Actinomycine AC: 2 L-proline, 1 L-thréonine, 1 D-alloisoleucine, 4 molécules d'acides aminés N-methylés (à répartir entre sarcosine et N-méthylvaline).

Actinomycine AA: même composition, mais D-valine au lieu de D-alloisoleucine.

Ces propositions nous paraissent en accord avec le bilan azoté ci-dessous (Tableau II) obtenu pour l'hydrolysat de l'actinomycine AC.

TABLEAU II

N total de l'hydrolysat	9.9%	théorique 10 *N*	10.3
N de la thréonine (dosage microbiol.)	1.0	1 *N*	1.03
N de la D-alloisoleucine (dosage microbiol.)	0.9	1 *N*	1.03
N de NH_2 (VAN SLYKE)	2.0	2 *N*	2.1
N de NH_3 (distillation)	1.5	2 *N*	2.1
N de la proline (dosage microbiol.)	2.0	2 *N*	2.1
N de $N-CH_3$	4.4	4 *N*	4.1

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PREPARATION AND PROPERTIES OF A COBALAMIN PROTEIN

by

HARRY G. WIJMENGA, KENNETH W. THOMPSON

Laboratories of N.V. Organon, Oss (Netherlands) and Organon Inc., Orange, N.J. (U.S.A.)

and

KURT G. STERN AND DANIEL J. O'CONNELL

Polytechnic Institute of Brooklyn, N.Y. (U.S.A.)

Employing extracts of hog gastric mucosa from the pylorus region and crystalline cyanocobalamin (vitamin B_{12}) as starting materials, a conjugated protein has been prepared in highly purified form. Electrophoretic analysis of the starting material by the moving boundary method revealed three principal protein components which have been separated by combining various techniques.

From a partially purified preparation obtained by fractional ethanol precipitation at low temperature and moderate ionic strength, two different paths to further purification were followed and the final products thus obtained were compared. The isolation of Fraction *A* * involved electro-

* Fraction *A* was preparation VII-c-2-B-α prepared by two of us (K.G.S., and D.J.O'C.).

phoretic separation by the moving boundary method at pH 7, and repeated ethanol fractionations at different pH values. The second preparation, Fraction B*, was obtained by ammonium sulphate fractionation followed by zone electrophoresis in a cellulose powder supporting medium.

In this way the protein of intermediate electrophoretic mobility was obtained in a high state of purity. This protein has a striking pink colour which is due to the presence of cobalamin in the molecule. The content of vitamin B_{12}, as determined by microbiological employing *L. Leichmannii*** and an *E. coli* mutant**, was 9.5 μg per mg of Fraction A and 12.3 μg per mg of Fraction B (both based on the dry weight of the preparation). All of the vitamin B_{12} was found in the "bound" form, so far as could be ascertained from these tests. Further, upon repeated dialysis the cobalamin moiety did not pass through a cellophane membrane. When the vitamin B_{12} content was calculated from the light absorption at 550 mμ, employing the extinction coefficient of free cyanocobalamin for the calculations, the following values were found: Fraction A: 10.4 μg per mg; Fraction B: 12.8 μg per mg.

The absorption spectra of these chromoprotein preparations exhibit several well-defined maxima in the ultraviolet and visible regions of the spectrum, nearly coinciding with those of vitamin B_{12}. The maximum at 278–279 mμ is much higher than in cyanocobalamin because of the presence of protein, whereas one of the peaks is found at 363 mμ instead of at 361 mμ as in the case of free cyanocobalamin. The absorption spectra of Fraction B, of free cyanocobalamin and of a partially purified preparation (I.F. 762) are reproduced in Fig. 1.

The analytical electrophoresis experiments on Fraction A, due to the small quantity available, were carried out in a micro-electrophoresis cell. While only one boundary was observed there was appreciable boundary spreading indicating a certain degree of heterogeneity. Fraction B, which was available in somewhat larger quantity, could be examined in a standard cell. There was an indication of the presence of an additional minor component of higher mobility in this preparation. All determinations were made in phosphate buffer at pH 7.7 and ionic strength 0.1.

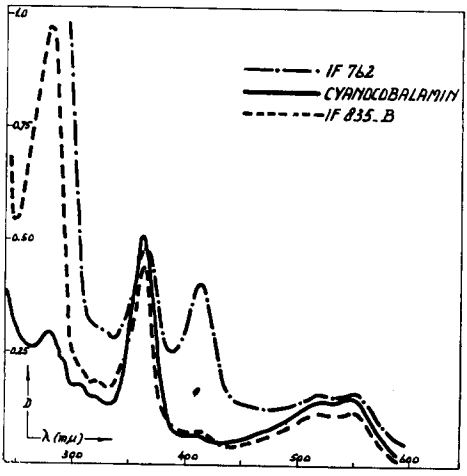

Fig. 1. Absorption spectrum of the most purified cobalamin protein preparation I.F. 835B, as compared with the spectra of a still clinically active cobalamin protein concentrate I.F. 762 and cyanocobalamin.

The mobilities of the boundary of the pink protein together with the results of diffusion experiments (Klett apparatus) and of ultracentrifugal examinations (Spinco ultracentrifuge) are given in Table I. This table also shows the protein concentrations and the molecular weight values calculated using the Svedberg equation, assuming a partial specific volume of 0.75 for the protein.

TABLE I

PHYSICAL-CHEMICAL PROPORTIES OF PURIFIED COBALAMIN PROTEIN PREPARATIONS

(solvent: phosphate buffer, pH 7.7, μ = 0.1)

Fraction	*A*	*B*
Electrophoretic mobility ($u \cdot 10^5$ cm^2/sec/v)	—2.0 (asc.)	—3.0 (asc.) —2.3 (desc.)
Protein concentration (%)	0.43	0.48
Sedimentation constant ($S_{20} \cdot 10^{13}$ cm/sec/dynes)	4.75	4.5
Diffusion constant ($D_{20} \cdot 10^7$ cm^2/sec)	3.6	4.3
Molecular weight (M_s calculated from S and D)	128,000	100,000

* Fraction B was preparation I.F. 835 B prepared by H.G.W. with collaboration of B. HURENKAMP, P. A. M. SWEEP AND J. W. DE VRIES.

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From the data thus far obtained it may be concluded that the pink protein possesses a molecular weight of the order of 100,000; the numerical differences between the values found for Fractions *A* and *B* are not considered to be significant. It might be mentioned that the sedimentation constant of Fraction *B*, when examined at 0.285% concentration, was found to be 4.6 *S*, indicating a small or negligible concentration dependence.

As judged by all available criteria, the pink protein represents a well-defined chemical compound; *viz.*, a cobalamin protein, rather than an adsorption complex. The vitamin B_{12} content is compatible with the assumption that each molecule of the conjugated protein contains one cobalamin residue. The protein moiety appears to be identical with the so-called "vitamin B_{12}-binding factor" present in hog gastric mucosa, and probably also in human gastric juice. Colour reactions performed on paper strip electrophoresis diagrams of Fraction *B* as well as the presence of hexosamine among the hydrolysis products of Fraction *A*, lend support to the assumption that this material is a mucoprotein.

From the absorption spectrum one may conclude that the cyano-group most probably is still present in the molecule. This would indicate that the cobalamin protein described herein is a substance that as such probably does not occur in nature under physiological conditions (cobalamin in normal food being linked to polypeptides or proteins rather than to a cyano-group).

The clinical activity in pernicious anemia patients in relapse upon daily oral administration, was followed at various stages throughout the purification. Partially purified preparations from which Fractions *A* and *B* were obtained showed such clinical activity; *Fraction I.F. 762*, the absorption spectrum of which is given in Fig. 1, and which has a vitamin B_{12} content of 3.7 μg per mg as determined with *L. Leichmannii*, provided a very good clinical response in two patients given doses of 2.0 and 3.0 mg per day respectively.

Fractions *A* and *B*, however, failed to show any clinical effect after the daily oral administration of amounts corresponding to 5 μg of bound vitamin B_{12}.

Although it would thus appear that the cobalamin protein herein described does not, *by itself*, exhibit hematopoietic activity, the question whether it represents a *component* of Castle's "Intrinsic Factor" is at present under investigation.

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EVIDENCE FOR A NEW INTERMEDIATE IN THE PHOSPHORYLATION COUPLED TO α-KETOGLUTARATE OXIDATION*

by

D. R. SANADI, DAVID M. GIBSON,
PADMASINI AYENGAR AND LUDOVIC OUELLET
Institute for Enzyme Research, University of Wisconsin, Madison, Wis. (U.S.A.)

The oxidation of KG** to succinyl CoA (reaction 1)[1] has been linked to the phosphorylation of ADP (reaction 2)[2,3]. The phosphorylation system has now been resolved into two separate enzymes,

$$KG + DPN^+ + CoA \rightarrow \text{Succinyl CoA} + DPNH + H^+ + CO_2 \quad (1)$$

$$\text{Succinyl CoA} + ADP + P_i \rightleftarrows \text{Succinate} + CoA + ATP \quad (2)$$

designated A and B, which catalyze consecutive reactions and involve a new intermediate. Enzyme preparation A is prepared from an acetone powder extract of beef heart mitochondria or of washed pig kidney residue by fractionation with ammonium sulfate and ethanol. Preparation B is obtained from an acetone powder extract of beef heart mitochondria. The activity of the individual and combined preparations in two different assays is shown in Table I.

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** The following abbreviations will be used: KG for α-Ketoglutarate, CoA or CoA–SH for coenzyme A, ADP for adenosine diphosphate, ATP for adenosine triphosphate, P_i for orthophosphate, DPN for diphosphopyridine nucleotide, THAM for trishydroxymethyl aminomethane, and –SH for sulfhydryl.