

oculated tubes of the toxin-broth mixture were of course incubated at the same temperature over the same period of time and subjected to the same filtering process in order to serve as controls. The unit amounts of toxin given in Table I are in terms of approximate m.l.d. values as determined on the control tubes after incubation.

The results obtained with these organisms do not reveal a very active destruction of the toxin (not nearly so great as results we have obtained with other organisms) but sufficient, we believe, to explain the fact that the ingestion of small numbers of spores of the organism of botulism leads to no apparent ill effects.

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The Labile Sulfur of Insulin.

VINCENT DU VIGNEAUD. (Introduced by John R. Murlin.)

From the Department of Vital Economics, University of Rochester.

All observers agree that insulin material contains sulfur and Abel¹ has shown that sulfur is present in a very labile form, being split off as a sulfide by boiling with weak alkali. Abel believes that the sulfur is roughly proportional to the hypoglycemic potency. Brand and Sandberg² have shown that although the sulfur of cystine is relatively stable, the linkage of other amino acids with cystine so affects the sulfur that it is easily split out by boiling with weak alkali. Cystine can be determined quantitatively by the uric acid reagent of Folin and Denis, as shown by Folin and Looney.³ The cystine is first reduced by sodium sulfite in saturated sodium carbonate to cysteine which then gives a deep blue color with the phosphotungstic acid reagent. Shonle and Waldo⁴ found that insulin preparations respond to this test for cystine.

I have observed that insulin preparations do not give the reaction directly with the uric acid reagent but do so after reduction with sulfite. The insulin material upon boiling for thirty minutes with 0.1 *N* Na₂CO₃ gives a positive reaction without previous reduction, due to the formation of sulfide ion. Acid hydrolysates of the insulin material give the reaction only after reduction with sulfite. They do not, however, give the reaction directly with the uric acid reagent after boiling with 0.1 *N* Na₂CO₃, showing that the sulfur has somehow become more stable in the fragment split off by acid hydrolysis. It is necessary to reduce first with sodium sulfite to obtain the reaction as in the case of the unboiled acid hydrolysate.

This difference in the stability of the sulfur in the original and in the acid hydrolysed material and the behavior towards the uric acid reagent can be explained on the assumption that a S-S linkage exists in the insulin material. In the acid hydrolysate the sulfur is in a more stable form but when the fragment containing the sulfur was linked with the other groups the sulfur became labile. This is similar to the effect which the linkage of other amino acids to cystine has on the stability of sulfur in cystine. The general behavior, therefore, is very much like what would be expected of an amino acid complex containing cystine. There is, as yet, however, no proof that insulin as such contains this sulfur; for ordinary proteins like casein, gelatine, egg albumin, and Witte's peptone behave similarly to insulin in their response to the uric acid reagent under the above conditions.

The reactions mentioned above are being studied on a quantitative basis to correlate, if possible, the potency of insulin preparations with the intensity of the reactions.

This is a preliminary report.

¹ Abel, John J., and Geiling, E. M. K., *J. Exp. Ther.*, 1925, xxv, 423.

² Brand, E., and Sandberg, Marta, *J. Biol. Chem.*, 1926, lxx, 381.

³ Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, li, 421.

⁴ Shonle, H., and Waldo, J. H., *J. Biol. Chem.*, 1924, lviii, 731.

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Method for Study of Muscular Activity of Intestinal Segment During Perfusion Through its Lumen.

JOHN B. POLANSKY. (Introduced by John R. Murlin.)

From the Department of Vital Economics, University of Rochester.

A method for the study of the muscular activity of the intestinal segment permitting a continuous flow through its lumen of a fluid which is readily replaceable by another is presented. A segment, suspended vertically, is attached by its cephalad end to a stationary glass inlet-tube and by its caudal end to a movable glass outlet-tube. The stationary tube immediately branches into two, each branch receiving fluid from a bottle which delivers at constant pressure. In both bottles the pressure is adjusted to the same value before the actual experiment is begun, so that, when one fluid replaces the other, any change in the muscular activity of the segment cannot be