Comments and Communications

Vitamin B_{12}, A Cobalt Complex

Vitamin B_{12} was described by the undersigned (*Science*, April 16, p. 396) as a crystalline red compound which is highly active for producing hematological responses in patients with pernicious and other anemias (R. West. *Science*, April 16, p. 398; T. D. Spies, *et al. S. med. J.*, 1948, **41**, 522, 523) and for the growth of *Lactobacillus lactis* (E. L. Rickes, *et al.* and M. S. Shorb. *Science*, April 16, pp. 396, 397), chicks (W. H. Ott, E. L. Rickes, and T. R. Wood. *J. biol Chem.*, 1948, **174**, 1047), and rats (G. A. Emerson, M. E. Zanetti, and T. R. Wood. To be published.) Independent isolation of an antipernicious anemia factor, presumably identical with B_{12}, has also been reported (E. L. Smith. *Nature, Lond.*, 1948, **161**, 638).

Information on the chemical nature of B_{12} is of primary interest. The following observations on B_{12} have been made which appear useful for other chemical and biological studies.

Emission spectrographic analysis[1] of B_{12} has shown the presence of cobalt.[2] Vitamin B_{12} appears to be a cobalt coordination complex which, having 6 groups about the cobalt atom, could involve one or more organic moieties. The red color of B_{12} appears to be at least in part associated with its cobalt-complex character.

The presence of cobalt in vitamin B_{12} reflects significantly upon many biological studies which have shown that cobalt is an essential trace element in nutrition, and perhaps upon suggestions concerning cobalt as a trace contaminant in iron therapy of anemias (E. J. Underwood. *Proc. Soc. exp. Biol. Med.*, 1937, **36**, 296). The nutritional significance of cobalt must be re-evaluated as the biological function of B_{12} is developed.

Cobaltous ion (1 μg/ml) was without activity for *L. lactis* as contrasted with the high potency of B_{12} (0.000013 μg/ml, half-maximal growth).

Randolph West has tested cobalt ion in two cases of pernicious anemia with negative results.[3] The average adult daily dietary intake of cobalt has been estimated at 100 μg (B. Ahmad and E. V. McCollum. *Amer. J. Hyg.*, 1939, **29A**, 24).

Spectrographic examination of B_{12} also showed the presence of phosphorus. Although nitrogen was found to be present, tests for sulfur were negative.

Microbiological assay of an aqueous solution of B_{12} (74 μg/0.5 ml) showed that autoclaving for 15 min at 121° C did not change the activity within the experimental error of $11.4 \times 10^6 \pm 0.6 \times 10^6$ u/mg.

Vitamin B_{12} in 0.015 *N* sodium hydroxide solution (0.2 μg/ml) was inactivated (microbiological assay) at room temperature as follows: 20% (0.67 hr), 45% (6 hrs), 90% (23 hrs), 95% (95 hrs); it was inactivated in 0.01 *N* hydrochloric acid solution (10 μg/ml) as follows: 18% (3 hrs), 75% (23 hrs), 89% (95 hrs).

The cobalt-complex nature of vitamin B_{12} is an outstanding property.

EDWARD L. RICKES, NORMAN G. BRINK, FRANK R. KONIUSZY, THOMAS R. WOOD, and KARL FOLKERS

Research Laboratories,
Merck & Co., Inc., Rahway, New Jersey

A Laboratory Test for the Virus of Poliomyelitis

The need for a reliable test for the presence of the virus of poliomyelitis other than expensive monkey inoculations requires no emphasis.

Preliminary experiments indicate that such a test might be satisfactorily obtained through an interference phenomenon resulting in the protection of mice inoculated with material suspected of containing the virus of poliomyelitis against a subsequent inoculation with the Lansing strain of virus.

Virus is extracted and concentrated from the supposedly infected material in the usual manner (P. Lépine. *C. R. Soc. Biol., Paris*, 1939, **131**, 573). Five mice, 4–6 weeks of age, are injected intracerebrally with the extracted materials. Two days later the mice are given another intracerebral injection with active Lansing mouse-adapted virus. Five control mice are similarly treated, each receiving about 50 LD_{50}.

The answer is drawn from the results observed on the 10th–11th day, when 80% (4 out of 5), at least, of the controls should be paralyzed or dead, and at least 3 out of the 5 mice inoculated with the suspected material should show a significant protection. It should be mentioned that this significant difference between the two groups of animals may be the result either of permanent protection or of merely delayed onset of symptoms in the protected group.

Our experiments have included (a) cord material from monkeys inoculated with one standard laboratory strain and (b) fecal material collected from patients during the summer of 1946 and preserved in the frozen stage since that time.

Extracts from normal human feces, or cord material inactivated after heating at 80° C for 15 min, showed no protective action.

Attempts to obtain interference protection by other means of inoculation (oral, nasal, intraperitoneal, intracutaneous, etc.) or with unconcentrated material, or through a longer interval between inoculations, have so far failed.

[1] We wish to thank Dr. Charles Rosenblum and Luise Anderson for this determination.

[2] Private communication from Dr. H. M. Walker, of Glaxo Laboratories, Ltd., discloses that Glaxo's factor also contains cobalt. E. L. Smith and L. F. J. Parker. *Proc. biochem. Soc.*, in press; E. L. Smith. *Nature, Lond.*, in press.

[3] Personal communication; levels of 500 and 150 μg of cobaltous ion administered subcutaneously as acetate and chloride, respectively.

It is hoped that these results will be found reproducible with strains of the virus of poliomyelitis other than the ones that have been tested.

PIERRE R. LÉPINE
Virus Division, Institute Pasteur, Paris

Facts and Theories About Sympathins

Marrazzi and Marrazzi (*Science*, November 28, 1947, pp. 520–521) have introduced in the discussion on sympathins a regrettable confusion between facts and theory.

The *fact* is that Cannon and Rosenblueth (*Amer. J. Physiol.*, 1933, **104**, 557) observed more excitatory than inhibitory effects as compared with adrenaline during humoral transmission of certain sympathetic nerve stimulations. They never *demonstrated* that there was a purely excitatory and a purely inhibitory sympathin; and they have never been able to obtain more inhibitory than excitatory effects!

The elaborate theory built around this fact was not convincing, although it was accepted by a majority of Anglo-Saxon physiologists. We pointed out as early as 1933 that it was possible to give different interpretations of the phenomenon observed by Cannon and Rosenblueth, without postulating a combination of adrenaline with a hypothetical compound E or I in the cell. We suggested the following possibility: sympathin I = adrenaline; sympathin E = nor-adrenaline.

It is now evident from the work of von Euler (*Science*, April 23, p. 422), and that of many others mentioned by him, that one finds in suitable extracts of mammalian nerves and tissues a powerful amine which mimics more closely the actions of nor-adrenaline than those of adrenaline. We have confirmed von Euler's observations (*Arch, int. Physiol.*, 1947, 55, 73) with the important addition, however, that in certain tissues (human coronary arteries and nerves, for instance) the substance extracted has the properties of adrenaline and not of nor-adrenaline (or arterenol or dimethylated adrenaline) (Fig. 1). It must not be forgotten that the power of the adrenal medulla of the vertebrates to synthetize *l*-adrenaline is not unique. The parotid gland of tropical toads is rich in *l*-adrenaline (and recent observations from this laboratory show that it may contain other phenolic amines); certain cells in the abdominal ganglion of annelids synthetize an amine which has not been isolated in chemically pure state, but possesses in the utmost detail the properties of adrenaline (J. F. Gaskell. *J. gen. Physiol.*, 1919, 2, 73; Z. M. Bacq. *Biol. Rev.*, 1947, 22, 73). Von Euler has confirmed the conclusion of Leowi (*Arch. ges. Physiol.*, 1936, **237**, 504) that the sympathomimetic substance extracted from the frog's heart is adrenaline, the methylated amine.

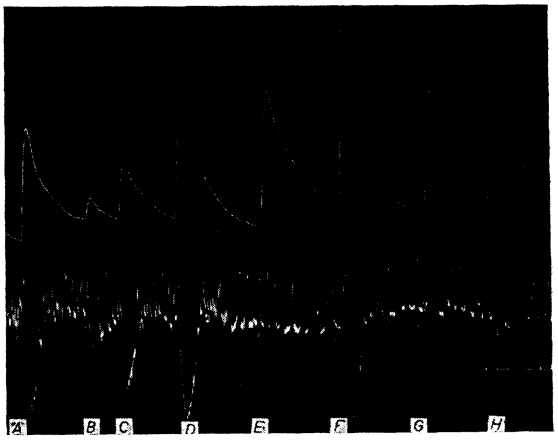

FIG. 1. Nonpregnant cat, Dial, curare. *Above*: nictitating membrane denervated 12 days previously. *Below*: uterus. Time in minutes. A, purified extract of 0.23 gm of human coronary nerves and arteries; B, *l*-adrenaline, 0.25 μg; C, *l*-adrenaline, 0.5 μg; D, *l*-adrenaline, 1 μg; E, *dl*-nor-adrenaline, 1 μg; F, G, H, purified extract of 0.75, 0.19, and 0.075 gm of horse spleen, respectively. This shows that the substance in the spleen acts very much like nor-adrenaline, but that the purified extract from human coronaries acts like adrenaline.

It seems to us that the best way to give a reasonable interpretation of these facts is to accept the idea that many tissues synthetize aminated derivatives of catechol, that the synthesis of adrenaline goes through arterenol—in other words, that the methylation of the nitrogen is the last step in this synthesis, according to Blaschko (*J. Physiol.*, 1942, **101**, 337), and that this methylation does not occur in certain tissues.

The facts (not the theory) point to the existence of two highly active sympathomimetic substances: nor-adrenaline and adrenaline. Thus, von Euler's suggestion that our nomenclature be changed from sympathin E and sympathin I to sympathin N and sympathin A is logical; it would avoid further confusion until our knowledge of the relation between adrenaline and nor-adrenaline has sufficiently improved to remove all doubt.

Z. M. BACQ
Laboratoire de Pathologie générale,
University of Liège, Belgium