Aus Aceton kristallösungsmittelfreie Prismen. Smp. 167° (Zers.) $[\alpha]_D^{20} = +421°$ ($\pm 4°$) (c = 2,0 in Chloroform) $[\alpha]_D^{20} = +483°$ ($\pm 4°$) (c = 2,5 in Pyridin). Für die Analyse wurde die Substanz bei 100° im Hochvakuum getrocknet. $C_{22}H_{27}O_3N_3$ Ber. C69,3 H7,1 O12,6 N11,0%; Gef. C69,3 H7,0 O12,7 N11,2%.

Unter energischen Bedingungen, Kochen in 10prozentiger KOH, wird das Alkaloid zu Lysergsäure und Valin verseift. Unter mildern Bedingungen, durch Stehenlassen in 0,1 *N* KOH bei 20° lässt sich das neue Alkaloid zu *d*-Lysergel-L-valin hydrolysieren. Nach zweimaligem Umkristallisieren, durch Lösen in verdünntem Ammoniak und Ansäuern mit Essigsäure auf pH 5,5, war das Produkt papierchromatographisch rein und in allen seinen Eigenschaften mit einem synthetisch dargestellten Vergleichspräparat identisch. Smp. 250° (Zers.). $[\alpha]_D^{20} = +60°$ ($\pm 3°$) (c = 0,5 in 2 *N* Ammoniak). Für die Analyse wurde bei 120° im Hochvakuum getrocknet. $C_{21}H_{25}O_3N_3$ Ber. C68,6 H6,9 N11,4%; Gef. C68,6 H7,0 N11,7%. Titration mit 0,02 *N* NaOH: Mol-Gew. Ber. 367,4 Gef. 372. Durch Verestern von *d*-Lysergyl-L-valin mit Diazomethan gelangte man wieder zu der mit dem natürlichen Alkaloid identischen Verbindung.

Summary. A description is given of the isolation from ergot of *d*-Lysergyl-L-valine methyl ester, a new minor alkaloid.

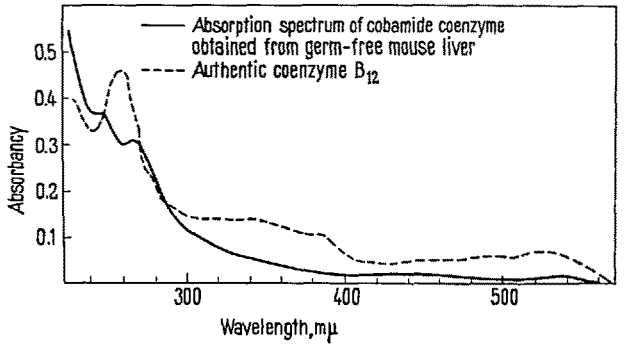
W. SCHLIENTZ, R. BRUNNER und A. HOFMANN

Pharmazeutisch-chemische Forschungslaboratorien, Sandoz AG, Basel (Schweiz), 24. Juni 1963.

Isolation of Coenzyme B_{12} from Liver of Germ-free Mice

Recent reports from BARKER's laboratory[1,2] have summarized studies leading to the discovery and characterization of several forms of cobamide coenzymes which are light-sensitive derivatives of vitamin B_{12} containing an adenosyl moiety attached to the cobalt atom in the corrin ring. The obligatory participation of cobamide coenzymes has been demonstrated in the enzyme-catalyzed isomerization of glutamate to β-methylaspartate[3], the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A[4–6], and the dismutation of 1,2-diols to the corresponding deoxyaldehyde[7]. The synthesis of B_{12} coenzymes by enzyme preparations obtained from several bacterial species has been amply demonstrated[8–11], and the presence of cobamide coenzymes in human, chicken, lamb and rabbit liver has been reported[12]. Recently the conversion of Co^{60}-labeled cyanocobalamin to its coenzyme form has been observed in the rabbit *in vivo*[13]; however, there has been no direct demonstration of the enzymatic synthesis of B_{12} coenzymes in preparations obtained from mammalian tissue *per se*. While the existence of such synthesis seemed likely, the possibility still remained that the formation of these materials might occur exclusively through the activity of intestinal flora, in view of the facile synthesis of cobamide coenzymes catalyzed by extracts of certain microorganisms. It seemed reasonable, therefore, to examine the livers of animals raised under germ-free conditions for the presence of B_{12} coenzymes.

The germ-free animals studied were drawn from a colony of white Swiss mice which has been maintained in steel isolators in the LGAR for approximately $4^1/_2$ years. The colony was obtained originally from the Lobund Institute, University of Notre Dame. The animals are fed a semi-synthetic diet[14] which is sterilized by steam at 255°F for 25 min. They are checked approximately every 2–3 weeks for bacteria, fungi, and parasites according to techniques described previously[15]. The control mice used for comparison were drawn from a colony which is maintained in an ordinary animal room, and which harbors a varied flora. This colony was derived from the germ-free colony. Breeders from the latter are removed periodically from the isolators and added to the control colony in an effort to keep the gene pools similar. These animals are fed the same sterilized diet given the germ-free.



Absorption spectrum of cobamide coenzyme isolated from germ-free mouse liver and authentic coenzyme B_{12}. The solution was adjusted to a final concentration of 0.01 *M* potassium phosphate buffer at pH 6.7. An exact comparison of the spectrum of the cobamide coenzyme obtained from the germ-free mouse liver tissue and crystalline coenzyme B_{12} cannot be made at this stage of purification[12]. The spectum shown here compares favorably with those reported by TOOHEY and BARKER[12] for the coenzymes obtained from lamb, human and rabbit liver.

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Several adult mice of both sexes from each colony were sacrificed and cobamide coenzymes were isolated from the pooled livers according to TOOHEY and BARKER[12]. Essentially the method consists of the preparation of an acetone powder followed by the extraction of cobamide coenzymes into hot 80% ethanol. The ethanol was removed and after extraction of phospholipids with ether, the solutions were passed through an anion exchange column, the cobamide coenzymes were concentrated with phenol, passed over a cation exchange resin at neutral pH and finally adsorbed and eluted from a 5 × 18 mm column of Dowex-50-Na^+H^+. The absorption spectrum of the purified coenzyme is shown in the Figure along with the spectrum of authentic coenzyme B_{12}. The amount of cobamide coenzyme in the various fractions was determined by the glutamate isomerase assay[16] (Table).

Determination of cobamide coenzyme in mouse liver. This assay was made after passing the coenzyme-containing solution through Dowex-50 at pH 7.0

Condition of mice	Control	Germ-free
Weight of pooled liver tissue in g	10	26
Cobamide coenzyme activity[a], mμmoles/kg	190	239

[a] The values are expressed in terms of the activity relative to that of crystalline coenzyme B_{12} in the glutamate isomerase assay system.

The results indicate that the livers of germ-free mice contain significant quantities of cobamide coenzyme and the values obtained compare very well with those obtained with mice harboring a flora. They also approximated values reported for human and lamb liver, which were 220 and 180 mμmoles, respectively, per kg of fresh liver at the same purification step. It was not possible to identify the specific base of the coenzyme obtained from the germ-free mice due to the limited quantity of tissue available.

The available information on the metabolic activities in tissues from germ-free animals has not indicated any profound abnormalities. Thus, one might have concluded that B_{12} coenzymes would be found in germ-free mouse liver if mammalian methylmalonyl-coenzyme A isomerase catalyzes an obligatory, or even a quantitatively significant, reaction for normal metabolic function.

Zusammenfassung. Die enzymatische Synthese von Cobamid-Coenzymen ist aus Extrakten, die von Mikroorganismen gewonnen wurden, gut bekannt, nicht aber von Säugetiergeweben. Unsere Befunde mit Cobamid-Coenzymen in der Leber von keimfreien Mäusen scheint anzuzeigen, dass die Verwandlung von Vitamin B_{12} in Coenzymform von Enzymen der Säugetiergewebe katalysiert werden kann.

R. O. BRADY and W. L. NEWTON

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Thyroxine Administration and the Uptake of Amino Acids by Liver Cell Nucleolus, Nucleus and Cytoplasm, and by Other Cell Types[1]

Several works show the important role of the thyroid hormone on growth in general (GEMMIL[2]; GROSS and PITT-RIVERS[3]; SCOW[4]) and protein synthesis in liver (SOKOLOFF and KAUFMAN[5]; STEIN and GROSS[6]). Others observed that the thyroid hormone induces an enlargement of the nucleoli in liver cells (STENRAM[7]), although the dry matter concentration of this organelle did not change (STENRAM[8]). The present work arose from an attempt to determine whether these various effects of thyroxine are related. The uptake of labelled amino acid was examined by radioautography in the various parts of the cells in liver and other organs.

Eight litter-mates, suckling rats, were used. Four of these rats received single daily intraperitoneal injections of 20 μg of *d-l*-thyroxine per 100 g of body weight, during 6 days. The other four animals served as controls. To label the newly formed proteins, 2 controls and 2 thyroxine treated rats each received one subcutaneous injection of 5 μc/g of body weight of *d-l*-tryptophane-H^3 (specific activity 658 mc/m*M*). The other 2 thyroxine injected rats and corresponding controls each received 5 μc/g of body weight of *d-l*-phenylalanine-H^3 (specific activity 126 mc/m*M*). Both amino acids were labelled in general and prepared by the Radiochemical Centre, Amersham, England. All rats were sacrificed 12 h after the amino acid injection. Organs were fixed in Bouin's fluid, embedded in paraffin, sectioned at 6 μ, and radioautographed with AR10 stripping film (Messrs. Kodak Ltd., London). Exposure time was from 22 to 105 days. Some radioautograms were stained, after processing, with basic fuchsin (BERGERON[9]) while others were left unstained.

The amount of radioactivity was estimated with the help of an ocular grid and grains were counted over 36 μ^2 of cytoplasm in each of 20 cells. In liver cells, the nuclear and nucleolar radioactivity was also assessed. All grains over each of 20 nuclei and nucleoli were counted, the respective areas estimated from the diameters, and the number of grains was also expressed per 36 μ^2 of each structure.

Figure 1 shows that, when injected into intact suckling rats, thyroxine accelerates the uptake of tryptophane-H^3 by liver cell nucleus and cytoplasm. The uptake by cytoplasm of other cell types, however, is decreased (Figure 2). Both amino acids used (tryptophane-H^3 and phenylalanine-H^3) gave similar results, which make us suppose

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