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Bacterial proteins.*

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The object of this work was the study of composition of bacteria so far as the proteins are concerned. It seemed to us of considerable importance to know the character of the proteins that exist in micro organisms, and further it was hoped to get definite information concerning the relative toxicity of these proteins. One of us (P) had available a supply of dried bacteria, which we had hoped would suffice for this study. Preliminary work on these dried bacteria showed the amounts on hand to be inadequate.

The first steps in the process consisted of the preparation of large amounts of bacteria. Three types of bacteria were used in this study, namely, the colon, pyocaneus, mucosa capsulatus. They were grown in pure culture on broth and were removed from the broth by centrifugalization in a Sharpless centrifuge. They were suspended in physiological salt solution and again thrown out by the use of the centrifuge, and the process repeated until they were washed free from the broth. They were then spread in thin layers on glass plates and dried.

A number of different methods were tried to disrupt bacteria, among them placing the bacteria under high pressure, then suddenly releasing this pressure, and grinding in a small glass ball mill, neither of these methods proved very successful. The method finally adopted was the grinding of the bacteria in a large porcelain ball mill in paste form with toluol for 48 hours, and subsequently with a solvent for another 48 hours. The resultant mixture was centrifugilized. The supernatant liquid was saturated to 33 per cent with ammonium sulphate. The filtrate from this precipitate was raised to 46 per cent, saturation with ammonium sulphate, filtered, and the filtrate raised to 64 per cent satu-

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ration. The filtrate from this was saturated with solid ammonium sulphate. The second extraction was made with the first solvent to remove the major portion of any water soluble protein that remained after the first extraction. The quantities obtained on the second extraction were always small in amount.

The residue from the centrifugalization was extracted for 48 hours in the ball mill with 10 per cent sodium chloride solution. After centrifugalization this extract was treated with ammonium sulphate in the same fashion as the water extract. A second extract was made with sodium chloride.

The next solvent used was 0.5 per cent sodium carbonate with which the residue was extracted for 48 hours in the ball mill. This was treated as the previous extracts. By this method we obtained evidence of the presence of albumin, globulin and alkali soluble protein. The amounts obtained, however, were not sufficient toxicity tests as shown by the negative results of our experiments with the available material.

In order to obtain sufficient material for toxicity tests a larger portion of the dried colon material was treated in the fashion indicated in the ball mill for the extraction of the water soluble protein. The following tests were made on this protein. After being dried this material was not completely soluble. The figures show the amount of soluble material in the solution used.

TOXICITY EXPERIMENTS.

Guinea pigs were used in these experiments. They each weighed around 250 gm.

I.

Dried Colon.

2.5 mg. suspended in .5 cc.—0.9 per cent NaCl—Sick.
5 mg. suspended in 1 cc.—0.9 per cent NaCl—Died 9½ hours later.
7.5 mg. suspended in 1.5 cc.—0.9 per cent NaCl—Died 5 hours later.
10 mg. suspended in 0.2 cc.—0.9 per cent NaCl—Died 6 hours later.
15 mg. suspended in 3 cc.—0.9 per cent NaCl—Died 5 hours later.
20 mg. suspended in 4 cc.—0.9 per cent NaCl—Died 3 hours later.

Colon Protein.

.22 mg. dissolved in 0.1 cc.—0.9 per cent NaCl—No effect.
.44 mg. dissolved in 0.2 cc.—0.9 per cent NaCl—No effect.
1.1 mg. dissolved in 0.5 cc.—0.9 per cent NaCl—No effect.
3.3 mg. dissolved in 1.5 cc.—0.9 per cent NaCl—No effect.
4.4 mg. dissolved in 2 cc.—0.9 per cent NaCl—Died 5 hours later.
6.6 mg. dissolved in 3 cc.—0.9 per cent NaCl—Died 5 hours later.
8.8 mg. dissolved in 4 cc.—0.9 per cent NaCl—Died 4 hours later.
13.2 mg. dissolved in 6 cc.—0.9 per cent NaCl—Died 2½ hours later.

II.

Colon Protein.

.75 mg. dissolved in 0.5 cc.—0.9 per cent NaCl—No effect.
1.5 mg. dissolved in 1 cc.—0.9 per cent NaCl—No effect.
2.25 mg. dissolved in 1.5 cc.—0.9 per cent NaCl—No effect.
3 mg. dissolved in 2 cc.—0.9 per cent NaCl—No effect.

4.5	cc.	dissolved in 3	cc.—0.9 per cent NaCl—No effect.	
6	mg.	dissolved in 4	cc.—0.9 per cent NaCl—Died several hours later.	

III.

.16	mg.	dissolved in .2	cc.—0.9 per cent NaCl—No effect.	
.4	mg.	dissolved in 5	cc.—0.9 per cent NaCl—No effect.	
.8	mg.	dissolved in 1	cc.—0.9 per cent NaCl—No effect.	
1.2	mg.	dissolved in 1.5	cc.—0.9 per cent NaCl—No effect.	
1.6	mg.	dissolved in 2	cc.—0.9 per cent NaCl—Died at the end of 48 hrs.	
2.4	mg.	dissolved in 3	cc.—0.9 per cent NaCl—No effect	
2.8	mg.	dissolved in 3.5	cc.—0.9 per cent NaCl—Died at the end of 46 hrs.	

CONCLUSIONS.

Our experiments show the presence of albumin globulin and alkali soluble protein in the colon.

The toxicity of the water soluble protein shows no marked deviation from that of the colon itself.

The work on the colon bacterial proteins will be continued on much larger amounts of the dried organism.

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The influence of posture of the neck on progression of the fowl (Gallus domesticus).

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In the course of our investigation of the body righting reflexes in the fowl, a detailed report of which will appear shortly, we had one animal which as a result of a cranial operation assumed a peculiar posture at certain times. It would keep its head down and tail up, so that the long axis of the body was at an angle about 60° to the horizontal. When in such a condition, the animal invariably walked backward and continued to do so till it would hit the wall of the room. But when it kept its head in a normal position it could and did walk forward.

In order to find out the mechanism of this phenomenon we studied a number of normal Leghorn and Sebright Bantam fowls in which the lower portion of the neck was kept flexed upon the trunk by means of a gauze bandage. We found that these ani-