TECHNICAL PAPERS

DDT for Powder-Post Beetle Control in Bamboo

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Scarcity of Eastern Hemisphere bamboo resulting from the war and continued shipping difficulties has created a rising interest in bamboo produced in the Western Hemisphere. About 30 species and varieties, comprising probably the largest collection of bamboo in this part of the world, have been introduced into Puerto Rico by the Federal Experiment Station in the 45 years since its establishment in Mayaguez. A number of these species have shown themselves to be well adapted to local conditions, and culms are now available in commercial quantities in Puerto Rico or other parts of the Western Hemisphere. However, the main limiting factor here, as elsewhere, in the utilization of these species has been the infestation of the dry culms by the widely distributed bamboo powder-post beetle (*Dinoderus minutus* F.).

Although none has yet been found to be immune, systematic testing over a period of years has shown that a number of species possess considerable natural resistance to this most troublesome and damaging pest. Much of this resistance was associated with lack of starch in the wood, and, where little starch was present, resistance was correlated with low moisture content and high specific gravity (*1*). The upper part of the culm was more resistant than the lower, and usually the older the culm, the greater was its total resistance to the beetle.

The best of various artificial methods to increase beetle resistance or to prevent attack either have been too cumbersome or too hazardous or have adversely affected the character of the wood for general use. Among some of the least cumbersome that have given good results have been harvesting culms of resistant ages and allowing freshly cut, untrimmed culms to dry or cure in the field. Recently, tests of the effect of "Bakelite-forcing" were conducted in cooperation with the Puerto Rico Development Company. While this impregnation of the wood with Bakelite did not entirely close the pores of the wood or change its reaction to the iodine starch test, it did harden the wood and imparted to it other commercially desirable qualities. Under the most severe conditions of infestation, pieces so treated remained immune to the beetle, while untreated wood of the same species and age was heavily attacked.

External application of DDT is proving more practicable than the foregoing and by far more effective than any other insecticidal treatment yet tried. In tests now in progress, one thorough brushing with a kerosene solution at the 5 per cent residual strength resulted in 94 per cent control of internodal infestation in highly susceptible, freshly harvested, one-year-old culms of *Bambusa vulgaris*. After treatment these culms were held in open storage away from rain and sun for 2½ months. Under the same conditions, a saturated solution of pentachlorophenol in kerosene and a 2 per cent solution of sodium pentachlorophenate in water plus 2 per cent of wettable sulfur produced 3 and 6 per cent control, respectively. Most of the few beetle holes in the DDT-treated culms were shallow. No internal infestation had developed that could be detected without splitting, while in the check and other treatments many culms were completely riddled. Heavy crystallization of DDT persisted on most of the internodes, and no living beetles (but many dead ones) were seen about the nodes where side branches had been removed. The practically colorless DDT solution did not discolor the wood or otherwise perceptibly affect its quality.

In a previous experiment freshly harvested wood was protected from beetle attack in a naturally ventilated cage for 6 months while air-drying to a moisture content of about 16 per cent. This treatment reduced infestation more than 83 per cent, indicating, with the results from field curing, that if protection against infestation can be provided for the first several months after harvest, the wood will increase considerably in beetle resistance.

From the foregoing tests it appears that DDT will supply this early protection and any that may be needed thereafter.

Reference

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Physiological Availability of Iodine in Dithymol Diiodide

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The insolubility of dithymol diiodide in water and brine and its chemical stability confer upon this compound distinct advantages over potassium iodide as an agent for iodizing salt blocks for cattle feeding. By means of radioactive iodine it has been shown that the iodine of dithymol diiodide is physiologically available to the thyroid of the albino rat.

Although a great many compounds of iodine have been tested with or without the use of radioactive iodine, there is apparently no experimental evidence that the iodine in dithymol diiodide in physiologically available. Dithymol diiodide is easy to synthesize and is commercially available. The material is sold under a number of trade names, including aristol, iodothymol, thymodin, thymol iodide, and others. Although the formula for dithymol diiodide is usually given as $(C_6H_2 \cdot CH_3 \cdot OI \cdot C_3H_7)_2$, with a molecular weight of 550.03, there is still some uncertainty as to just how the iodine is bound. In 1918 Bougault proposed a formula in which the iodine was connected directly to an aromatic carbon in place of the hypoiodite type of linkage; and the two thymol molecules were

linked through the OH groups, thus explaining the stability of the compound and the suppression of the phenol function (*1*). In 1935, Leclercq, in reviewing the tests and assays for dithymol diiodide, recommended the formula of Bougault as correct (*3*). If the iodine in dithymol diiodide is bound directly to the phenolic ring, there is some similarity between the compound and thyroxine of the thyroid. Joliot has already shown that thyroxine administered to rabbits was dissociated in the body, as evidenced by the subsequent formation of diiodotyrosine and ionic iodine (*2*).

In order to determine the physiological fate of iodine in dithymol diiodide, the compound was synthesized using I^{131} obtained from the Radioactivity Center at the Massachusetts Institute of Technology. The compound was prepared and fed to albino rats as described below. Various organs were then tested for radioactivity, which was followed by means of a Geiger-Müller counter and by radioautographs. The latter method requires no special equipment, although at best, the results are only semiquantitative.

Using the method of preparation described in the Merck Index (5th ed.), radioactive dithymol diiodide was prepared from 50 mg. of potassium iodide containing 1 millicurie of I^{131}. The product which was obtained as a light red-brown precipitate was washed free of iodide ions and dried over P_2O_5.

Iodized salt for cattle blocks usually contains 0.007 per cent iodine; consequently, dithymol diiodide corresponding to this amount of iodine was thoroughly mixed with 100 mg. of tricalcium phosphate and then added to 10 grams of iodine-free salt. The tricalcium phosphate is generally used for the dispersion of iodine compounds in salt. The iodized salt was then ready for incorporation in a normal diet. A second batch of salt was prepared in which this iodine content was 100 times the normal amount. The remaining dithymol diiodide was made into a water slurry of 10 mg./ml. to be administered orally by syringe to albino rats.

The diet fed to the rats in these experiments had the following composition: corn meal, 33 per cent; oat flour, 33 per cent; skim milk powder, 25 per cent; dried brewer's yeast, 3 per cent; Mazola oil, 5 per cent; and salt (iodized with the dithymol diiodide), 1 per cent.

Diet #1 contained salt with the normal amount of iodine (0.007 per cent). Diet #2 contained salt with 100 times the normal amount of iodine (0.7 per cent). The first pair of rats were starved for 24 hours and then given diet #1. Both rats in this pair weighed 450 grams, and both ate approximately 50 grams of feed before they were sacrificed at the end of 36 hours.

A similar procedure was followed with the second pair of rats, which were fed the iodine-enriched diet #2. These rats also consumed approximately 50 grams of feed before they were sacrificed at the end of 36 hours.

A third pair of rats were fed a normal diet, but each was given by syringe three oral administrations of $\frac{1}{3}$ cc. each of the suspension of dithymol diiodide in water.

At the end of the 36-hour feeding period the thyroid, kidney, spleen, and liver were removed from each rat, fixed in a 10 per cent solution of formaldehyde for 2 hours, washed in 95 per cent and in absolute alcohol, and allowed to dry. Radioautographs of all the organs were made by pressing them against an Agfa no-screen X-ray film contained in a black, lightproof envelope, the beta and gamma rays emitted by the radioactive iodine, if present, exposing the film. In such a process, a total of approximately 2,000,000 beta particles/cm.^{2} are required to "expose" the film. The results are best seen in the accompanying photographs in which the thyroids badly overexposed the films; in identical exposure times the kidney, spleen, and liver produced no exposure whatsoever.

In Fig. 1A, the radioautograph of a few minute specks of the freshly synthesized dithymol diiodide, there is evidence that the radioactive iodine had combined with the thymol, since the product had been washed thoroughly to remove all soluble iodine or iodides.

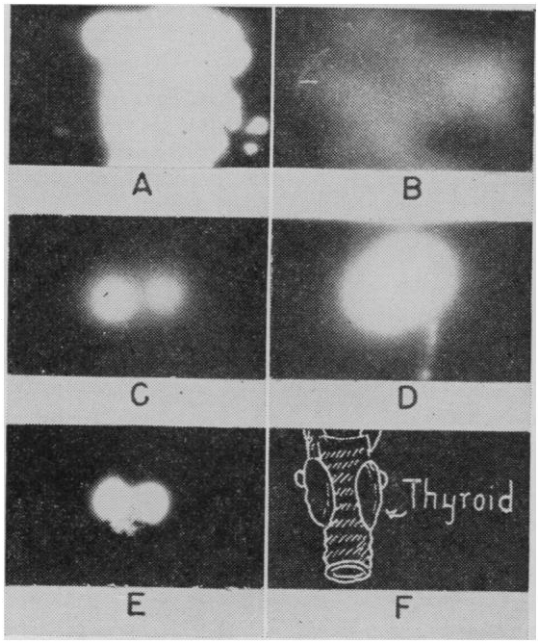

FIG. 1. Radioautographs and diagram of thyroid of albino rat: A, radioactive dithymol diiodide; B, rat diet; C, thyroid, rat #1; D, thyroid, rat #3; E, thyroid, rat #5; F, diagram thyroid, esophagus 2× actual size.

Fig. 1B shows the rat diet after the admixture of the salt containing the radioactive dithymol diiodide. The picture was taken to verify the fact that the radioactivity was evenly distributed throughout the diet by the mixing procedure used.

Fig. 1C is the radioautograph of the thyroid from one of the rats fed the diet containing the normal amount of iodine. The picture was obtained by cutting lengthwise the portion of the esophagus holding the thyroid glands and flattening the tissue against the enveloped film with a microscope slide. The radioactivity had very evidently accumulated in the thyroid.

From radioautographs of the various organs, plus those of the urine and feces, it was estimated that from 25 to 50 per cent of the iodine consumed had concentrated in the thyroid. The radioautographs shown in Fig. 1D and E were obtained in a similar manner, using the thyroids from the second and third pair of rats, which, respectively, had been fed the iodine-rich diet and had been given the dithymol diiodide slurry orally.

The fact that the thyroids of rats given the diet with 100 times the normal amount of iodine accumulated more iodine than those of the rats given much larger doses orally by syringe bears further investigation. The rats given dithymol diiodide by syringe excreted a greater percentage of the material in the feces than did the others. Some radioactivity was found in the

feces as well as the urine of all the rats consuming dithymol diiodide.

The radioautographs afford graphic evidence that the iodine in the water-insoluble dithymol diiodide is physiologically available to the thyroid of the albino rat.

References

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Plasma L-Methionine Levels Following Intravenous Administration in Humans[1]

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In connection with a study of amino acid metabolism in hepatic disease the observations here reported have been made on 11 normal human male subjects used as controls.

Methionine determinations were made by a microbiological method using plasma from heparinized blood. Removal of proteins from the plasma was accomplished by the following method, which avoids the use of heavy metal precipitants. To 1 volume of plasma in a 15-cc. centrifuge tube was added 1 volume of water and the tube placed for 1–2 minutes in a boiling water bath. One drop of 5 per cent acetic acid per cc. of plasma originally taken was then added and the contents of the tube thoroughly mixed, the tube remaining in the water bath for approximately 3 additional minutes. The tube was then centrifuged and a water-clear supernatant decanted for assay. The addition of tungstic acid precipitant to such a filtrate produces a very faint additional turbidity, indicating that removal of protein by the heating procedure was essentially as complete as that obtained with the tungstic acid precipitant.

Microbiological assay of the methionine in these filtrates was carried out with *Leuconostoc mesenteroides* P-60 in a medium in which an oxidized gelatin hydrolysate supplemented with tyrosine, tryptophane, and cystine served as the amino acid source. Methionine in the hydrolysate was completely removed, as evidenced by low blanks, by oxidation with 30 per cent hydrogen peroxide, using a method previously reported for the treatment of peptone (*2*). The response to added increments of methionine was equivalent to that reported for this organism by other workers.

Following a 12-hour fast a blood sample was drawn for determination of the level of plasma methionine. Fifty cc. of a 3 per cent solution of DL-methionine[2] was then injected intravenously over a 5-minute period, blood samples being drawn 15, 30, 60, 120, and 180 minutes after the injection. Methionine determinations in the urine excreted during the test period as well as during the 12 hours preceding the test were also made. In several cases urine samples to correspond to the periods when blood was drawn were also collected and analyzed.

The character of the variation in plasma methionine concentration after the intravenous injection is illustrated by the typical curve shown in Fig. 1. It is apparent that there

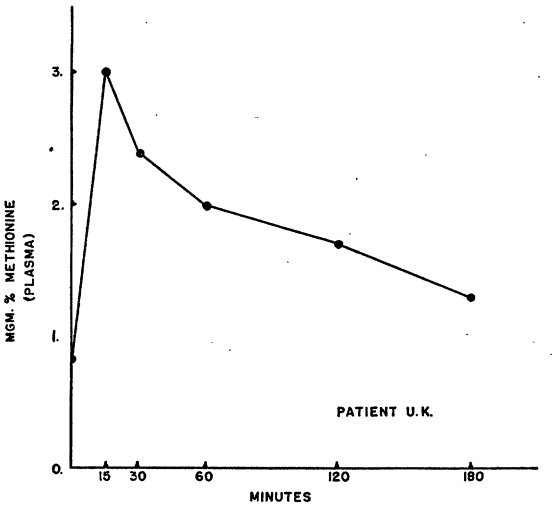

Fig. 1. Rate of disappearance of intravenously administered methionine in normal young adult.

was a sharp rise immediately following the injection which decreased to lower levels rapidly during the first 30 minutes and then much more slowly during the subsequent periods. In no case did the plasma level return to the previous fasting value in the 3-hour period of observation. In the entire series of 11 normal individuals, considerable variation in the absolute values for the various points on the curve were observed. The fasting levels ranged from 0.46 to 1.48 mg./100 cc. with an average of 0.85 ± .09. The observed "peak" value (at 15 minutes) ranged from 2.06 to 4.44 mg./100 cc., and the decrease in methionine concentration between 30 and 180 minutes varied from 0.82 to 1.36, except for a value of 2.14 in one case. Calculating the hourly rate of disappearance of methionine in the plasma from the 30- to the 180-minute period gave values ranging from 0.33 to 0.54 mg./100 cc./hour with an average of 0.41 ± .03, with the exception of the one case mentioned (not included in the average), in which a figure of 0.85 was obtained.

The excretion of methionine was most rapid during the first 15–30 minutes, when the blood levels were highest, but the amount excreted at any time was quite low when compared with the original quantities administered. For the entire 3-hour postinjection period of observation only 1.6–6.5 mg. of methionine were excreted. Fasting excretion values of approximately 0.1 mg./hour were uniformly observed. Thus, it is apparent that excretion accounted for the disappearance from the plasma of only a negligible portion of the 750 mg. of the L isomer administered, and that at these plasma levels no evidence of a limiting rate of renal tubular reabsorption for methionine was observed. This is in accord with the observations on dogs reported by Wright, *et al.* (*3*).

In view of the report of Dunn, *et al.* (*1*) on the response of various lactobacilli to the isomers of methionine, these assays as carried out with *Leuconostoc mesenteroides* P-60 are in-

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[2] The DL-methionine used in these experiments was supplied by Mead Johnson Co. through the courtesy of Dr. Warren Cox.