showed strong, long-lived ($> 10^{-4}$ sec) emission spectra, usually in the green-yellow region of the spectrum, but this phosphorescence was tracked down in every case to the anesthetics that had been used before operation. Despite the subsequent use of samples from patients who had been subjected to a restricted anesthetic program, the anesthetic emission was always so strong that the search for any fluorescence characteristic of tumor tissue was abandoned.

The emission was observed with substances as widely different as barbiturates, morphine, hyoscine, sodium pentothal, syncurine, and *d*-tubocurarine chloride, and it was stronger than that which could be obtained from the drugs alone in concentrations as high as the maximum possibly present within the tissue.

This observation lends strong support to the view that the molecules of anesthetic act as traps for energy absorbed elsewhere in the tissue. I have already put forward the idea, in connection with a discussion of certain aspects of radiation damage (*3*), that most of the biologically important prosthetic groups act as such traps. The molecules of an anesthetic may thus provide traps competitive with those normally operative, and this leads to the prediction that radiation damage may be less severe on irradiation of anesthetized tissue than it is on unanesthetized tissue.

It is noteworthy that, while the triplet-level explanation is probably not directly applicable to small molecules with anesthetic properties (cyclopropane, ether), these molecules, with no exceptions so far as I am aware, are in turn oxidized to products that show phosphorescence with an exceptionally high quantum yield. We are now embarking on a more quantitative study of these phenomena.

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29 November 1956

Blocking Effect of Brain Extract on Lysergic Acid Diethylamide Reaction in Siamese Fighting Fish

The method used to study the blocking effect of beef brain extract is based on the criteria described by Abramson and Evans (*1*) for the reaction of the Siamese fighting fish to lysergic acid diethylamide (LSD-25). However, these criteria were modified for the purposes of this investigation (*2*) and are listed in Table 1. The main criterion utilized was the nose up-tail down position of the fish, at an angle of approximately 45 deg or more. Beef brain extract was prepared in a fashion similar to that described by Florey and McLennan (*3*). In general, the experiments reported here were all run similarly.

Table 1. Inhibition of LSD-25 effect by brain extract. The readings recorded in rows 1a, 2a, and 3a were made 1 hour after brain extract was added to the container, but before LSD-25 was added. The readings in rows 1b, 2b, 3b, and 5b were made 1 hour after LSD-25 was added.

Treatment	Response (No. of fish)			
	Nose up, tail down	Kink in tail	Top of water, tail up	Bottom of container
Experimental containers				
1a Brain extract (2 mg/ml)	0	0	4	0
1b LSD-25 (2 μg/ml)	4	4	1	2
2a Brain extract (0.2 mg/ml)	0	0	9	0
2b LSD-25 (2 μg/ml)	8*	9	0	2
3a Brain extract (0.02 mg/ml)	0	0	2	4
3b LSD-25 (2 μg/ml)	9	10	0	1
Water control				
4a No brain extract	0	0	3	2
4b No LSD-25	0	0	2	4
LSD-25 control				
5a No brain extract	0	0	3	3
5b LSD-25 (2 μg/ml)	10	10	0	0

* One fish dead.

Five bottles containing 200 ml of distilled water each were used to observe 50 fish simultaneously, with ten fish in each bottle. Readings were made with two different methods every 15 minutes for a period of 4 hours or more. The effects of brain extract (2 mg/ml, 0.2 mg/ml, and 0.02 mg/ml) on the reaction of the fish to 2 μg of LSD-25 per milliliter of solution were compared with a water control (no brain extract) and an LSD-25 control (no brain extract). Table 1 presents, in general, the method of bioassay currently employed.

Until LSD-25 was added, none of the fish showed the typical nose up-tail down position except as a normal movement. Fifteen minutes after the LSD-25 was added to the five vessels, all the fish in the LSD-25 control showed the nose up-tail down position and continued to do so throughout the experiment. Essentially similar results were obtained in the bottle containing 0.02 mg of crude brain extract per milliliter. In the bottle containing 2 mg of brain extract per milliliter, by contrast, not only was the initial excitatory phase absent, but there was also a lag, with one fish showing the nose up-tail down behavior at 45 minutes and only four fish showing this behavior at 1 hour. Table 1 gives illustrative data obtained at the end of the first hour.

Using unpublished reaction-time curves, it can be readily shown that the fish in the bottle containing 2 mg of brain extract per milliliter acted as if approximately 0.2 μg of LSD-25 per milliliter were present. In other words, the brain extract blocked the appearance of the LSD-25 effect, and when this effect finally did make its appearance it resembled that of a much weaker solution of LSD-25 than was actually added.

The question naturally arose: Is the serotonin present in brain extract responsible for the action of the brain extract? Two hours after the fish were exposed to 2 mg of serotonin per milliliter, 2 μg of LSD-25 was added. Serotonin does not block the LSD-25 reaction.

Negative results were also found with histamine and γ-aminobutyric acid, as well as with the following amino acids: *l*-hydroxyproline; *l*-serine; 3,5-diiodo-*l*-tyrosine; *dl*-α-aminobutyric acid; *dl*-cysteine hydrochloride; *l*-lysine hydrochloride; *dl*-methionine; *l*-tyrosine; *dl*-valine; *dl*-tryptophan; *l*-leucine; *dl*-phenylalanine; acetyl glycine; *l*-arginine hydrochloride; *dl*-threonine; *l*-histidine hydrochloride; *l*-glutamic acid; *l*-valine; acetyl-*dl*-phenylaline; glycine; and *l*-proline. There was possibly slight blocking in *l*-histidine hydrochloride, for all the fish did not react to LSD-25 immediately.

Lack of material (the result of the difficulty of preparing the crude brain extract in quantity) has prevented our determining where the blocking substance acts or what it is. Conceivably, it could act in several places: (i) outside the fish, forming a loose compound with LSD-25; (ii) at the gill membrane; or (iii) inside the fish itself, as a true pharmacologic inhibitor. The data indicate that some type of equilibrium is set up in which the inhibitory action is dependent on the concentration of the LSD-25 blocking substance present in the liquid.

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.

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31 December 1956

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*).

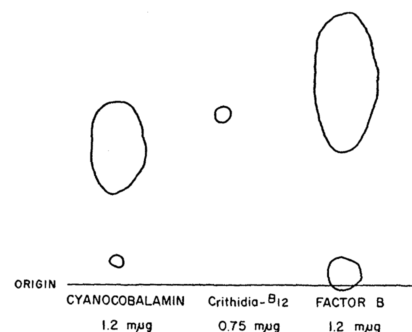

Fig. 1. Descending chromatogram of *Crithidia* vitamin B_{12}, with electrophoretically pure cyanocobalamin and factor B as standards; solvent system: *n*-butanol, ammonium hydroxide and water (100/1/50). Activity of the *Crithidia* material was assayed, before application, with *Euglena.* Development 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.

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