TABLE 3

EFFECT OF PIPERONYL CYCLONENE ON CONVERSION OF DDT TO DDE

	DDT applied µg/fly	Piperonyl cyclonene µg/fly	Internal DDT recovered µg/fly	Internal DDT recovered Percent of amount applied	Internal DDE recovered µg/fly	Internal DDE recovered Percent of amount applied
Laton strain						
(a) dead	0.5	10.0	0.115	23.0	0.030	6.0
(b) living ...	0.5	0.0	0.0	0.0	0.250	50.0
Bellflower strain						
(a) dead	5.0	25.0	0.793	15.8	0.173	3.4
(b) living ...	5.0	0.0	0.376	7.5	1.654	33.1

detoxification process. Typical data for the Laton and Bellflower strains are shown in Table 3. Similar results were found with other resistant strains.

An indication that DDE is not the only decomposition product formed in some cases is given by attempts to account for all applied DDT. With survivors from the resistant strains, the sum of external DDT, internal DDT, internal DDE calculated back to DDT, plus these compounds excreted or brushed off in the container usually was at least a third less than the amount of DDT originally applied.

The conversion of absorbed DDT to DDE may be an enzymatic process, for flies first killed by heating in air at 80° C for a few minutes absorbed large amounts of DDT but converted none to DDE.

Work on this problem is continuing and will be reported on more fully later.

Biosynthesis of Radioactive Vitamin B_{12} Containing Cobalt^{60}

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Vitamin B_{12} obtained from liver extracts (*6, 9, 11*) and by fermentation from *Streptomyces griseus* (*8*) has been characterized as a cobalt complex (*1, 7*). In a paper by Hendlin and Ruger (*3*), it has been shown that the production of LLD activity and vitamin B_{12} can be increased during fermentation processes by the addition of small amounts of cobalt ions. The addition of radioactive cobalt to a broth inoculated with *Streptomyces griseus* should yield radioactive vitamin B_{12}. Fermentations[2] conducted with this organism using a broth to which cobalt^{60} sulfate[3] had been added actually have yielded vitamin B_{12} containing radioactive cobalt. The vitamin content of the vitamin B_{12} concentrate was evaluated by means of an 8-stage countercurrent distribution, using the system benzyl alcohol-water.[4] In addition to measuring color intensities in the several tubes, the β-radioactivity of aliquots was determined by means of a thin window counter, employing evaporation residues in steel planchets. The counting efficiency of our equipment was $\simeq 6\%$. Activities are reported as measured, i.e., without efficiency corrections.

Preliminary experiments were undertaken to ascertain the feasibility of preparing the radioactive vitamin by fermentation. For this purpose radioactive cobalt, having a specific activity of 1.94×10^5 cpm/mg was added to a fermentation broth at the level of 2 ppm. After fermentation, isolation, and partial purification, a vitamin B_{12} solution with a total activity of 2000 cpm was obtained which was analyzed spectrophotometrically and by countercurrent radioactivity distribution. The vitamin B_{12} content of this solution equivalent to the observed intensity of the 5500-A band (*1, 2*) was computed to be 0.147 mg vitamin B_{12} or 6.6 µg Co (*7, 10*) and therefore would be expected to have a maximum activity of 1280 cpm if added cobalt were utilized preferentially by the organism. This would indicate that the radioactive vitamin B_{12} constituted not more than $\frac{1280}{2000} \times 100$, or 64% of the product (if all radioactive components were of approximately equal molecular weights). The material available was insufficient for a countercurrent color distribution. From countercurrent radioactivity distribution analysis, however, it was ascertained that 19% of the total activity concentrated in the fourth tube as compared to a theoretical value of 29% for pure vitamin B_{12}. This analysis indicates that a maximum of $\frac{19}{29} \times 100$, or 66% of the radioactive cobalt was present as vitamin B_{12}, which maximum agrees with the value of 64% computed from the spectrophotometric determination. The agreement between the maximum vitamin B_{12} contents calculated by these independent methods in experiments of this type clearly pointed to the formation of radioactive vitamin B_{12}.

A subsequent experiment, performed with cobalt of higher specific activity, permitted the isolation of $\simeq 0.5$ mg of once-crystallized vitamin. The visual appearance of these radioactive crystals was similar to that of normal vitamin B_{12}, and the spectrum in aqueous solution exhibited all the absorption bands (*1, 2*) reported for the nonradioactive product, despite the presence of another absorbing component. The presence of activity in the

[1] The authors are greatly indebted to Robert G. Denkewalter and George B. Hughey for their interest in this work.

[2] Fermentations were performed under the supervision of E. O. Karow and B. L. Wilker.

[3] The radioactive cobalt sulfate was obtained from Tracerlab, Inc., on allocation from the Isotopes Division, U. S. Atomic Energy Commission.

[4] A manuscript including details of this analytical method is in preparation and will be published separately by F. A. Bacher *et al.*

crystals was confirmed by means of the radioautograph technique.

The yield of crystalline material was sufficient to permit a countercurrent analysis for vitamin B_{12} content based both on color (5500-A band intensity) and on radioactivity distribution. The fraction of total color distributed in the fourth and fifth tubes, where vitamin B_{12} concentrates, was equal within experimental error to the fraction of total radioactivity found in these tubes. This correspondence between color and radioactivity definitely establishes the identity of the radioactive vitamin B_{12} with the normal vitamin. The intensity of absorption at 5500 A in the fourth and fifth tubes amounted to 22% and 19% of total, and the corresponding radioactive cobalt content was 23% and 18.2%, respectively, as compared to theoretical values of 29% and 24.7% for the pure vitamin. Color distribution results thus indicate a purity of 76% for both tubes, while the activity measurements show that 79% and 73% (average 76%) of the radioactive cobalt is present as vitamin B_{12}. As judged by the color and activity distributions and the absorption spectrum of the crystals, the second component (also radioactive) appears to be largely vitamin B_{12a} (*4*) or vitamin B_{12b} (*5*).

TABLE 1

COUNTERCURRENT DISTRIBUTION OF CRYSTALLINE RADIOACTIVE VITAMIN B_{12}

Tube	Water phase % of total		Benzyl alcohol phase % of total	
	Color	Activity	Color	Activity
1	1.9	2.5	1.1	1.0
2	5.9	6.2	4.6	4.2
3	11.7	11.4	9.8	10.0
4	14.6	15.3	12.9	12.5
5	11.9	12.1	10.6	10.2
6	6.4	5.6	5.4	5.2
7	1.4	1.5	1.6	1.7
8	(0.1)	0.2	(0.1)	0.4

In view of this preliminary investigation, larger scale fermentations were conducted and about 100 mg of high purity, crystalline, radioactive vitamin B_{12} was isolated. The absorption spectrum of the product was characteristic of the normal vitamin in all its detail (*1*, *2*) and indicated a vitamin B_{12} content of 97%. The results of an 8-stage countercurrent distribution analysis are reported in Table 1, which lists the percentage of total color and of radioactivity found at each stage and in both phases.

This distribution of radioactivity is identical within experimental error with the color distribution and corresponds closely with that expected for vitamin B_{12}, thus definitely establishing that the preparation is truly radioactive vitamin B_{12}. The activity of the present crystalline product is $\simeq 0.25$ μc/mg. By starting with cobalt sulfate of higher specific activity than employed in this work, radioactive vitamin B_{12} of much higher specific activity than 0.25 μc/mg can be produced.

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Culturing *Crepidula plana* in Running Sea Water

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The simple method of culturing *Crepidula plana* described here is designed to prevent waste of material and lessen the expense of collection at seaside laboratories, where the eggs of this small gastropod are in demand for embryological work.[1]

The shells of hermit crabs may contain attached communities of *C. plana* numbering several dozens of individuals of all sizes and sexual phases from tiny spat to old females. Immature specimens and males are more numerous and are usually discarded and lost, although they are all potential egg-producers. If all the individuals are detached and transferred to 4-in. finger bowls under running sea water they will attach themselves to the glass, and the young will grow to maturity and provide eggs, requiring no more attention than an occasional dumping of debris from the bowls.

At Woods Hole, males removed from the communities in June and cultured may reach the adult female phase by the end of August. Small spat, 2–5 mm in length, may not produce eggs until the next summer season, but these animals survive the winter with very low mortality. The natural tendency to community sex adjustment in *C. plana* insures that any artificial community, unless it consists exclusively of adult females, will eventually contain both sexes. It is immaterial whether it is started with sexless young, immature males, adult males, regressive males, or any mixture of these phases. Adult females should be accompanied by small individuals which will develop the male phase and fertilize the eggs.

The number of individuals which may be cultured in one dish is limited only by the area available for attachment and growth. Dense crowding limits size and increases the proportion of males. A continual movement of unfiltered sea water over the animals is important, since it provides the microscopic food organisms necessary. A stack of finger bowls separated by squares of coarse galvanized iron wire netting, with the sea water

[1] Other workers have conducted laboratory experiments on a related species.