pseudo-*D*-glycal ($C_{11}H_{13}N_5O_3$; calculated: 50.18% C, 4.98% H, 26.61% N; found: 49.97% C, 5.01% H, 26.95% N).

This assignment could be confirmed by independent synthesis. Pseudo-*D*-glycal diacetate[4-6] was treated with ethereal hydrogen chloride and the non-crystalline residue which we assume contains the chloride of pseudo-*D*-glycal diacetate V was allowed to react with chloromercury benzamidopurine. The product was subjected to methanolysis as above and chromatographed on Whatman No. III paper in the system *n*-butanol-ethanol-water; elution of the appropriate spot afforded material identical with compound III, prepared by the method outlined above.

The anomalous nucleosides[7] described in this note were found to exhibit marked cancerostatic effects. In particular compound III was highly effective against experimental leucaemia in mice (strain AKR): ten doses (250 mg/kg) i.p. protected as much as 80% of the animals from leucaemia, using a small number of cells (1000) for inoculation.

A detailed account of these findings will be published in due course in Collection of Czech. Chem. Commun.

Zusammenfassung. Synthesen von 9-(2-Deoxy-1-*D*-glukosyl)-adenin und 1-(9-Adenyl)-pseudo-*D*-glukal werden beschrieben. Diese Substanzen wirken bei der Maus erheblich antileukämisch.

J. J. K. NOVÁK and F. ŠORM

Department of Organic Synthesis, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague (Czechoslovakia), November 25, 1961.

4 M. BERGMANN, Liebigs Ann. *443*, 223 (1925).
5 M. BERGMANN and W. FREUDENBERG, Ber. dtsch. chem. Ges. *64*, 158 (1931).
6 M. BERGMANN, Liebigs Ann. *434*, 79 (1923).
7 Czechoslovak patent application No. 5892-61.

The Site of Binding of Injected H^3-Norepinephrine

H^3-Norepinephrine, after injection into animals, was taken up and bound by certain tissues (heart, spleen, adrenals, etc.)[1]. Once bound, the H^3-norepinephrine slowly disappeared over a period of days. The bound material seemed protected to a large extent from metabolic alteration whereas the circulating H^3-norepinephrine was rapidly inactivated, predominantly by O-methylation[1,2].

That the site of binding of the H^3-norepinephrine is at or near the sympathetic nerve ending was indicated by the fact that the ability to bind was lost after postganglionic sympathetic denervation[3]. HERTTING and AXELROD[4] also demonstrated that the bound H^3-norepinephrine was released upon sympathetic stimulation. It remains to be determined, however, if the uptake and binding of exogenous H^3-norepinephrine is similar to the uptake and binding of endogenous norepinephrine. The findings presented in this report indicate that endogenous and bound H^3-norepinephrine 4 to 8 h after administration are released at the same rate following reserpine treatment; this suggests their presence in a common pool at this time.

Male guinea pigs weighing 200 to 220 g were divided into groups of 6 and injected in a hind leg vein with 1.4 μg of *dl*-β-H^3-norepinephrine (20 μC/μg). 4 h later, after the tissues had taken up and bound the H^3-norepinephrine, the groups were injected intraperitoneally with 30 μg of reserpine per kg. They were sacrificed 4, 4.5, 5, 6, and 8 h after the injection of H^3-norepinephrine. Control animals given only H^3-norepinephrine were sacrificed at the same intervals. The hearts were removed immediately after sacrifice, rinsed, blotted, weighed, and homogenized with a sufficient volume of 5% trichloracetic acid to yield a final volume of 10 ml. The assay for total norepinephrine was carried out according to the method of CROUT, CREVELING, and UDENFRIEND[5].

To measure the H^3-norepinephrine a 1 ml aliquot of the eluate from the alumina column containing the norepinephrine was added to 10 ml of a scintillation solution[6] and counted in a liquid scintillation counter. Correction for quenching was made by adding an internal standard of H^3-toluene to each sample.

The specific activity was calculated for each heart; the results are expressed as the mean for each group (± standard error of the mean).

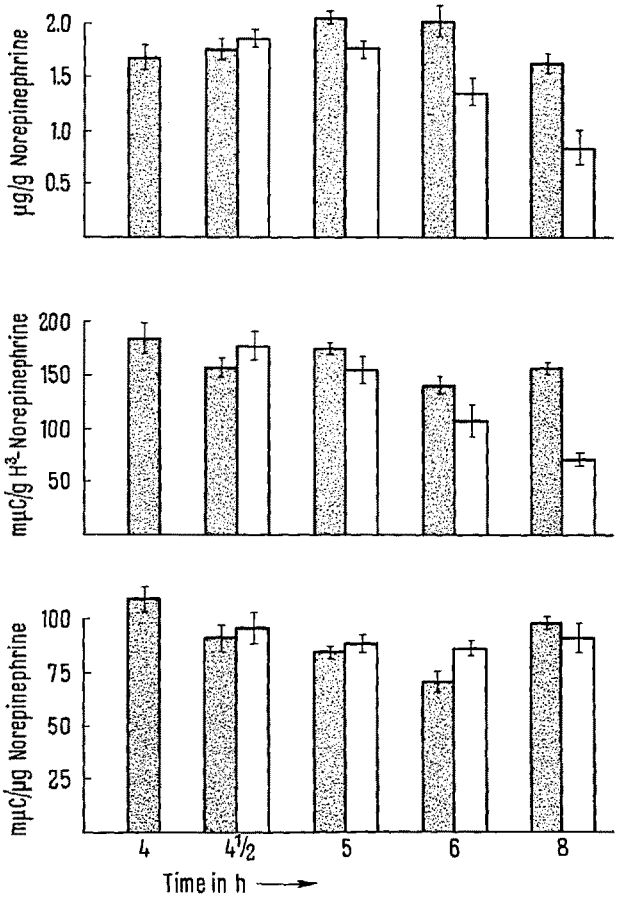
The results of this experiment have been summarized in the Figure. The total concentrations of norepinephrine found in the hearts (μg/g) at each time interval are shown in the uppermost series of bars. The concentrations of H^3-norepinephrine (mμC/g) found in the groups of hearts are shown in the second row of bars, and the specific activities for the groups appear in the third row of bars.

The specific activities presented in the third series of bars are the means of the individual specific activities in each group and are expressed as mμC/μg of norepinephrine.

The specific activity of the injected H^3-norepinephrine was sufficiently high (20 μC/μg of norepinephrine) so that the H^3-norepinephrine present in the heart was truly a trace amount which labeled the endogenous norepinephrine. The top row of the Figure reveals that the concentrations of norepinephrine in the hearts of animals treated with reserpine had dropped to almost one-half of the control values in 4 h (i.e., 8 h after the injection of H^3-norepinephrine). The concentration of labeled norepinephrine in the 4 h group treated with reserpine was found to be about half the concentration of the control group for the same period. Thus the specific activities remained unchanged. Similar changes with respect to the ratios of the concentrations of labeled and endogenous norepinephrine were found in the other groups indicating relatively constant specific activities during the entire period in which the reserpine was acting to release norepinephrine.

With the exception of the 6 h groups, the specific activities of the experimental and control groups in each period

1 L. G. WHITBY, H. WEIL-MALHERBE, and J. AXELROD, J. Pharmacol. exp. Therap. *132*, 193 (1961).
2 J. AXELROD, Science *126*, 400 (1957).
3 G. HERTTING, J. AXELROD, I. J. KOPIN, and L. G. WHITBY, Nature *189*, 66 (1961). – B. C. R. STROMBLAD and M. NICKERSON, J. Pharmacol. exp. Therap. *134*, 154 (1961).
4 G. HERTTING and J. AXELROD, Nature *192*, 172 (1961).
5 R. CROUT, C. R. CREVELING, and S. UDENFRIEND, J. Pharmacol. exp. Therap. *132*, 269 (1961).
6 G. BRAY, Anal. Biochem. *1*, 279 (1960).



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.