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# Parasiticidal Properties of the Proteolytic Enzyme Ficin.*

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For centuries natives of Tropical America have used the crude fresh latex of *Ficus glabrata* and *F. doliaria* as an anthelmintic, especially for the removal of the human whipworm *(Trichocephalus trichiurus)*.[1] However, unless refrigerated or chemically preserved, the latex ferments rapidly. Robbins[2] isolated the anthelmintic principle *ficin* in amorphous form, while more recently Bliss[3] has prepared a semi-refined crystoid and Walti[4] a pure crystal product.

The present study was undertaken to compare the parasiticidal properties of the refrigerated and chemically preserved (0.1-1.0% sodium benzoate) latex, the semi-refined crystoid (sealed *in vacuo*) and the amorphous ficin.†

*In vitro* tests were made on egg albumin coagulated by heat in capillary tubes 25 mm long by 2.5 mm inside diameter, on living pig *Ascaris* and on living dog whipworms. The products tested *in vitro* were the undiluted refrigerated and preserved crude latex, 1, 2, 3, 4, 5, and 10% dilutions of these products in 0.85% sodium chloride, and 3 and 10% dilutions in artificial gastric juice; likewise 0.01, 0.1, 1, 5, and 10% solutions of the crystoid in distilled water, 0.85% sodium chloride and artificial gastric juice. The test objects were immersed in the samples to be tested, were incubated at 36-37°C, and were examined at regular intervals up to 24 hours. The amount of digestion of egg albumin from the ends of the tubes provided an index of proteolytic efficiency of the product, while the lessened activity of the worm and state of destruction of its cuticle served as criteria of anthelmintic potency. Controls in physiological salt solution were set up in each experiment.

*In vivo* observations were made on 80 dogs, infected with hook-

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1 Thomen, L. F., *Am. J. Trop. Med.*, 1939, **19**, 409.

2 Robbins, B. H., *J. Biol. Chem.*, 1930, **87**, 251.

3 Bliss, S. W., personal communication.

4 Walti, A., *J. Am. Chem. Soc.*, 1938, **60**, 493.

† With the exception of one sample of refrigerated latex supplied by Dr. H. C. Clark, Director of the Gorgas Memorial Laboratory, Panama, R. de P., and preserved latex obtained from Higueronia Laboratories, Mexico, D. F., all of the products tested have been obtained from Merck and Company.

worms, whipworms, ascarids, tapeworms, and *Endamoeba histolytica.* Tests were conducted with refrigerated and preserved latex, the crystoid, and the amorphous ficin. The study included pre-treatment and post-treatment Stoll egg counts of hookworms and whipworms. Measured amounts of the drugs were administered on the basis of body weight. Tests were conducted with and without pre-treatment purgation or enemata. After therapy and follow-up studies, each animal was anesthetized and the entire digestive tract examined to determine the results of the treatment

All of the *in vitro* tests demonstrated some degree of digestive action upon the test objects. The refrigerated unpreserved samples of latex were most active, digesting one mm length of coagulated albumin in 60 minutes, digesting the cuticle of *Ascaris* within 30 minutes in a dilution up to 1:10 in 0.85% sodium chloride and disintegrating the whipworms within 2 hours in a 1:20 dilution. The Higueronia product was about 25% less efficient. A 10% solution of the freshly opened crystoid in 0.85% sodium chloride was comparable to the undiluted or 10% sample of refrigerated latex. In all cases 0.85% sodium chloride used as a diluent was found to provide a higher potency than distilled water, while gastric juice appeared partially to inactivate the enzyme.

The *in vivo* experiments provided certain conclusive results. Administration of one ounce of the refrigerated latex to each of 5 unpurged dogs removed all of the whipworms. Similar results were obtained with preserved latex (5 animals) following pre-treatment purgation. With the freshly opened crystoid single doses of 0.6 to 3 g per kilo of body weight (9 animals) or equivalent amounts in 2 divided doses (6 animals) or 4 divided doses (2 animals) were equally effective. Lesser amounts of the crystoid (2 animals) were 25 to 70% efficient. However, when exposed to air for only 2 or 3 days the crystoid (7 animals) rated only 0 to 25%. The amorphous ficin (24 animals), although relatively stable on exposure to air, required a total minimum of one gram per kilo of body weight, in single or divided doses, to produce 100% removal of whipworms, but if the bowel was obstructed by tapeworms or feces, as much as 4 g per kilo were ineffective.

Comparable ratios of efficiency among the several test products were obtained on hookworms but the specificity was invariably less than on whipworms. Intragastric intubation of a solution of the crystoid or amorphous ficin was usually more efficient than oral administration in gelatin capsules. The action of no product was consistently efficient for dog ascarids; the efficiency for tapeworms

was essentially *nil.* In 10 animals treated with the fresh crystoid and 14 with the amorphous ficin, trophozoites of *E. histolytica* in the wall of the large bowel quite uniformly became dwarfed and inactive, and at times had died *in situ.*

*Summary.* The unpreserved, refrigerated crude latex was consistently the most efficient parasiticide tested, the preserved latex somewhat less effective. A 10% solution of the freshly opened crystoid compared favorably with the refrigerated latex but exposure to air rapidly diminished its potency. The amorphous ficin, while relatively stable, proved to be only about half as efficient as the fresh crystoid. The most specific action of these products occurred on whipworms, provided the large bowel was previously free of feces. The effect on hookworms was almost uniformly less satisfactory. Considerable amebostatic and, at times, amebicidal action was demonstrated.

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## Determination of Ascorbic Acid in Whole Blood.*

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It has been found that ascorbic acid is oxidized by the oxygen set free from oxyhemoglobin when whole blood is deproteinized with $HPO_3$, and that this oxidation can be prevented by displacing the oxygen of oxyhemoglobin with CO, $N_2$, and $H_2$.[1-3] Butler and Cushman[4] have reported a method for the determination of ascorbic acid in whole blood involving saturation of the blood with CO and precipitation of the proteins under an atmosphere of CO.

We have found that a very convenient method to prevent the oxidative effect of oxyhemoglobin is alternate removal of oxygen by evacuation and treatment with $CO_2$ under pressure. A combination of these two steps gives an efficient and practical way of reducing oxyhemoglobin which is adaptable to clinical laboratory use.

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3 Huzita, A., Ebihara, T., and Numata, I., *Biochem. Z.,* 1939, **301**, 245.

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