

THE INACTIVATION OF TRYPSIN.

IV. THE ADSORPTION OF TRYPSIN BY CHARCOAL.

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It has been frequently observed that trypsin is more or less completely removed from solution by charcoal. The reaction has been studied in detail by Hedin¹ who found that the amount removed depended on the time of standing, the order in which the components of the solution were mixed, and, in general, behaved as a typical heterogeneous reaction. Hedin also studied the inhibiting action of serum on trypsin and concluded that it was analogous to the action of charcoal. Hussey and the writer² have found, however, that the reaction between the inhibiting substance and trypsin could be accurately calculated by the laws of homogeneous reactions. The reaction was found to be completely and instantly reversible and independent of the order in which the components were mixed. The discrepancy between these results and those obtained by Hedin is due to the fact that Hedin allowed the mixture of trypsin and plasma to stand some time before measuring the amount of trypsin combined. Under these conditions the trypsin undergoes a secondary irreversible inactivation which complicates the results. It is quite true that under these conditions the order of mixing, etc., plays an important part, as Hedin found. This is due, however, as the writer showed³ in the case of the equilibrium between trypsin and the inhibiting substance (formed by its action on proteins), to a secondary irreversible inactivation of the trypsin and is not a property of the reaction between the trypsin and

¹ Hedin, S. G., *J. Physiol.*, 1904-05, xxxii, 390; *Z. physiol. Chem.*, 1906-07, 1497; *Biochem. J.*, 1906, i, 484; 1907, ii, 81; *Ergebn. Physiol.*, 1910, ix, 433.

² Hussey, R. G., and Northrop, J. H., *J. Gen. Physiol.*, 1922-23, v, 335.

³ Northrop, J. H., *J. Gen. Physiol.*, 1921-22, iv, 261.

the inhibiting substance. In order to confirm this explanation and to be sure that the reaction with charcoal differs from that with serum and the other inhibiting substances studied, it seemed important to repeat the experiments with charcoal under the same conditions that were used in the experiments with serum. The present paper is a report of these experiments. The results obtained with charcoal confirm those of Hedin, but are qualitatively different from those with serum. There is no analogy therefore between the two reactions and therefore no reason for considering the reaction with serum as heterogeneous.

Experimental Methods.

The trypsin was determined, as described by Hussey and the writer,⁴ by noting the time required to cause a 10 per cent change in the viscosity of a standard gelatin solution. The charcoal used was a preparation manufactured by the General Chemical Company and labelled "decolorizing charcoal." It was finely ground and washed with water and acetone before use.

A 2 per cent solution of Fairchild's trypsin in glycerin was used as a stock solution. It was diluted 20 times with water or buffer solution.

The relation between the amount of trypsin removed from solution and the quantity of charcoal is shown in Fig. 1. The curve is of the general form obtained either in adsorption reactions or with reversible homogeneous equilibria. It can be calculated fairly well on either basis but since the adsorption formula is without theoretical interest and the reaction cannot be considered as a reversible equilibrium, it is unnecessary to give the figures. The experiment shows, however, that very little can be deduced from the results of experiments in which the amount of one of the components alone is varied. The results, as a rule, can be calculated either as homogeneous or heterogeneous reactions. The reversibility of the reaction is a much more useful test. Heterogeneous reactions, as a rule, are slowly and *incompletely* reversible, whereas homogeneous equilibria, and especially ionic reactions, are rapidly and completely reversible. As was stated above, the

⁴ Northrop, J. H., and Hussey, R. G., *J. Gen. Physiol.*, 1922-23, v, 353.

reaction between trypsin and the inhibiting substance in serum is rapidly and completely reversible, while the reaction with charcoal is not.

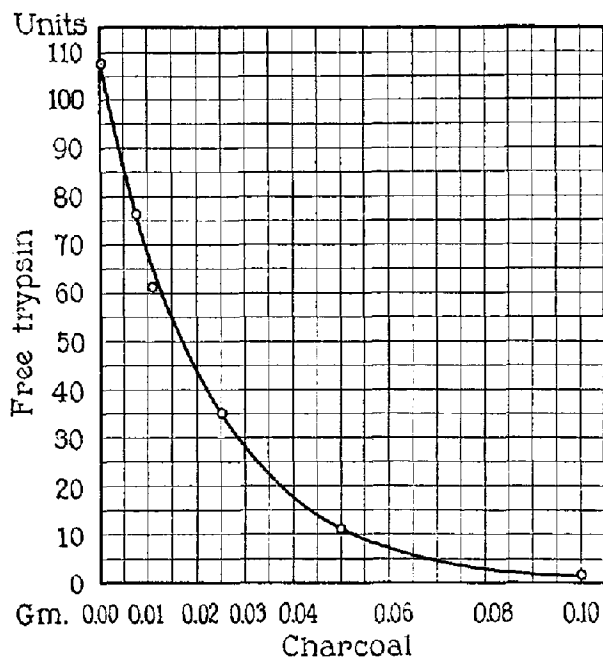


FIG. 1. The effect of increasing amounts of charcoal on the removal of trypsin from solution. 5 cc. of a 0.1 per cent trypsin solution in glycine phosphate acetate buffer pH 8.0 added to the amounts of charcoal indicated. Kept 0.5 hour at 25° C., centrifuged, and trypsin determined in 0.2 cc. of the supernatant liquid.

Effect of the Acidity of the Solution.

The result of an experiment at various pH is given in Table I. The amount of trypsin combined with charcoal is evidently independent of the pH within a wide range. This is entirely different from the results obtained when pepsin combines with solid protein.⁵ Unpublished experiments show that trypsin also combines with solid protein and that the combination is markedly affected by the pH.

⁵ Northrop, J. H., *J. Gen. Physiol.*, 1919-20, ii, 113; 1920-21, iii, 211.

The mechanism is apparently entirely different from the reaction with charcoal and is closely connected with the Donnan equilibrium.

Effect of the Order of Mixing the Components.

The amount of trypsin removed depends entirely on the order in which the components are mixed, as is shown in Table II. If the trypsin and charcoal are mixed first, a very much greater amount of

TABLE I.

Effect of the pH on the Adsorption of Trypsin by Charcoal.

5 cc. of glycine phosphate acetate buffer of pH noted + 0.5 cc. of trypsin solution + 0.0125 gm. of charcoal. Kept at 20°C. for 5 minutes, centrifuged, and 0.2 cc. supernatant added to 10 cc. gelatin.

| pH..... | 9 | 7 | 5 | 3 | 7 Control, no charcoal. |
|---|------|------|------|------|-------------------------------|
| Time for 10 per cent change in viscosity, hrs..... | 0.52 | 0.49 | 0.50 | 0.48 | 0.15 |

TABLE II.

Effect of Order of Mixing.

| Method of preparing mixture. | Time required for 10 per cent change in viscosity. hrs. |
|---|---|
| 1. 1 cc. of H ₂ O + 0.2 cc. of trypsin, kept 5 min. at 20°C., 10 cc. of gelatin added..... | 0.41 |
| 2. 1 cc. of charcoal suspension (\approx 0.1 gm. of charcoal) + 0.2 cc. of trypsin, kept 5 min. at 20°C., 10 cc. of gelatin added..... | > 3.00 |
| 3. 10 cc. of gelatin + 1 cc. of charcoal suspension kept 5 min. at 20°C., 0.2 cc. of trypsin added..... | 0.42 |

the enzyme is combined than if the charcoal is first mixed with the gelatin. This is typical of a heterogeneous reaction and contrary to most homogeneous equilibria. It is exactly the opposite of the results obtained with serum or other inhibiting substances when the experiment is carried out under the same conditions.

The experiment also shows that the combination is not reversible and that the trypsin combined with charcoal is inactive.

It might be supposed that this result is due to the fact that the trypsin is combined with the gelatin in conformation with the usual hypothesis of an intermediate compound between substrate and enzyme. The failure of the trypsin to combine with the charcoal when the enzyme has been previously mixed with gelatin would then be considered as evidence for the existence of such a compound between trypsin and gelatin. This explanation, however, is incorrect as may be seen from Table III, which gives the result of an experiment in which the charcoal was previously treated with gelatin. Under these conditions the trypsin is only slightly removed although the charcoal had been washed. The gelatin evidently forms a film on the surface of the charcoal and prevents the trypsin from combining. If trypsin and gelatin combined, it might be expected that the previous treatment of the charcoal with gelatin would increase the amount of trypsin combined instead of decreasing it.

TABLE III.

Effect of Previous Gelatin Treatment of Charcoal on the Adsorption of Trypsin.

| | Units of trypsin per 0.2 cc. of solution. |
|--|---|
| 1. 0.1 gm. of charcoal added to 10 cc. of H ₂ O. Centrifuged, 5 cc. of dilute trypsin added; centrifuged after 5 min. and trypsin determined in 0.2 cc. of supernatant. | <0.10 |
| 2. 0.1 gm. of charcoal added to 10 cc. of 3 per cent gelatin pH 7.4. Centrifuged, washed with 10 cc. of H ₂ O, and trypsin added, etc., as for (1). . . | 1.0 |
| 3. Control, no charcoal. | 1.3 |

SUMMARY.

1. Charcoal removes trypsin from solution. The amount removed depends on the order in which the solutions are mixed. The reaction is not reversible and is almost independent of the pH of the solution.

2. Charcoal which has been previously treated with gelatin does not remove trypsin from solution.

3. The reaction is not analogous either to the reaction between trypsin and the inhibiting substance of serum or to the reaction between solid protein and either pepsin or trypsin.