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Chemical Nature of the Cyst Wall in Human Intestinal Protozoa.

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The chemical nature of the cyst wall of human intestinal protozoa was studied on fresh material in human stools of cysts of *Endamoeba histolytica, Endolimax nana, Councilmania lafleuri,* and *C. dissimilis,* and *Giardia lamblia.*

Notably in *Councilmania lafleuri* with reagents of high osmotic pressure such as 50% H_2SO_4 and concentrated lactic acid there is at first a caving in at the pore. Later there is a return to normal contour. In lactic acid the pore is thereafter evident. In 50% HNO_3, 50% acetic acid, and 50% H_2SO_4, there is an early extension of the plug of the pore in the amoeba and in *Giardia* at the smaller end.

Various authors have referred to the cyst wall as being probably chitin, pseudo-chitin(?), or cellulose. The following tests made by us for chitin were negative: Zander's[1] iodine-zinc chloride test, Kuhnelt's[2] sulfuric acid-iodine test, and the picro-nigrosine stain. The amyloid test for cellulose was also negative.

Untreated cyst walls give a violet color reaction with Mayer's muchaematin stain. Cysts treated with dilute basic solutions to remove extraneous mucus no longer gave the mucin reaction. If the cyst wall is covered with a thin layer of mucin (secreted by the protozoan itself), the methods used to remove extraneous mucus will also remove the mucin. There is no possibility of extending this part of the investigation, since it is impossible to obtain mucus-free cysts. When the extraneous mucus and possibly the mucin is removed (use of basic solutions) there still remains the cyst wall apparently unaltered.

Ujihara[3] states that the cyst wall is possibly lipoidal in nature. The following lipoid solvents were used with negative results; ether, chloroform, alcohol, and xylol. The cysts were also unaffected by heating for 2 hours at a temperature of 140° C in 2% KCN (Topley and Wilson[4]).

[1] Zander, E., *Arch. f. Physiol. von Pflüger,* 1897, **66**, 545.

[2] Kuhnelt, W., *Biol. Zentralbl.,* 1928, **48**, 374.

[3] Ujihara, K., *Zeitschr. f. Hyg.,* 1914, **77**, 229.

[4] Topley, W. W. C., and Wilson, G. C., "The principles of bacteriology and immunity," 1929, **2**, 837.

The protein nature of the cyst wall was demonstrated by the positive xanthoproteic reaction; Millon's reagent also gave a faint positive reaction. We found the cyst wall to be insoluble in the following dilute acids: acetic, lactic, hydrochloric, and sulfuric. It was only soluble after boiling or a prolonged treatment in the following strong acids: hydrochloric, nitric, and sulfuric. In the strong acids a slight swelling was noticeable previous to hydrolysis. Insolubility of the cyst wall was demonstrated in the following bases: KOH, NaOH, and NH_4OH. A slight swelling was also noticeable in these reagents.

The following enzymes were used: Trypsin (Pfanstiehl), Bactotrypsin (Digestive Ferments Co.), Pepsin (Merck) (acidulated with HCl), Pancreatin (Eli Lilly & Co.), and Steapsin (Eimer & Amend). With the exception of pepsin no visible changes were evident; however in pepsin a pore was made distinctly visible.

There is no evidence to show that the cyst wall is of a carbohydrate, lipoidal, or chitinous nature. Due to the behavior of the cyst wall in the various reagents it seems only possible that the cyst wall belongs to the albuminoid or scleroprotein group. The albuminoid or scleroprotein group contains the following proteins: the keratins, fibroin, elastin, spongin, collagen, and sericin.

The following of the scleroprotein group are extremely insoluble: keratin (scales, feathers, hair, wool, horns, hoofs, nails, and silk), fibroin (substances covering the core of silk fibers), elastin, spongin (skeleton or ordinary bath sponges), and sericin (silk gelatin, forms the outer coating of the silk fiber). Since the cyst wall is extracellular, and primarily supporting or protective in function, it seems appropriate to place the cyst wall either in the group of connective tissue proteins, namely elastin, or in the group of epidermal proteins, namely, the keratins. Boiling in water and tests with acetic acid eliminate the possibility of collagen.

According to Lloyd[5] elastin does not swell in acid or basic solutions, and is rapidly digested by trypsin, while the keratins are not attacked by pepsin, trypsin, or bacterial tryptases. Keratins swell in the presence of strong basic solutions, while elastins do not exhibit this phenomenon.

According to Robertson's criteria[6] for the identification of proteins, the behavior of the cyst wall in various reagents shows evidences of great insolubility. The slight swelling of the cyst wall in basic solutions and its resistance to trypsin tends to differentiate

[5] Lloyd, D. J., "Chemistry of proteins," 1926, pp. 51-54.

[6] Robertson, T. B., "Principles of biochemistry," 1924, p. 134.

elastin from the keratins. As to the physical properties shown by micromanipulative studies the walls show flexibility as well as resilience or toughness.

Our studies of both the chemical and the physical properties of the cyst wall show that this structure has the properties of the group of keratins more nearly than those of any other group of scleroproteins. A positive test for unoxidized sulfur or for a high cystine content would convincingly demonstrate that the cyst wall is keratin; however, due to the small quantity of material present in the cyst wall, it is at present impossible to apply such tests for cystine.

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Digestible Constituents in Tubercle Bacillus.

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In 3 investigations, Loeffler,[1] Robinovitsch,[2] and Day and Gibbs[3] found that living tubercle bacilli were killed in about 48 hours when mixed with pancreatic enzymes. According to Loeffler, the live bacilli were digested, according to Day and Gibbs they were not. None of the foregoing papers records the use of quantitative biochemical methods for detecting tubercle bacillus digestion. Nor was consideration given to the possibility that this might take place without complete dissolution of the bacterial cell.

One of the objects of the present work was to determine by quantitative methods, the extent to which tubercle bacilli undergo digestion by pancreatic enzymes acting together. In all the experiments, human and bovine strains grown on Long's synthetic agar medium were used. The nearly dry masses of bacilli were easily lifted off the agar, weighed and mixed at once with commercial pancreatic enzyme preparations.

Digestible Carbohydrate. Series of June, 1928-May, 1929. Suspensions of tubercle bacilli in chloroform Ringer solution were incubated with and without the addition of pancreatic enzyme powder. In most of the experiments sufficient chloroform and toluol was added to promptly kill the bacilli. At convenient intervals of a few

[1] Loeffler, F., *Deutsche Med. Wochenschr.*, 1913, **39**, 1025.

[2] Robinovitsch, L. G., *Endocrinology*, 1926, **10**, 602.

[3] Day, A. A., and Gibbs, W. M., *J. Infect. Dis.*, 1930, **46**, 26.