TABLE I. Dose Response Curves and Potency for Estrone, Estradiol-17β, 18-Norestrone and 18-Norestradiol-17β.

Compound	Curve	Potency*
Estrone	Uterine wt = $-.2 + 30.5$ (log 100 dose)	100%
18-norestrone	" " = $5.9 + 11.6$ (log dose)	.028
Estradiol-17β	" " = $17.4 + 12.6$ (log 1000 dose)	2500
18-norestradiol-17β	" " = $8.3 + 13.0$ (log dose)	.058

* At response equivalent to about 100% increase in uterine wt over oil-treated controls.

twice as potent as the estrone. The more remarkable finding, however, is decrease in slope of dose-response curve for 18-norestrone, which was similar to that for estradiol-17β. Why such structural modifications as removal of angular methyl group at carbon 13 or incorporation of oxygenated functions at carbon 6 or carbon 16 should produce decreases in slope of dose-response curve is unclear at present time.

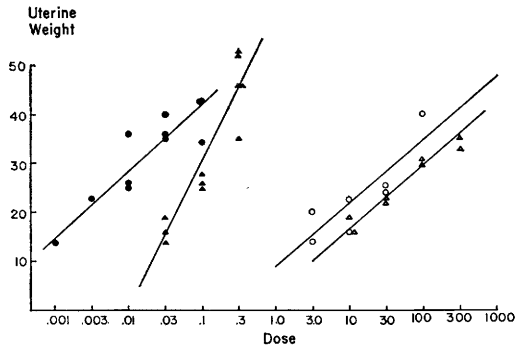

FIG. 1. Effects of estrone (▲), estradiol-17β (●), 18-norestrone (△) and 18-norestradiol-17β (○) on uterine growth in immature intact female mice. Each point represents one mean of 8-10 mice. Curves were fitted by the method of least squares.

Summary. Removal of the angular methyl group of estradiol-17β to form 18-norestradiol-17β results in a decrease in potency. A similar structural modification of the estrone molecule, forming 18-norestrone, produces both potency decreases and alterations in slope of the dose-response curve.

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Absence of Sialic Acids and Other Carbohydrates in Crystalline Papain.* (26086)

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Studies from this laboratory have been concerned for some years with the enzymic activity and structure of crystalline papain (1). Studies of the composition of this protein by Smith, Stockell and Kimmel(2) indicated that non-amino acid constituents were probably absent since the recovery of amino acids accounted satisfactorily for the weight and nitrogen content of the protein. Moreover, both Close, Moore and Bigwood(3) and Smith *et al.*(2) could detect no carbohydrate by the carbazole method. Recently, Loo, Lee and Flemming(4) reported that crystalline papain contains sialic acid by the direct Ehrlich method. It would be surprising indeed if sialic acid were present in a protein which

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gives a negative carbazole test, since sialic acid is generally associated with other carbohydrate constituents. Nevertheless, it was essential to reexamine papain for possible presence of various carbohydrate constituents. A study of the complete amino acid sequence of papain is currently in progress in this laboratory(1) and the presence of carbohydrate constituents could be of major importance.

Materials. Recrystallized papain was prepared from papaya latex by the method of Kimmel and Smith(5) and dialyzed against water. Specific activity (C_1) of this preparation was 2.0, among the highest found for any preparation(1). The purity of the enzyme has been established by a variety of methods(1).

Methods. Papain was heated in 0.05 N sulfuric acid at 90°C for 1 hour by the method used by Svennerholm(6) for determination of sialic acids. For chromatography, the hydrolysate was neutralized with barium hydroxide, and the barium sulfate precipitate and the soluble protein were separated by dialysis against several changes of water. The combined dialysates were collected and concentrated under vacuum below 28°C to a convenient volume for chromatography.

Aliquots were spotted on Whatman No. 1 paper and the chromatograms developed in the N-butanol-pyridine-water (6:4:3) solvent of Jeanes *et al.*(7). The chromatograms were sprayed with the thiobarbituric acid reagent of Warren(8) or the orcinol-trichloroacetic acid reagent of Klevstrand and Nordal (9), which was used for detection of sialic acids by Blix *et al.*(10).

Papain was also tested for sialic acids by both the direct Ehrlich method and by Bial's orcinol method as described by Werner and Odin(11).

Colorimetric analysis for hexoses was performed by the orcinol-sulfuric acid method, essentially as described by Winzler(12) for determination of protein-bound hexoses in serum proteins.

Results and discussion. A color yield corresponding to a sialic acid content of 1.2% was obtained by the direct Ehrlich method in agreement with the results of Loo *et al.*(4) who reported 1.1% sialic acid by the same method. However, sialic acids could not be detected chromatographically in aliquots of papain hydrolysates equivalent to 0.7 to 0.8 μmoles of papain, either by the thiobarbituric acid reagent or the orcinol-trichloroacetic acid reagent. In addition, the sialic acid content of papain was found to be less than 0.05% with Bial's orcinol method.

Examination of papain by the orcinol-sulfuric acid method showed that this protein contains less than 0.05% hexose when compared with a standard solution containing equimolar quantities of galactose and mannose.

Both the direct Ehrlich method and Bial's orcinol method for the determination of sialic acids lack specificity. Bial's orcinol reagent develops significant color with pentoses and, to a lesser extent, with hexoses. The orcinol-sulfuric acid reagent produces color reactions with hexoses, pentoses and uronic acids. The negative results with these last two tests indicate the absence of all these sugars as well as of sialic acids from papain.

It is evident from our previous and present studies that papain appears to be a simple protein devoid of carbohydrate constituents.

Summary. Studies of crystalline papain indicate that this protein does not contain carbohydrate. A report that sialic acid is present in this protein could not be confirmed by methods which are specific for these substances.

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Fluorescent Antibody Technic for Sero-diagnosis of Schistosomiasis in Humans.* (26087)

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The diagnostic value of fluorescent antibody procedures has been adequately demonstrated in a wide variety of infectious diseases (1,2,3,4,5,6). Except for a study by Jackson (7,8), conducted to locate antibody binding sites in or about *Trichinella spiralis* and *Nippostrongylus muris* in infected animals, the fluorescent antibody technics have not been applied heretofore to the field of helminthology.

This report describes a fluorescent antibody test developed for laboratory diagnosis of schistosomiasis. The procedure was evaluated with human sera and results compared with those obtained by the slide flocculation test described recently by one of us(9).

Materials and methods. During preliminary investigations, eggs, miracidia, cercariae and adults of *Schistosoma mansoni* were stained by fluorescein tagged antisera. Brightest fluorescence and greatest uniformity of results were obtained with cercariae. Therefore, *S. mansoni* cercariae, freshly emerged from laboratory infected *Australorbis glabratus* snails, were used throughout these studies.

Human sera from 227 well-documented cases were tested by the fluorescent antibody test. Of these, 206 were also tested by the slide flocculation test. All schistosomiasis sera were obtained from persons in whom the diagnosis had been established by demonstrating the eggs in stools or urine. Some of the sera had been treated with merthiolate and others showed obvious signs of bacterial contamination. However, neither merthiolate in the amounts used for serum preservation nor the contamination seemed to interfere greatly with the reactions with either test. To determine the specificity of the reaction, sera from individuals with proven trematode, cestode, nematode, protozoan, mycotic and bacterial infections were tested. To test whether the presence of auto-antibodies would give rise to false positives, sera of patients with lupus erytemathosus were included. To test whether the positive reactions obtained in schistosomiasis patients might be due to nonspecific factors deriving from liver damage, a few sera from persons with other diseases of the liver were also included. Normal sera were obtained from healthy donors undergoing physical examination as candidates for appointment to the U. S. Military Academy. All sera were heated at 56°C for 30 minutes before being tested.

The indirect technic using a labelled goat antihuman globulin was employed. Dilution of sera and globulins were prepared in 0.01 M phosphate buffered saline at a pH of 7.2 (PBS). Labelled antihuman globulin was prepared by conjugation with fluorescein-iso-

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