bands, however both show definite N–H stretching peaks[9] at 3450 cm^{-1} and 3470 cm^{-1} respectively. BU'LOCK and HARLEY-MASON failed to obtain any crystalline fluorescent product from the alkaline hydrolysis of the deiodinated acetyl derivative[7], however, hydrolysis of 3,5,6-triacetoxyindole with 1 N sodium hydroxide in the presence of a small quantity of sodium hydrosulphite gave, in our hands, after acidification with glacial acetic acid, a yellow-orange solid, which afforded noradrenolutin (5,6-dihydroxyindoxyl, V: R = H) in bright yellow needles (m.p.—totally decomposed by 248°) on recrystallization from hot water (containing a small quantity of sodium hydrosulphite). (Found: C, 58.43; H, 4.49; N, 8.48. $C_8H_7O_3N$ requires C, 58.18; H, 4.27; N, 8.48%). No crystalline product could be isolated when the hydrolysis was carried out in the absence of sodium hydrosulphite[7]. Noradrenolutin obtained by the method described above dissolves in water or methanol to give intensely fluorescent solutions. The ultraviolet and visible absorption spectra were measured in water (λ_{max}: 248; 284; 315; 368 mμ). The fluorescence characteristics[10] of noradrenolutin were also measured in aqueous solution and showed that maximum fluorescence apparently occurred at 490–500 mμ with excitation at 380–390 mμ.

These studies which form part of a comprehensive study of the chemistry of aminochromes are continuing and further details will be published elsewhere.

Zusammenfassung. Herstellung und einige Eigenschaften von kristallinem Noradrenolutin (5,6-Dioxyindoxyl), dem gelbgrün fluoreszierenden Oxydationsprodukt von Noradrenalin, werden beschrieben.

R. A. HEACOCK and B. D. SCOTT

Psychiatric Research Unit, University Hospital, Saskatoon (Saskatchewan, Canada), May 15, 1961.

[9] The infrared spectra were measured in chloroform solution.

[10] Measured on an Aminco-Bowman spectrophotofluorometer, through the courtesy of Dr. V. WOODFORD.

Uncoupling of Oxidative Phosphorylation in Antigen-Antibody Reactions

It is now an accepted fact that cells are damaged by antigen-antibody reactions, probably by intervention of complement.

Although the exact mechanism by which the damage is produced is so far unknown, we can infer, from indirect data and by comparison with the phenomena observed in sensitized erythrocites, that the lesion begins in the cell membrane.

It is usually accepted that blood vessel endothelia and mesenchymal cells are the site of the antigen-antibody reaction; parenchymal injury is thought to be secondary, caused by toxic substances liberated by the cells at the site of reaction. Numerous data bear out this hypothesis, but one cannot entirely reject the possibility that parenchymal cells may be site of antigen-antibody reaction, either in abnormal conditions or because the antigen, having first been modified by the cells of the reticuloendothelial system, then passes into the cells of the parenchyma.

It is not known which cellular structures take part in the antigen-antibody reaction, nor whether it takes place at the cell surface or inside the cell itself. It seems logical to suppose that the antigen penetrates into those cells which produce the antibodies, as this is the only way protein syntheses may be stimulated and directed according to the chemical structure of the antigen; it is also possible that the antigen may be adsorbed at the surface of cells which are not directly involved in antibody production. The fact that lesions in serum sickness begin as soon as antibodies appear in the blood stream (HAWN and JANEWAY[1]; GERMUTH[2]; GERMUTH, PACE, and TIPPETT[3]) while the antigen is still present, is in accordance with this hypothesis. Adsorption of the antigen at the cell surface and its subsequent binding to the antibody appears therefore to be the damaging factor of cell structures.

In this study I have considered oxidative phosphorylation, which is a most precise and sensitive index of the function and structure of mitochondria, which are concerned with some of the most important aspects of cellular life.

Investigations were carried out in three directions, i.e.: (1) Action of specific antiserum on oxidative phosphorylation in mitochondria obtained at various intervals from animals injected with corresponding antigen. (2) Influence of the antigen-antibody complex on oxidative phosphorylation after removal of the precipitate. (3) Behaviour of oxidative phosphorylation in mitochondria after antigen adsorption and subsequent treatment with corresponding serum.

Methods. Animals: Albino rats weighing on the average 100–120 g were used. The rats were fed on a standard diet and were killed by decapitation.

Isolation of mitochondria: Mitochondria were obtained by centrifugation (Servall SSI centrifuge) from 10% homogenate of liver and kidney prepared in a cold room at + 2°C with 0.25 M sucrose and 0.02 M Tris-HCl buffer pH 7.4. The mitochondrial sediment was then resuspended in 0.25 M sucrose and 0.02 M Tris buffer. The system used for the study of oxidative phosphorylation consisted of: 0.013 M Na_2HPO_4-KH_2PO_4 pH 7.4; 0.01 M Tris buffer pH 7.4; 0.005 M $MgCl_2$; 0.01 M KCl; 0.00001 M $MnCl_2$; 0.02 M ATP; 0.02 M KF; 0.02 M glucose; 0.03 M sucrose;

[1] Z. VAN HAWN and C. A. JANEWAY, J. exp. Med. *97*, 257 (1953).

[2] F. G. GERMUTH, J. exp. Med. *97*, 257 (1953).

[3] F. G. GERMUTH, M. G. PACE, and J. C. TIPPETT, J. exp. Med. *101*, 135 (1955).

0.00001 *M* cytochrome; 0.01 *M* K glutamate; 0.03 *M* yeast hexokinase (Sigma's Type II) and mitochondria corresponding to 100 mg fresh tissue.

Preparation of immune serum. Crystalline albumine prepared from horse serum (kindly provided by I. V. Toscano Sclavo) was used as antigen. Immune serum was obtained from rabbits, following injection of 350 mg albumin, divided into five subcutaneous injections which were given in the course of one month. Ten days after the last injection, the precipitin titer of the serum was determined by the zone reaction, which was positive at a maximum antigen dilution of 0.001 to 10 ml saline.

Experimental. (1) *In vivo:* 10 albino rats were each injected with 2 mg crystalline albumin diluted in 1 ml saline. They were then killed 2, 6, 12, 24 h and 2 days after injection. Mitochondria were prepared from liver and kidney. 0.20 ml anti-albumin-serum were added to them in the experimental conditions described for the study of oxidative phosphorylation. The aim of this investigation was to see whether the injected antigen was fixed by mitochondria and whether the added antibody would react with it, thereby uncoupling the P:O ratio.

(2) *In vitro:* 0.50 ml of antialbumin serum was added to 10 mg albumin dissolved in 2 ml 0.88% NaCl. The mixture was incubated for 1 h at 37°C. The resulting precipitate was then removed by high speed centrifugation (30 min at 20000 Xg Servall SSI) 0.5 ml of the supernatant was added to mitochondria suspension. The aim of this investigation was to see whether a toxic substance capable of modifying the P:O ratio was produced by the antigen-antibody reaction.

(3) *In vitro:* mitochondria obtained from 1 g rat liver and kidney were washed in 0.25 *M* sucrose containing 3% crystalline albumin. The mitochondria were suspended in a suitable medium and mixed with anti-albumin serum, with and without the addition of complement. The aim of this investigation was to see whether the antigen was adsorbed on mitochondria surfaces and whether the specific immune serum caused the uncoupling of the P:O rate by reacting with it.

The results are shown in the Tables and point out that mitochondria of animals injected at various times with horse albumin do not show any appreciable modification of oxidative phosphorylation when mixed with their respective antibody. The most plausible explanation is that the antigen has not come into contact with the antibody, either because it is not present in mitochondria or because it is not in a suitable form to react. It is very likely that cell and mitochondrial membrane permeability to protein molecules is involved in the phenomenon.

Tab. I. Oxidative phosphorylation in rat mitochondria. The animals were injected with 2 mg albumin and were killed after variyng intervals. Liver and kidney mitochondria were mixed with 0.20 ml anti-albumin serum.

No. Exp.	Mitochondria isolated from	Time after injection	P:O rate
1	liver	$^1/_2$ h	2.87
2	liver	2 h	2.70
3	liver	24 h	2.40
4	kidney	2 h	2.10
5	kidney	21 h	2.90
6	kidney	48 h	3.10

Tab. II. Oxidative phosphorylation in rat mitochondria after addition *in vitro* of 0.50 ml antigen-antibody solution after removal of precipitate.

No. Exp.	1	2	3	4	5
Mitochondria isolated from	liver	liver	liver	liver	liver
P:O rate	2.10	2.20	2.70	2.85	2.15

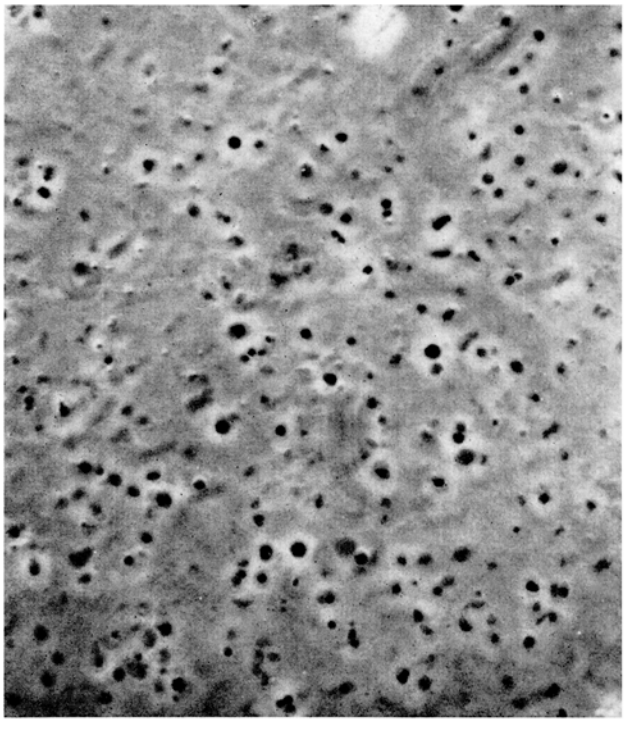

Fig. 1. Mitochondria from normal rat liver suspended in 0.25 *M* sucrose containing 3% crystalline horse serum albumin.

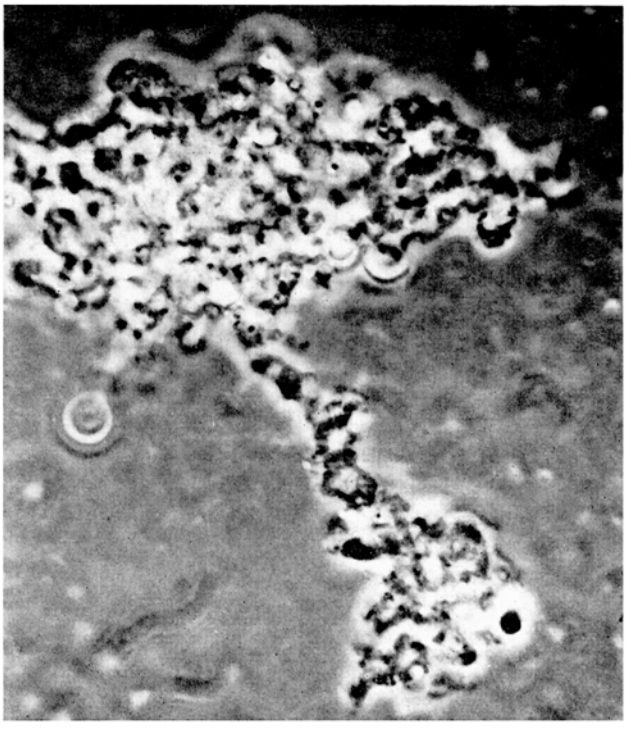

Fig. 2. The same mitochondria as in Figure 1, after addition of the specific anti-albumin serum.

The antigen-antibody complex plays no part in oxidative phosphorylation once the precipitate has been removed. On the contrary, as shown by Table III, mitochondria which have adsorbed the albumin antigen and are then mixed with their respective antibody show a clear uncoupling of phosphorylations from oxidations, which seems to be enhanced by the addition of complement.

Mitochondria clearly show a high adsorbing power towards a heterologous protein such as horse serum albumin; the antigen is fixed by them even after washing with 0.25 *M* sucrose. The mitochondrial membrane is obviously damaged by the antigen-antibody reaction, as shown by the swelling, agglutination (Fig. 1 and 2) and by uncoupling of the P:O rate.

Riassunto. Mitocondri di fegato e rene di ratto adsorbono l'albumina del siero di cavallo; facendo agire su questi in secondo tempo l'immunsiero specifico anti-albumina, i mitocondri presentano: agglutinazione, rigonfiamento e dissociazione delle fosforilazioni dalle ossidazioni. L'autore mette in rilievo che la reazione antigene-anticorpo al livello mitocondriale, ne danneggia gravemente la struttura e la funzione.

Tab. III. Oxidative phosphorylation in mitochondria after adsorption of antigen, subsequently mixed with specific antibody.

No. Exp.	Mitochondria isolated from	Additions	P:O rate
1	liver	0.2 ml immun serum	1.1
2	liver	0.2 ml immun serum	1.80
3	liver	0.2 ml immun serum	1.25
4	liver	0.2 ml immun serum	1.40
5	liver	0.2 ml immun serum + 0.1 complement	0.50
6	liver	0.2 ml immun serum + 0.1 complement	0.53
7	liver	0.2 ml immun serum + 0.1 complement	0.35
8	liver	0.2 ml normal serum	2
9	liver	0.2 ml normal serum	2.69
10	liver	0.2 ml normal serum	2.09
11	liver	0.2 ml normal serum	2.25
12	liver	0.2 ml normal serum + 0.1 complement	2.20
13	liver	0.2 ml normal serum + 0.1 complement	1.85

L. MICHELAZZI

Department of General Pathology, University of Genova (Italy), April 4, 1961.

Nachbargruppeneffekt des Pyridinringes beschleunigt alkalische Ester-Hydrolyse

Im Zusammenhang mit Untersuchungen über die Wirkungsweise von PAM (Pyridin-2-aldoxim-methojodid) war es notwendig, das reaktive Verhalten von Pyridin-analogen Cholinestern bei der alkalischen Hydrolyse kennenzulernen. Durch potentiometrische Titration bei konstantem pH[1] fanden wir, dass der Benzoesäureester I von 2-Hydroxymethyl-N-methylpyridiniumchlorid mit OH^--Ionen mehr als doppelt so schnell reagiert als der «aktivierte Ester»[2] Benzoylcholin II (vergleiche Tabelle I). Dieses Resultat schien zunächst überraschend; es wäre nämlich zu erwarten gewesen, dass sich der elektrostatische Effekt[2] beim Cholinester stärker auswirkt als bei I, weil wegen der vielseitigen Resonanzmöglichkeiten im N-Alkyl-pyridiniumsystem[3] die positive Ladung am Stickstoff teilweise intramolekular kompensiert ist. Der elektrostatische Effekt allein vermag also die gefundenen Tatsachen nicht zu deuten. Berücksichtigt man jedoch ausserdem, dass der Pyridinium-Ester, nicht aber der Cholinester zur nucleophilen Addition von Anionen befähigt ist[4], so kommt man zu einer befriedigenden Erklärung. Wir nehmen an (vergleiche Figur 2), dass zunächst die Ester-Carbonylgruppe von der positiven Ladung angezogen und in räumliche Nachbarschaft zum Stickstoff gebracht wird (III) (elektrostatischer Effekt); dadurch wird die Polarisation der C=O-Gruppe noch verstärkt und der Angriff durch das OH^--Ion erleichtert. Gleichzeitig erfolgt intramolekulare Addition des Carbonylsauerstoffs

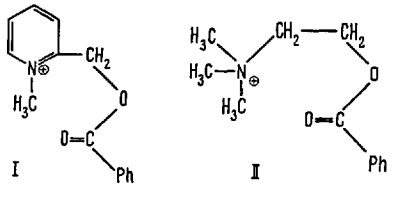

Fig. 1

Tab. I

Versuch Nr.		1	2	3	4
		$k_1 \cdot 10^2$ (min^{-1})			
Ester	I	10,74	9,47	9,81	11,60
	II	3,24	3,43	3,65	3,18

pH: 11,00 ± 0,03; Temperatur: 19,6°; c_0 = 0,025 Mol/l (Anfangskonzentration des Esters in bidest. Wasser); Ionenstärke p = 0,5 Mol/l NaCl. Aus dem Verlauf der Titrationskurven wurde nach dem zweiten (halbgraphischen) Verfahren von GUGGENHEIM[6] die Reaktionsgeschwindigkeitskonstante erster Ordnung k_1 ermittelt.

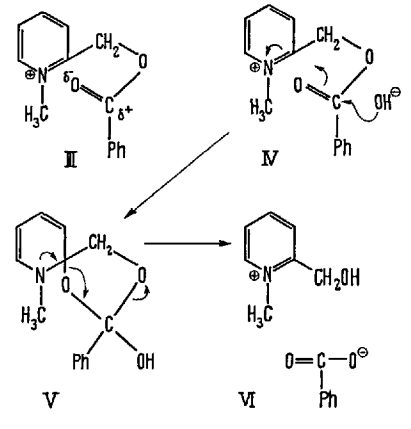

Fig. 2

[1] Zur pH-Stat-Methodik vgl. C. F. JACOBSEN, J. LÉONIS, K. LINDERSTRÖM-LANG und M. OTTESEN, Methods of Biochemical Analysis *4*, 171 (1957).

[2] E. SCHÄTZLE, M. ROTTENBERG und M. THÜRKAUF, Helv. chim. Acta *42*, 1708 (1959).

[3] F. KRÖHNKE, Angew. Chem. *65*, 605 (1953).

[4] Vgl. die ausführliche Arbeit von K. WALLENFELS und H. SCHÜLY, Liebigs Ann. *621*, 86 (1959).