

The above cited tests though encouraging are by no means conclusive or definite as yet. Much more work must be done to be sure of the specificity of the flocculation test for scarlet fever serums and toxins, as in the case of diphtheria.

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Note on the relationship between insulin and trypsin.

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Epstein¹ and his co-workers have recently claimed that insulin is not digested by trypsin and pepsin. It is supposed to form an inactive addition product with trypsin, which is stable at an alkaline pH, and from which the insulin can be reactivated by acidifying. A similar reaction occurs with pepsin, but under opposite conditions as far as the pH is concerned. The physiological and clinical aspects of these *in vitro* experiments were followed up by Epstein² and his co-workers by *in vivo* experiments and perfusion studies of the pancreas.

Epstein's results, however, as far as the perfusion experiments go, were contradicted by W. S. Collens,³ and his *in vivo* experiments could not be supported by D. A. Scott.⁴ As to Epstein's *in vitro* experiments, Scott was able to confirm that insulin forms an inactive addition compound with trypsin, from which it can be reactivated. But he found, in accord with previous investigators, that trypsin digests insulin. The fact that acid reagents reactivate insulin from its inactive addition compound with trypsin, led Scott to point out other results which indicate that insulin may exist in the pancreas and in the body in an inactive form.

¹ Epstein, A. A., and Rosenthal, N., *J. Am. Med. Assn.*, 1924, lxxxii, 1990; Epstein, A. A., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxii, 9; Epstein, A. A., and Rosenthal, N., *Am. J. Physiol.*, 1924, lxx, 225.

² Epstein, A. A., and Rosenthal, N., *Am. J. Physiol.*, 1925, lxxi, 316.

³ Collens, W. S., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 367.

⁴ Scott, D. A., *J. Biol. Chem.*, 1925, lxxiii, 641.

The finding that more insulin is extracted from tissues by acid reagents than by water cannot be taken as evidence that insulin exists in the tissues in an inactive form. This phenomenon is a consequence of the chemical properties of amphoteric colloids.

Scott suggests that the primary action between trypsin and insulin is a mutual adsorption phenomenon, which is followed by complete proteoclastic destruction. He states that this is in accord with the theory of enzyme action, referring to the textbook of Bayliss.⁵ Bayliss, from a colloidchemical point of view, supports the idea of enzyme and substrate forming an adsorption compound, and bases their relation on the so-called adsorption-isotherm. Diametrically opposed to this view are the more recent views of the majority of investigators who consider the reaction between enzyme and substrate as of a purely chemical nature following the mass law. For those who do not accept the view of Bayliss, the theory of Scott is also not acceptable.

We were interested in the problem of the insulin-trypsin reaction from quite a different point of view from that of Epstein and Scott. In this connection we should like to mention a few experiments which were carried out about a year ago. Recently, Willstätter and Waldschmidt-Leitz were able to decide between the different theories on the action of enterokinase on trypsin, in favor of the so-called complement theory. Waldschmidt-Leitz showed definitely, confirming previous investigators, that the reaction between kinase and trypsin involves the formation of a loose addition compound, and that this combination follows stoichiometrical laws. These interesting results and the inexplicable phenomenon of certain inhibitors of the tryptic system which are present or formed in the pancreas, induced us to study the influence of insulin on tryptic digestion. We used in our experiments a trypsin preparation prepared from dried pancreas according to Willstätter,⁶ which already showed the so-called spontaneous activation. We found no change in the tryptic activity⁷ either in the presence of insulin or after previous treat-

⁵ Bayliss, W. M., *The nature of enzyme action*, New York, 3rd edition, 1914.

⁶ Willstätter, R., and Waldschmidt-Leitz, E., *Ztschr. f. physiol. Chem.*, 1923, cxxv, 132.

⁷ In our experiments we used the method of Willstätter and Waldschmidt-Leitz, *Ztschr. f. physiol. Chem.*, 1924, cxxxii, 181. This method, however, has to be modified according to the latest results of Willstätter, cf. Willstätter, R., and Persiel, H., *Ztschr. f. physiol. Chem.*, 1925, cxxxiii, 245.

ment with insulin at different hydrogen ion concentrations. At that time we saw no necessity for publishing these results.

Epstein's first communications caused us to take this question up again. While the interpretation of our experiments seemed to permit the formation of an addition compound between insulin and trypsin, it did not agree with Epstein's finding that trypsin does not digest insulin. The formation of an insulin-trypsin compound without subsequent digestion would mean that the combination between trypsin and insulin is of a different nature from the combination between trypsin and gelatine, or trypsin and casein. In this case, according to theoretical considerations,⁸ insulin might be expected to act as an inhibitor of tryptic digestion, while, in our experiments, it did not act in this way. In the first place our preliminary experiments showed, in accord with Scott, and contrary to Epstein, that trypsin digests insulin. This result eliminates the necessity of assuming that the trypsin-insulin compound is of a different nature from the trypsin-gelatine compound.

Many of the earlier investigators as well as more recent authors like Michaelis, Euler, Willstätter and Kuhn consider the intermediate compound between enzyme and substrate the necessary base for enzyme action. Northrop,⁹ however, in a series of exact and mathematically analyzed investigations on pepsin and trypsin has found that his results could not be accounted for by the assumption of an intermediate compound between enzyme and substrate.

The insulin-trypsin and insulin-pepsin reactions now seem to furnish the first biological evidence of the existence of the intermediate compound between enzyme and substrate. It is significant that the intermediate compound between enzyme and substrate in the case of trypsin and insulin is apparently formed involving the physiologically active group of insulin.

Summary.

1. Insulin combines with trypsin not as an inhibitor of trypsin.
2. Trypsin combining with insulin inhibits the action of the

⁸ cf. Michaelis, L., and Rona, P., *Biochem. Ztschr.*, 1914, lx, 62.

⁹ Northrop, J. H., *J. Gen. Physiol.*, 1922, iv, 487; Northrop, J. H., *Die Naturwissenschaften*, 1923, xi, 713; Northrop, J. H., *J. Gen. Physiol.*, 1924, vi, 239.

latter at first reversibly and later irreversibly, probably by digestion.

3. It is possible that further study of the nature of the insulin-enzyme reaction will help to decide whether proteolytic enzymes can act without the formation of an intermediate compound between enzyme and substrate.

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Influence of Ultra-violet radiations on basal metabolism in children.

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When malnourished children are exposed to ultra-violet radiations, there is often an improvement in their appetite and a gain in weight. The cause for this phenomena has not been satisfactorily explained. Until recently the impression has been that there is a concomitant rise in the basal metabolism. Otto Kestner¹ and his co-workers have demonstrated that there is an immediate rise in the basal metabolism, which, as far as they investigated, remains elevated for only a short time. Therefore the present work was undertaken to ascertain whether this rise in the basal metabolism extended over a long period of time, and whether it bore any relation to the clinical results.

The investigation was carried on from the beginning of December to the end of March, a period when the intensity of the ultra-violet rays from the sun is not sufficiently strong to influence results. Three children on the Pediatric Service of the Mount Sinai Hospital were studied. Two were free from all organic defects, while the third had a simple goitre. To obtain comparative figures these children were observed in the hospital for a control period of one month. They were on a fixed calculated diet, and their basal metabolism was determined three times

¹ Kestner, Peemoller, Plaut, *Klin. Therap. Wchnschr.*, No. 44, Oct., 1923, p. 2018.