Determination of DDT by Bioassay

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The widespread use of the insecticide DDT has brought about the problem of its residue in milk and other foods (*1, 3*). As a result, a simple rapid procedure for quantitative measurement of small amounts of DDT is urgently needed.

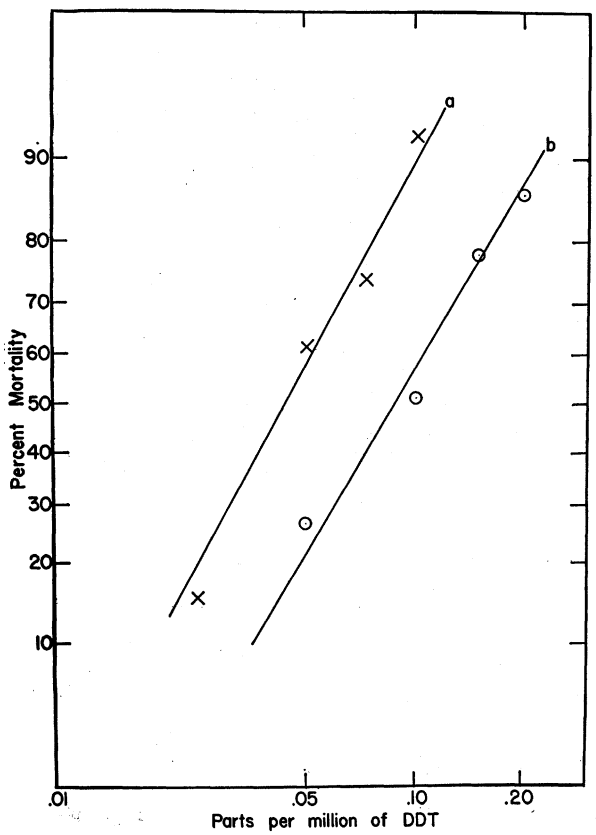

FIG. 1. Dosage-response curve of DDT as obtained with guppies (*Lebistes reticulatus* Peters).

A biological method (*2*) in which guppies are used as the test animal for determining the toxicity of certain insecticidal plants was modified to adapt it for detecting small amounts of DDT. Preliminary trials using technical DDT in water were made to establish the toxicity range. Five replicates of ten fish each were used at the following concentrations of DDT: 0.025, 0.050, 0.100, 0.150, and 0.200 ppm. Mortality counts, at the end of 24 hr, gave data that plotted a straight line on log probability paper (Fig. 1).

Fish from two different sources were used in these trials. Line *a* shows the dosage-response curve obtained with fish from a city ditch, while line *b* was obtained with fish caught in a brook high in the mountains. The temperature differential in these two locations may be a factor in the observed difference in resistance. It should be noted that even though there is a significant difference in susceptibility to the poison, the lines are parallel, which indicates a similar response to varying dosages. The lower limit of sensitivity when the most susceptible fish were used was around 0.025 ppm, which concentration of DDT produced a kill of approximately 15% in 24 hr.

A practical application of this technique was made by determining DDT residues on vegetables. The experimental procedure followed was to apply a 0.1% solution of wettable DDT powder[1] (50%) to whole fresh vegetables. Four 500-g samples of both tomatoes and string beans were weighed separately into quart Mason jars. The DDT suspension was then applied from a pipette at the rates of 0, 8, 12, and 16 ppm. The samples were allowed to stand for 1 hr and then extracted using the technique described by Wichmann *et al.* (*4*). Acetone was substituted for benzene in this extraction procedure, as benzene is toxic to the fish. The acetone extracts were then evaporated to 100 ml so that the concentrations of the test solutions would be 0.00, 0.04, 0.06, and 0.08 mg/ml, respectively, assuming that no losses occurred in the extraction procedure. These solutions were tested on the fish, and the results obtained are presented in Table 1. The results show good recoveries for all concentrations of DDT in both vegetables, with an error within limits of biological methods.

TABLE 1

RECOVERIES OF DDT OBTAINED FROM DDT RESIDUES ON TOMATOES AND BEANS

Samples	DDT in test solution mg/ml	Fish mortality %	DDT found mg/ml	Recovery %
Tomatoes	blank	0	—	—
	0.040	16	0.035	87.4
	.060	56	.059	98.4
	.080	92	.092	115.0
Beans	blank	0	—	—
	.040	20	.038	95.0
	.060	42	.050	83.4
	.080	74	.070	87.4
Standards* (Controls)	blank	0	—	—
	.040	24	—	—
	.060	58	—	—
	.080	84	—	—

* Standard test solutions of DDT were prepared from an acetone extract of 50% DDT wettable powder.

The high sensitivity of the fish to DDT suggested the application of this biological test in detecting traces of DDT in milk. However, when diluted milk contaminated with DDT was used as the test medium, erratic results were obtained. The milk fat in some way slowed down the rate of penetration or poisoning action of DDT. This necessitated extending the exposure time to 48 hr, with the results that the controls had a high mortality.

[1] Chipman DDT (50%) spray powder.

The inconsistent results obtained with prolonged exposure in milk indicated that some factor other than the DDT was also toxic to the fish. This was substantiated by the fact that on occasions when mortality in the controls was low, the kill obtained in treated samples was proportional to the concentration of DDT. Bacteriostatic and surface-active agents, aeration, and homogenization were of no benefit in producing consistent results.

References

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Tyrosinase in Human Skin: Demonstration of Its Presence and of Its Role in Human Melanin Formation

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The mechanism of formation of melanin in human skin has been the subject of extensive investigation and vigorous controversy for the past 50 years (*14*). In 1895, Bourquelot and Bertrand (*5*) found in the mushroom an enzyme called tyrosinase, which catalyzed oxidation of the amino acid tyrosine to melanin. A few years later, tyrosinase was demonstrated in many plant, insect, fungus, and marine animal tissues. At this time, it was assumed that tyrosinase was also present in mammalian skin and that this enzyme catalyzed the oxidation of tyrosine to melanin. Although there was some experimental evidence to support this view, skepticism concerning the presence of tyrosinase in mammalian skin arose when reports demonstrating tyrosinase in rabbitskin could not be confirmed.

Bloch and his co-workers (*4*) found that on incubation of sections of human skin in a solution containing dihydroxyphenyl-L-alanine (dopa), under appropriate conditions, black granules were deposited in the melanoblasts of the basal layer of the skin. This process was shown to involve the enzymatic oxidation of dopa to melanin. The investigators (*4*) found that incubation of skin with compounds other than dopa, for example tyrosine and epinephrine, did not result in formation of melanin in the melanoblasts. Therefore, Bloch concluded that the melanoblasts in human skin contained a specific "dopa oxidase" which could catalyze the oxidation of dopa only to melanin.

[1] Abridgment portion of a thesis submitted by Dr. Fitzpatrick to the faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Master of Science in dermatology and syphilology.

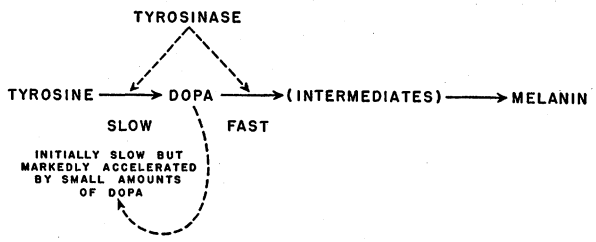

FIG. 1. Enzymatic oxidation of tyrosine to melanin by mammalian tyrosinase.

Several years later, Raper (*17*) showed that dopa was the first product formed in the enzymatic oxidation of tyrosine to melanin by tyrosinase of plant and insect sources. He also demonstrated that plant and insect tyrosinases could catalyze the oxidation of dopa, as well as of tyrosine, to melanin; that is, plant and insect tyrosinase possesses tyrosinase and dopa-oxidase activities.

In the past 8 years, it has been demonstrated that extracts from melanomas of mice (*9, 11, 15*), horses (*7*), and human beings(*10*) display both tyrosinase and dopa-oxidase activities. Furthermore, it has been shown (*15*) that, under certain conditions, a true distinction cannot be made between tyrosinase and dopa-oxidase activities in mammalian tissue. It was recommended (*15*) that the term *tyrosinase* be used for this enzymatic activity, instead of the separate terms *tyrosinase* and *dopa oxidase.* Dopa was also found to be a catalyst in the enzymatic oxidation of tyrosine (*15*). This concept is diagramed in Fig. 1. If the view represented in Fig. 1 is correct, as opposed to Bloch's hypothesis, it should be possible, under appropriate conditions, to demonstrate tyrosinase activity in human skin. The experimental results demonstrating the presence of tyrosinase activity in human skin form the basis of this report.

It has been reported (*3*) that under certain stimuli—for example, ultraviolet or roentgen rays—the human dendritic melanoblasts enlarge, become branched, and give a more pronounced dopa-oxidase reaction than when unirradiated skin is used. For this reason we performed experiments in which we used nonpigmented skin of normal individuals, which had been irradiated for 8 days with erythema doses of ultraviolet radiant energy obtained from a quartz mercury vapor lamp.[2] The results presented in this paper represent a study of the skin (normal before irradiation) of 30 human volunteers.

On the 8th day, a specimen for biopsy was taken, by means of a punch, from the irradiated site and was fixed immediately in 10% solution of formalin for 1 hr at 5° C. Slices of the fixed tissue, 1–2 mm thick, were placed in 25 ml of 0.005 M l-tyrosine made up in 0.1 M phosphate buffer at pH 6.8[3] where they remained for 24 hr at 5° C. The slices of tissue were then immersed in fresh tyrosine-phosphate buffer again, and were left in

[2] Alpine Sun Lamp, Luxor Model, Hanovia Chemical and Mfg. Co., Newark, N. J.

[3] This value is the optimal pH for the mammalian tyrosine-tyrosinase reaction (*15*). There was no change in the intensity of the reaction between pH 6.8 and 7.38.