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On the nature of the anti-tryptic action of serum and its biologic significance.

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The power which serum possesses to inhibit tryptic digestion is a well known phenomenon, but the nature of the process is not well understood. This is evidenced by the fact that the anti-tryptic power of serum has been variously ascribed to the proteins, the lipoids, and the nitrogenous crystalloids of the serum, and more recently to the saponified unsaturated fatty acids present in it.

The observations to be reported were made in the course of a study begun two years ago on the isolation of the different pancreatic ferments. For reasons which will be discussed at another time, the anti-tryptic action of serum was investigated. At first an inquiry was made into the particular substance in the serum which was endowed with the power to inhibit tryptic digestion. Our results were in conformity with those who found that the albumin fraction of the serum proteins was chiefly responsible for the phenomenon. It may be added that other substances contribute to the total inhibitory action of serum. The globulins, for example, possess the power to a limited degree, and only when present in concentration. While studying this phenomenon with whole serum, in great dilution, it was found that the degree of inhibitory action was proportionate to the quantity of albumin present. Under such conditions other substances apparently played no part in the reaction. The inhibitory action obtained from the fatty substances present in the serum is insignificant, when contrasted with the remarkable power which whole serum or its albumin fraction possesses.

The next question investigated was the nature of the inhibitory action. You will recall that when the anti-tryptic action of serum was first observed it was thought to resemble the action of the specific anti-body of the immune system. This view was soon abandoned and the multiplicity of opinions concerning the

particular substance which causes the inhibition, existing at present, left this question entirely unsettled.

The first point considered was the fate of trypsin in mixtures with serum. Several effects seemed possible; (1) that trypsin was destroyed by the serum and that the action was purely toxic; (2) that the reaction between trypsin and serum was one of physical adsorption; and (3) that the reaction might be of a chemical nature.

In some previous work reported before this society¹, it was found that trypsin could be easily isolated from fresh and commercial extracts of the pancreas in a state of great purity by means of colloidal iron. It seemed possible, therefore, that this method of isolating the ferment might prove of service in studying the problem in hand; and indeed such was the case.

By means of adequately chosen experiments it was found that trypsin could be recovered quantitatively from mixtures with serum even after prolonged periods of incubation. Other means of isolating trypsin were also found, for example, by treating mixtures of trypsin and serum with adequate amounts of ethyl alcohol, in which case the proteins of the serum are precipitated and the trypsin remains in solution. It might be added that the latter method is not quantitative.

These experiments indicate, I believe, the following: that the anti-tryptic action of serum is not due to a destruction of the ferment by the serum, but that the inhibitory action of serum is either due to an adsorption of the trypsin by the serum, or that the reaction is a chemical one, of a reversible nature.

When mixtures of trypsin and a digestible substrate such as casein are treated with alcohol, after a brief incubation, some of the trypsin can be recovered. After digestion of the casein is completed none of the trypsin can be recovered. When such mixtures are treated with colloidal iron the trypsin can not be isolated by reason of the fact that the reaction takes place between the casein and the iron, forming casein-ferrate. When, however, serum is first added to a mixture of trypsin and casein, in sufficient amount to cause inhibition of digestion, and this is then treated with colloidal iron, both the serum proteins and the casein are precipitated and the trypsin remains in solution. The

¹ Epstein, Albert A., *PROC. SOC. EXPER. BIOL. AND MED.*, 1921, xix, 3.

same result is obtained when such mixtures are treated with ethyl alcohol. These experiments justify the conclusion that the inhibitory action of serum on trypsin is in the nature of an interference phenomenon. That is to say, that the serum protein, either by virtue of a chemical reaction with the casein or a simple mechanical process acts as a protective colloid. This conclusion, however, does not entirely rule out the possibility that a chemical reaction takes place between trypsin and serum which is readily dissociable.

In the communication on trypsin alluded to above¹, I stated that the ferment appears to be of a strongly acid character. As is known it has a positive electric charge, whereas serum albumin which is chiefly concerned in the anti-tryptic phenomenon, is said to have a negative charge, so that a reaction between the two is conceivable.

As to the biologic significance of the anti-tryptic action of serum, I will say only a few words at the present time. Serum possesses diastatic and lipolytic ferments; tryptic ferments on the other hand are not ordinarily demonstrable. Because of the anti-tryptic action, failure to demonstrate trypsin in serum does not necessarily imply that no trypsin is present. However, by the methods suggested trypsin can be recovered from serum. Therefore the question arises as to the source of all these ferments and the relation which the pancreas bears to them. There are two possibilities, namely: (1) that the ferments arise from the pancreas, and (2) that they arise from the tissues and that the pancreas serves as an organ of excretion. This phase of the subject will be taken up at another time.