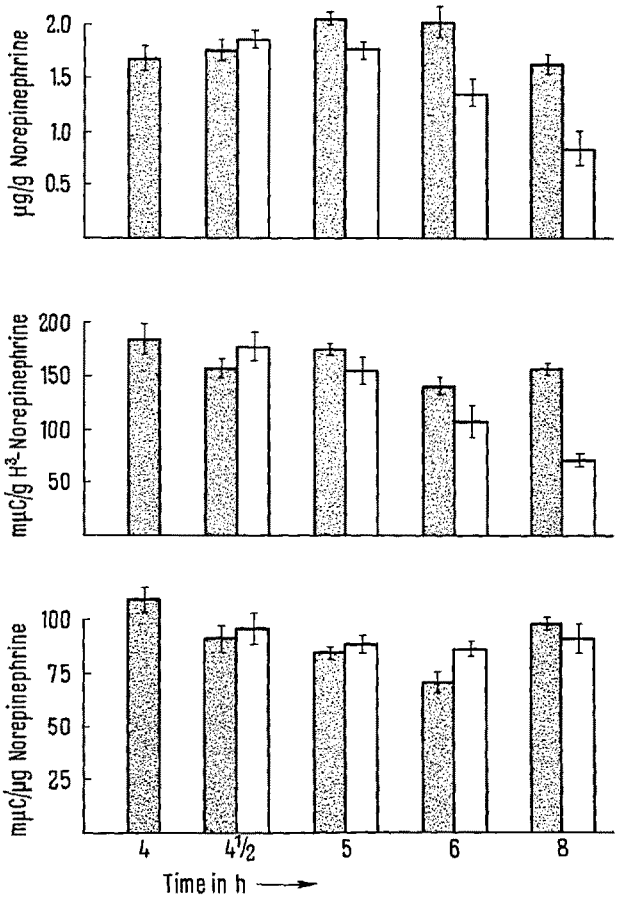


Norepinephrine content in the hearts of guinea pigs after the injection of H^3-norepinephrine and reserpine. Guinea pigs were injected intravenously with 1.4 µg of *dl*-β-H^3-norepinephrine (20 µC/µg). 4 h later the test groups received intraperitoneal γ 30 µg of reserpine per kg. The time intervals in the Figure are the periods after the H^3-norepinephrine injection when the animals were sacrificed and the norepinephrine content of the hearts determined.

Each bar represents the mean of the values for 6 animals assayed separately. The solid bars represent the control groups which received H^3-norepinephrine but no reserpine. The hatched bars represent the test groups. The standard error of the mean is indicated at the top of each bar.

were essentially constant. In the case of the 6 h groups, the difference between the specific activities fell just outside the standard error of the mean.

The presence of a single pool of norepinephrine in the heart comprised of endogenous and exogenous amines injected 4 to 8 h earlier was suggested by the following considerations: The rate of release of the tiny amount of H^3-norepinephrine as determined by the scintillation counter was identical to the rate of release of the relatively large amount of endogenous norepinephrine as determined fluorometrically. This result in view of the tremendous disparity in the amounts of the two types of norepinephrine present is best explained by the assumption that they were released from a common pool by the same mechanism.

With the exception of the 6 h interval, the specific activity, mµC/µg, of norepinephrine in each period was essentially the same for the control and reserpine treated animals. The substantially greater amounts of norepinephrine in the hearts of the control animals had the same specific activity as was found in the smaller amounts of norepinephrine remaining in the hearts of the animals treated with reserpine, after that drug had released a significant amount of the norepinephrine. This, too, indicates a common pool.

Zusammenfassung. Tritium-markiertes nor-Epinephrin, welches im Meerschweinchenherzen 4 bis 8 h nach intravenöser Injektion gebunden war, wurde mit der gleichen Geschwindigkeit wie körpereigenes Catecholamin nach Verabreichung von Reserpin freigesetzt. Es kann daher angenommen werden, dass injiziertes nor-Epinephrin sich im Herzen mit der Masse des endogenen Hormons vermischt.

G. HERTTING[7] and S. M. HESS[8]

National Institute of Mental Health and National Heart Institute, Bethesda (Maryland USA), March 5, 1962.

Present address: Pharmakologisches Institut der Universität Wien (Austria).

[8] Present address: Squibb Institute for Medical Research New Brunswick (New Jersey U.S.A.).

Agar Electrophoresis of Soluble Proteins Isolated from Cellular Fractions of CCl_4-Treated Rat Liver

It is well known that in the course of some degenerative processes of the liver, such as steatosis and cloudy swelling, severe biochemical alterations occur. Among these, the changes of mitochondrial soluble proteins have been deeply subjected to investigation[1-6].

In this note results are reported on the fractionation, by means of agar gel electrophoresis, of the soluble proteins, isolated from the nuclea and mitochondria of normal and CCl_4-treated liver cells.

Material and Methods. Male rats of Wistar strain and about 150–170 g, were treated with CCl_4, in two groups (1 ml/kg, subcutaneously, for 1 and 5 days respectively). The liver was perfused with the following solution: NaCl 0.094 *M*, phosphate buffer at pH 7.4 0.012 *M*, ethylene diaminetetra acetic acid 0.011 *M*, glucose 0.046 *M*.

Nuclear and mitochondrial fractions have been obtained by differential centrifugation, according to HOGEBOOM and SCHNEIDER[7]. After the isolation of sedimented mitochondria, the supernatant, containing soluble proteins and microsomes, has been collected and has been designated 'cytoplasmic fraction'. The 'mitochondrial fraction' includes the soluble proteins after repeated freezing and thawing of mitochondria.

[1] E. CIARANFI and G. DI SABATO, Pubbl. Staz. Zool. Nap. *28*, 269 (1956).
[2] M. U. DIANZANI, Biochem. biophys. Acta *14*, 514 (1954).
[3] M. U. DIANZANI, Biochem. J. *65*, 116 (1957).
[4] G. UGAZIO and L. CONGIU, Boll. Soc. Ital. Biol. sper. *36*, 759 (1960).
[5] G. UGAZIO, Exper. *16*, 349 (1960).
[6] L. CONGIU, G. Biochim. *9*, 123 (1960).
[7] G. H. HOGEBOOM, *Methods in Enzymology* (Academic Press Inc, New York 1955), vol. I, p. 16.

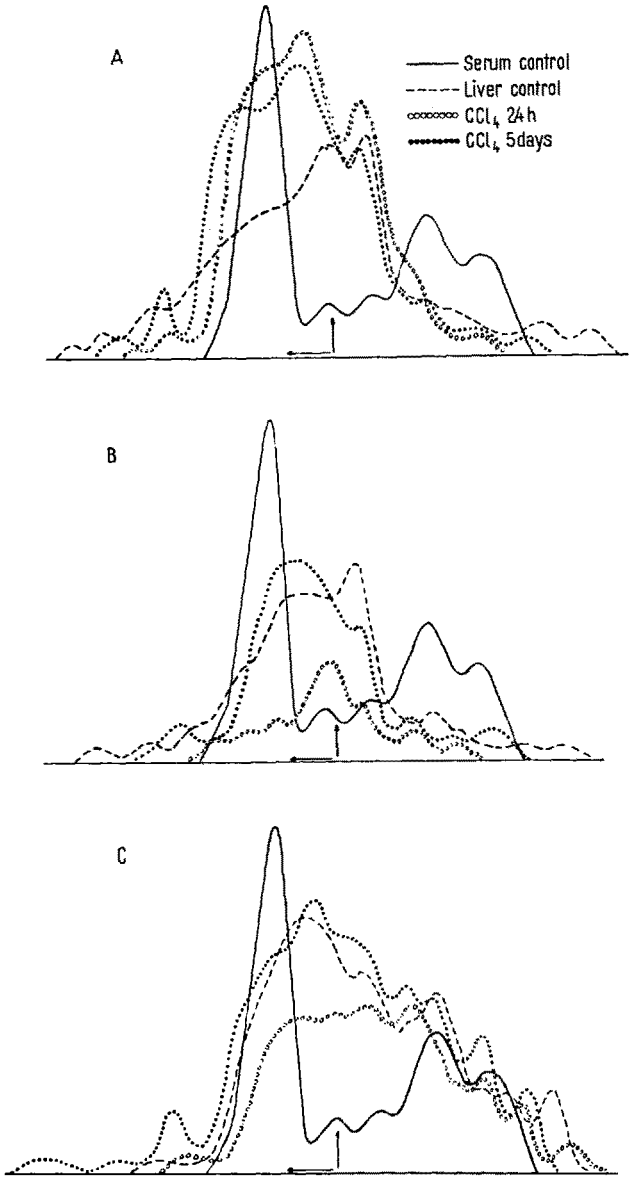


Fig. 1. Electrophoretic patterns of the soluble proteins isolated from liver cells of normal and CCl_4-treated rats. Preparation according to HOGEBOOM and SCHNEIDER[7]. Buffer: sodium barbiturate 0.065 M; HCl 0.017 M (pH 8.2, ionic strength $\mu = 0.05$). V/cm 6.4; mA/cm 2.5, for 7 h. Staining with Amidoschwarz 10 B.
A = nuclear fraction. B = mitochondrial fraction. C = cytoplasmic fraction. The vertical arrow indicates the point of deposition; the horizontal one the sense of migration.

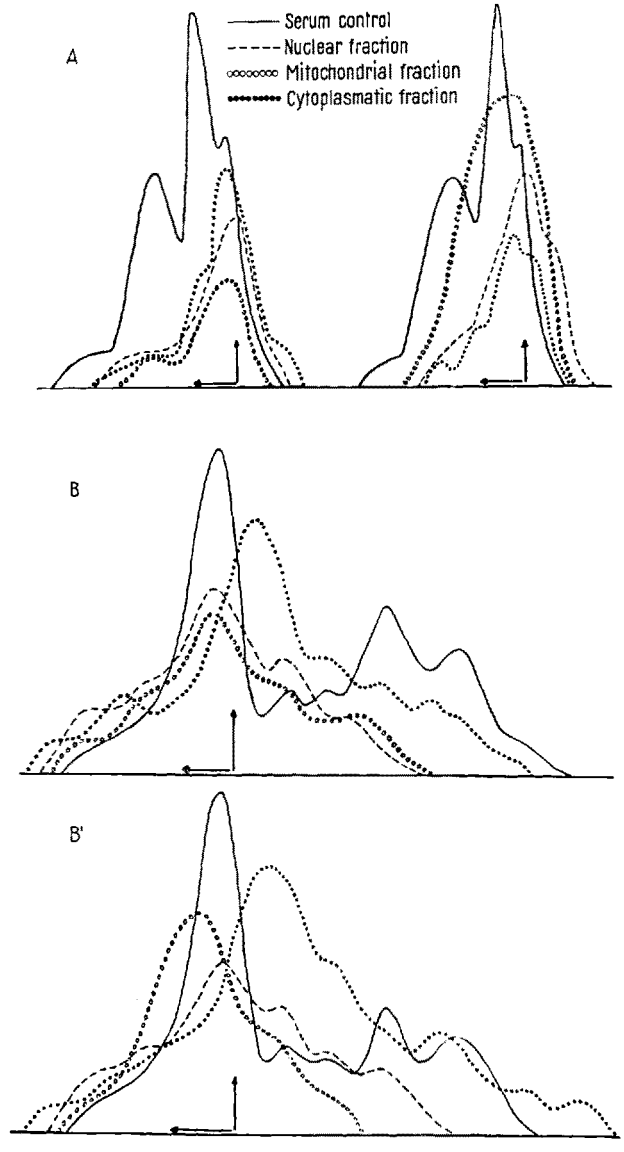


Fig. 2. Electrophoretic patterns of soluble lipo- and glycoproteins isolated from liver cells of normal and CCl_4-treated rats. Preparation according to HOGEBOOM and SCHNEIDER[7]. Buffer: sodium barbiturate 0.065 M; HCl 0.017 M (pH 8.2, ionic strength $\mu = 0.05$). V/cm 6.4; mA/cm 2.5, for 7 h.
A = staining with Sudanschwarz. At the left: normal rats; at the right: after 5 days of treatment with CCl_4. B and B' = staining with PAS reaction: normal (B) and CCl_4-treated rats (B'). The vertical arrow indicates the point of deposition; the horizontal one the sense of migration.

The electrophoretic runs were performed in agar gel at 1.5%, of 2.5 mm thickness, employing a barbiturate (0.065 M), HCl (0.017 M) buffer, at pH 8.2 and with ionic strength at 0.05 μ. About 1.5 mg of proteins (3–4 mg for the glycoproteins and lipoproteins) were run for 7 h, with 6.4 V/cm and 2.5 mA/cm.

The plates were fixed in acetic acid 2% for 12 h, dried under filter paper in the oven and stained with Amidoschwarz 10B, with Sudanschwarz and PAS reaction, according to [8] and [9].

Results. We have called A the prealbumin zone, while from B to F are included all the peaks corresponding respectively to serum albumin, α-1, α-2, β-, and γ-globulins, and G the tail. The displacement and the amounts of the different peaks, for nuclear, mitochondrial and cytoplasmic fractions, are reported in Figure 1 and in the Table. No change in cytoplasmic fraction occurred; both in the nuclear and in the mitochondrial preparation, after 24 h, the percentage of the slowest and fastest components was found deeply decreased, with a typical grouping in a zone with an intermediate mobility (the C zone, corresponding to α-1-serum-globulins).

For longer treatment with CCl_4 (5 days), while the prealbumin-like components showed a partial tendency to

[8] G. URIEL, Clin. chim. Acta *3*, 234 (1958).
[9] G. URIEL and P. GRABAR, Ann. Inst. Pasteur *90*, 427 (1956).

normal values, the most marked grouping of the proteins was observed in the B zone, corresponding to serum albumin. The number of different identifiable components was significantly decreased in the treated animals: the nuclear fraction appeared resolved into 7–8 (after 1 and 5 days respectively) components, against 9 of the controls; the mitochondrial one into 7–6 and 10 respectively (Figure 1).

In our opinion the patterns observed can be considered as an expression of protein denaturation, as firstly suggested by DAVIS, HOLLANDER, and GREENSTEIN[10].

As regards the glycoproteins, we have found significant changes for the mitochondrial fraction: after 5 days a typical grouping of components in the albumin-like zone was observed (Figure 2a).

It is of interest that no difference of lipoprotein pattern for the nuclear and cytoplasmic fractions were noted, while for the mitochondrial we observed only one β-lipoprotein-like peak, instead of the two well defined peaks corresponding to the normal mitochondrial pattern (Figure 2b).

Percentual amounts of the soluble proteins isolated from rat liver nuclear, mitochondrial and cytoplasmic fractions, in CCl_4 induced fatty change

	A	B	C	D	E	F	G
	Nuclear fraction						
Normal liver	16.8	32.2	19.9	13.7	8.5	4.5	4.4
After 24 h	1.4	37.4	32.9	19.6	6.7	2.0	—
After 5 days	11.2	52.7	24.4	5.1	5.2	1.4	—
	Mitochondrial fraction						
Normal liver	6.7	44.4	24.4	16.5	6.2	1.5	0.3
After 24 h	1.2	18.5	52.9	20.1	7.3	—	—
After 5 days	4.8	59.4	24.1	5.1	4.5	2.1	—
	Cytoplasmic fraction						
Normal liver	1.8	29.5	20.9	12.2	20.8	10.1	4.7
After 24 h	1.5	16.2	23.6	20.8	24.4	10.2	3.3
After 5 days	11.1	24.7	22.7	9.8	19.7	12.0	—

Riassunto. Gli autori hanno studiato, mediante elettroforesi su agar, il comportamento delle proteine solubili isolate dalle diverse frazioni cellulari di epatociti di ratto, in corso di steatosi da CCl_4.

A carico delle frazioni nucleare e mitocondriale è stata osservata una diminuzione dei componenti più e meno dispersi, con un raggruppamento in zone a motilità intermedia, pari a quella delle α_1-globuline sieriche o delle albumine, accanto ad una riduzione del numero dei componenti proteici chiaramente individuabili.

Analoghe modificazioni sono state rilevate a carico dei componenti glico- e lipoproteici – nella sola frazione mitocondriale.

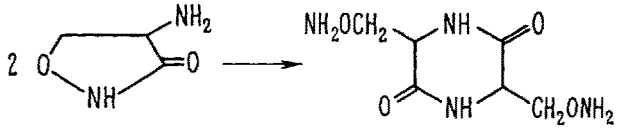
F. R. SONNINO and P. P. GAZZANIGA

Istituto di Patologia Generale della Università, Roma (Italy), January 19, 1962.

[10] B. D. DAVIS, A. HOLLANDER, and J. GREENSTEIN, J. biol. Chem. *146*, 663 (1942).

Über die tuberkulostatische Aktivität von 2,5-Bis-(aminooxymethyl)-3,6-diketopiperazin, eines Umwandlungsproduktes des Cycloserins

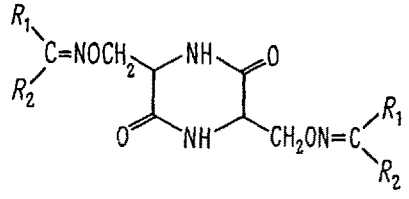
In den wässrigen[1], wässrig-alkoholischen oder essigsauren[2] Lösungen fällt 4-Amino-3-isoxazolidon (Cycloserin) bis zu einem gewissen Grad einem interessanten Dimerisierungsvorgang anheim. Das Endprodukt dieses intermolekularen Umamidierungsprozesses ist 2,5-Bis-(aminooxymethyl)-3,6-diketopiperazin (DKP)[1]. Je nach der Darstellungsweise und dem Reinigungsverfahren enthalten sämtliche Handelsprodukte des Cycloserins bestimmte Mengen von DKP.



Aus den wässrigen Lösungen lässt sich DKP leicht in Form von Dihydrochlorid isolieren. Aus diesem kann freie Base auf übliche Weise zurückgewonnen werden. Das DKP wurde weiter als Monosulfat und Dipikrat charakterisiert.

Als Träger von zwei Aminooxygruppen reagiert DKP sowie sein Dihydrochlorid oder Sulphat mit überraschender Leichtigkeit mit einer Reihe von Oxoverbindungen zu den entsprechenden 2,5-Bis-(alkyliden- bzw. aralkylidenaminooxymethyl)-3,6-diketopiperazinen.

Diese Kondensationsprodukte, von denen manche eine biologische Aktivität aufweisen, werden an anderer Stelle näher besprochen. Es ist jedoch von Interesse, dass die oben angeführten Derivate des DKP auch direkt vom



Cycloserin ausgehend durch Einwirkung verschiedener Oxoverbindungen in wasserfreiem Alkohol zugänglich gemacht werden konnten. Ihre Struktur wurde auf Grund der Ergebnisse der katalytischen Hydrierung und der Hydrogenolyse gesichert; zum Beispiel konnte 2,5-Bis-(benzylidenaminooxymethyl)-3,6-diketopiperazin auf diese Weise zum Serinanhydrid und Benzylamin gespalten werden. Die Bildung der entsprechenden Schiffchen Basen konnten wir unter den gegebenen Reaktionsbedingungen in keinem Falle beobachten.

Der Wirkungsmechanismus des Cycloserins wird von verschiedenen Autoren[3–5] unter anderem eben auf die Bildung einer Schiffschen Base von Cycloserin mit Pyridoxal bzw. Pyridoxal-5-phosphat zurückgeführt.

[1] PH. H. HIDY, E. B. HODGE, V. V. YOUNG, R. L. HARNED, G. A. BREWER, W. F. PHILLIPS, W. F. RUNGE, H. E. STAVELY, A. POHLAND, H. BOAZ und H. R. SULLIVAN, J. Amer. chem. Soc. *77*, 2345 (1955).

[2] H. BRETSCHNEIDER und W. VETTER, Mh. Chem. *90*, 779 (1959).

[3] T. AOKI, Kekkaku *32*, 605 (1957); Chem. Abstr. *52*, 7427 (1958).

[4] N. K. KOTSCHETKOW, R. M. CHOMUTOW, M. J. KARPEISSKI, E. J. BUDOWSKI und J. S. SSEVERIN, Dokl. Akad. Nauk UdSSR *126*, 1132 (1959).

[5] N. K. KOTSCHETKOW, Österr. Chem. Z. *62*, 276 (1961).