Future experiments are being designed on mammals and on man to estimate whether the brain extract inhibits the LSD-25 reaction as it does in the fish and whether it will affect the course of clinical psychoses. In view of the small amount of material obtained from beef brain, our present methodology is being scrutinized in an effort to obtain more of the inhibitory substances from beef brain or from other tissues and other animals. Whether the LSD-25 blocking substance is similar to Florey's synaptic inhibitor remains to be determined.

H. A. ABRAMSON, B. SKLAROFSKY,
M. O. BARON, H. H. GETTNER
Biological Laboratory, Cold Spring Harbor, New York, and *Research Division, State Hospital, Central Islip, New York*

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Properties of Vitamin B_{12}-like Material from Crithidia fasciculata

Several naturally occurring pseudovitamins of the B_{12} series have been recognized (*1*). Others have been prepared by chemical degradation of cyanocobalamin (*2*) and by directed biosyntheses with bacterial mutants (*3*). The pseudovitamins B_{12} differ from cyanocobalamin in that selected purines and benzimidazoles replace 5,6-dimethylbenzimidazole, the naturally occurring nitrogenous base of cyanocobalamin.

Although vitamin B_{12} (and vitamin B_{12b}) appears to be the sole naturally occurring form that is metabolically active, recent data (*4*) obtained in our laboratory on the effect of certain biosynthetically prepared pseudovitamins B_{12} on the growth and metabolism of parasitic protozoa suggested that in certain of these organisms there exists a functional equivalent to vitamin B_{12}, which is distinct from cyanocobalamin. This report (*5*) describes properties of a naturally occurring vitamin B_{12}-active material found in the hemoflagellate, *Crithidia fasciculata.*

Extracts of *Crithidia* containing the vitamin B_{12}-active material were prepared by mild acid hydrolysis (*6*) of washed cell concentrates. The liberated material, after removal of protein, replaces vitamin B_{12} in the growth of *Escherichia coli* 113–3 (*7*), of the soil microbacterium referred to as "Lochhead 38" (*8*), and of *Euglena gracilis* (*9*). Although these organisms have been used at various times for assay of vitamin B_{12}, they do not respond specifically to this factor. In addition to responding to vitamin B_{12}, the *Escherichia coli* mutant responds to pseudovitamins of the B_{12} series, as well as to various products of hydrolysis of nucleic acids, and to methionine (*8*).

The "Lochhead 38" organism in our hands responds also to various pseudovitamins B_{12} as well as to factor B [the B_{12} molecule minus the 5,6-dimethyl-1-(α-β-ribofuranosyl) benzimidazole-3′-phosphate moiety (*10*)] but not to methionine or nucleic acid fragments. In addition to responding to cyanocobalamin, *Euglena gracilis* responds to various pseudovitamins B_{12} (*8*).

The active principle in the *Crithidia* extracts does not support growth of the chrysomonad protozoan, *Ochromonas malhamensis;* this organism has been repeatedly shown to be specific for cyanocobalamin, or materials, such as factor III (*11*), which are clinically similar to cyanocobalamin in effectiveness as antipernicious anemia factors (*8*). The vitamin B_{12}-functioning material in *Crithidia* thus behaves on microbiological assay as a typical pseudovitamin B_{12}.

The vitamin B_{12}-functioning material has been separated by paper chromatography, and the activity has been made visible by bioautography of the chromatograms on agar plates seeded with *Escherichia coli* 113-3. Figure 1 shows a typical separation obtained in a solvent system composed of water, ammonium hydroxide, and *n*-butanol (50/1/100) at *p*H 10.7. In this system, vitamin B_{12} and factor B undergo slight degradation, releasing unidentified fragments that support the growth of the *E. coli* mutant. The *Crithidia* vitamin B_{12}-functioning material is also labile in this solvent system. Chromatograms developed for various times show a continuing decrease in recovery of the *Crithidia* material that was originally applied to the paper: after development for 24 hours, essentially no activity is recovered. However, destruction of the factor, unlike the alkaline degradation of cyanocobalamin and factor B, does not release microbiologically active fragments.

The *Crithidia* material also differs from vitamin B_{12} in its lability to acid. Chromatography in a *sec*-butanol and acetic acid solvent system at *p*H 3.5 for 24 hours, which has no adverse effect on cyanocobalamin or on factor B, completely destroys activity of the vitamin B_{12}-functioning material from the hemoflagellate.

All the vitamin B_{12}-like activity produced by *Crithidia* is contained within the cell. Concentrates of the medium in which the organisms are cultured ("the final synthetic" medium described by Cowperthwaite *et al.,* *12*) are lacking in vitamin B_{12} activity for *Euglena* and "Lochhead 38" as well as for *Ochromonas* (*9, 13*).

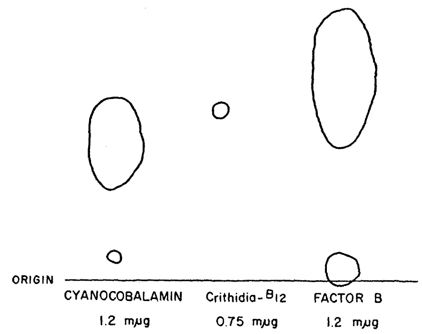

Fig. 1. Descending chromatogram of *Crithidia* vitamin B_{12}, with electrophoretically pure cyanocobalamin and factor B as standards; solvent system: *n*-butanol, ammonium hydroxide and water (100/1/50). Activity of the *Crithidia* material was assayed, before application, with *Euglena.* Development was for 18 hours at 25°C. Since the solvent runs off the paper during the extended development, R_F values cannot be calculated.

The marked acid lability of *Crithidia*-vitamin B_{12} and the alkaline degradation without release of microbiologically active fragments like those obtained with factor B and cyanocobalamin (Fig. 1) indicate that this material does not contain factor B groups, which are common to all the known vitamins B_{12}. These observations suggest that there exists in nature an entire series of new vitamin B_{12}-functioning materials present in organisms which lack the specific vitamin B_{12}, cyanocobalamin. Preliminary results in our laboratory with materials separated from various protozoans, both free-living and parasitic, attest to this generalization.

GERALD R. SEAMAN
FRED SANDERS
Carter Physiology Laboratory, University of Texas Medical Branch, Galveston

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21 November 1956

Fallout and the Strontium-90 Hazard

In a recent paper (*1*) Andrews discussed the hazard from Sr^{90} where the total fission products from a nominal atomic bomb have fallen on one small area. His calculation relating to food assumes uniform dispersal of the Sr^{90} over an area of 2 square miles, and his calculation dealing with water is based on complete mixing in Lake Mead (volume, 600×10^9 cubic feet). He estimates that, to accumulate the maximum permissible body burden of Sr^{90}, a man would have to consume the fission products deposited on 4 square feet of food, or drink 50,000 cubic feet of the Lake Mead water. The latter figure has recently been cited by another author (*2*).

Andrews' conclusion that there is a negligible Sr^{90} hazard might be correct, but both of the calculations on which it is based are in error by two orders of magnitude. The maximum permissible body burden quoted from Handbook 52 (*3*) should be 1.0 microcurie (0.005 microgram) of Sr^{90}, not 1.0 microgram as he states. Andrews' estimates are therefore low by a factor of 200, and the corrected figures for human consumption are 3 square inches of food and 250 cubic feet of water.

I. L. Ophel
Atomic Energy of Canada Limited, Chalk River, Ontario

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9 November 1956

Root-Nodule Bacteria of Prosopis stephaniana

The most dominant of the leguminous plants that grow wild in Iraq is *shok* or *kharnub, Prosopis stephaniana* (Willd.) Spreng. This plant is found in desert, in open fields, along irrigation ditches, on river banks, in orchards, and in the foothills of the Iraqi mountains. It is a perennial plant with long roots. Some of the roots grow deeper than 2 m, and they branch in all directions. Some of the branches are more than 5 m long. The top shoot sheds its leaves in December, and new leaves and branches are formed in May; the plant blooms in late June and the fruits are green in color in late July, turning reddish-brown in late August.

Prosopis stephaniana seems to be a very ancient native of Mesopotamia. The old records of the Sumerians (3600–3000 B.C.) mentioned this plant and called it *eri-til-la,* meaning "the plant of the city of life." The Akkadians (3000–2300 B.C.) called it *kharubu,* which is very similar to the Arabic name *kharub* or *kharnub* (*1*). It is likely that the plant was in Mesopotamia earlier than is indicated by the written records so far discovered.

Winsherst (*2*) mentioned *Prosopis* and considered it to be an indicator of a good soil. He suggested the presence of nodulous bacteria, but he was unable to find nodules on the roots. I was able to grow *Prosopis* from seeds (*3*), and seedlings grown under greenhouse conditions had nodules when they were examined 3 months after planting. Microscopic examination of the nodules showed the presence of *Rhizobium* bacteria. A search was made to find young roots which might have nodules in the field. One-year old roots were found to have nodules which are reddish in color. Old roots were also found to have nodules, but they were not as conspicuous as those on the young roots. The bacterium found was motile and rod-shaped.

Rhizobium species from *Prosopis* are not mentioned in Bergey's *Manual* (*4*), and this could be a new species that has not been described before; its host is *Prosopis stephaniana.* There is another leguminous plant that is usually associated with *Prosopis*—camel thorn, *Alhagi maurorum* Medic., but the bacteria isolated from the nodules of *Alhagi* are different from those isolated from *Prosopis.* Further study is needed for the determination of these *Rhizobium* species.

Preliminary tests showed that *Prosopis* nodules contain large amounts of nitrates (*5*), the presence of which is attributed to fixation of the atmospheric nitrogen by bacteria. Large amounts of nitrates are being added every year to the soils of the Tigris and Euphrates valley through direct derivation from nodules and from the leaves that are shed every winter. The addition of nitrates to the soil increases the fertility of the land. The land of Mesopotamia, which has been under cultivation for more than 5000 years, is still fertile because of the constant supply of nitrogen provided by *Prosopis* plants. Winsherst in 1920 even suggested that *Prosopis* should be cultivated in lands where it does not grow in order to increase the fertility of the soil.

The Iraqi farmers have always used the fallow system. They do not use chemical fertilizers or the crop-rotation system to enrich their lands. In the fallow system, they cultivate half their land for 1 year and the other half the second year. *Prosopis* grows on the fallow land and adds to the fertility of the soil.

A. K. Khudairi
Department of Botany, College of Arts and Sciences, Baghdad, Iraq

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14 September 1956

Ultrasonic and Electron Microscope Study of Onion Epidermal Wall

The wall structure of the cortical cells of the root of the onion, *Allium cepa,* as observed under light and electron microscopes, has recently been described in detail (*1*). In this report, certain results of similar studies on the epidermis of the onion leaf are summarized. The structure of the onion leaf has been described by numerous anatomists, including Hayward (*2*). The gray "bloom" conspicuous on the older green blades consists of ubiquitous, minute wax rodlets about 2 to 4 μ in length, the majority 1 to 2 μ in diameter, and a minority, random in distribution, about twice this thickness. The underlying cuticle stains clearly with Sudan III.

The entire cell wall, as indicated by standard microchemical tests, consists in the main of cellulose and pectic substances. The latter are particularly abundant in a thin layer immediately beneath the cuticle. As is usual in the epidermis, the external wall of the cell is 2 or 3 times as thick as the inner tangential and the anticlinal walls. Within the living protoplasts, refringent, minute droplets of fat stainable with Sudan black (*3*) are seen to be most numerous next to the outer wall—that is, comparatively near the cuticle.