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Influence of Iodination on Proteolytic Activity and Blood Pressure Lowering Ability of Trypsin.*

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It has been pointed out by Silva[1] and by Dragstedt and his associates[2-5] that the intravenous administration of trypsin results in a decided fall in blood pressure, and attention has been called to the similarity between trypsin shock and anaphylactic shock.[6] During the course of observations dealing with the influence of intravenously-administered trypsin on the blood pressure of dogs, it was observed that the iodination of trypsin serves to decrease its influence on blood pressure with a smaller decrease in proteolytic activity.[7]

Methods. Crude trypsin was iodinated with 0.01 N or 0.1 N iodine in the presence of starch at 38°C with the solution buffered at pH 7 with phosphate buffer. The iodine was added in successive small quantities sufficient to maintain only slight to moderate starch-iodine color in the iodinating solution. The time required varied between 30 minutes and 4 hours, according to the degree of iodination. Following final decoloration the solutions were allowed to remain at 38°C for one additional hour and ascorbic acid was added as a precaution against the presence of free iodine. Control preparations were treated in exactly the same manner except for the addition of iodine.

The concentrations of starch, excess potassium iodide, and phosphate buffer, which are known to influence the rate of iodination, were maintained constant. Blood pressure recordings were made in the usual manner and complete recovery was allowed following each injection into the femoral vein.

Since the amount of substrate digested in a given period is not directly proportional to the concentration of the enzyme, the proteolytic activities of the various preparations were estimated by determining the relative amount of each preparation required to accomplish the same degree of casein digestion as a basic amount of the untreated enzyme under standardized conditions at pH 7 and 38°C. The undigested casein was precipitated with trichloracetic acid and was estimated by the Kjeldahl procedure.

TABLE I.
Influence of Partially Iodinated Trypsin on Blood Pressure.

Milliequivalents of iodine per mg of trypsin	Decrease in blood pressure upon injecting 1.5 mg of trypsin per kg, mm
0	62
0.2×10^{-3}	55
0.4 "	60
0.8 "	39
1.2 "	6
1.6 "	7
2.0 "	4
0	58

Results. The data of Table I indicate the blood pressure changes in a single dog following the injection of a series of trypsin preparations differing in degree of iodination. There is a decided decrease in the hypotensive effect with an increase in iodination. It was further observed that limited iodination which is insufficient to decrease the hypo-

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TABLE II.
Influence of Large Doses of Iodinated and Non-iodinated Trypsin on Blood Pressure.

Dog No.	Milliequivalents of iodine per mg of trypsin	Amt of trypsin inj., mg/kg	Iodinated trypsin: Fall observed, mm	Iodinated trypsin: Duration of decrease, min	Non-iodinated trypsin: Fall observed, mm	Non-iodinated trypsin: Duration of decrease, min
10	1.6×10^{3}	20	10	2	80	35
12	2.0 "	20	32	2	80	fatal
14	2.0 "	15	5	0.25	85	50
15	2.0 "	20	10	1	70	40
16	2.0 "	15	6	1	54	35
19	2.0 "	15	11	1	62	25
17	2.0 "	10	5	1	96	75

TABLE III.
Comparison of Proteolytic Activity and Hypotensive Effect.

Dog No.	Milliequivalents of iodine per mg of trypsin	Ratios of concentrations of iodinated to untreated trypsin required to obtain same proteolysis and hypotensive effect obtained with untreated trypsin: Proteolysis	Hypotensive effect
20	1.3×10^{-3}	2.5	30-35
21	1.3 "	3.0	30-40
22	1.4 "	2.0	30
23	1.5 "	4.0	40
25	1.6 "	3.5	45
26	1.7 "	4.5	40-50
27	1.8 "	4.5	40-60
28	1.9 "	5.0	50-60
29	2.0 "	6.0	60
30	2.0 "	5.0	30

tensive effect of 1.5 mg of trypsin per kg of body weight does, however, serve to decrease the effect of smaller amounts of trypsin such as 0.5 mg per kg.

The data given in Table II show that the administration of relatively large doses of extensively iodinated trypsin does not cause the profound hypotensive changes noted with the same amount of untreated trypsin. In all cases, the iodinated enzyme was injected before the noniodinated and the blood pressure was allowed to return to the normal level before the second injection. The injections were intentionally made rather rapidly in 30 to 75 seconds according to the size of the dose and at about the same rate in each case.

The data presented in Table III refer to the results of the simultaneous comparison of proteolytic activity and hypotensive effect of each of 10 different preparations. The decrease in hypotensive activity of trypsin is accompanied by a smaller decrease in proteolytic activity. In each case the severity of shock resulting from 1 mg of untreated trypsin per kg was first determined by several injections, allowing for complete recovery after each injection. The iodinated preparations were then administered in increasing amounts until a hypotensive effect comparable, in degree of fall and duration, to that resulting from the untreated trypsin was obtained. Following this 1 mg per kg of the untreated enzyme was again administered and it was found that the final hypotensive effect was consistent with that produced originally by this amount. The untreated trypsin was then injected in amounts equal to the largest amount of the iodinated enzyme used and this invariably resulted in decided shock lasting for a period of 20 to 50 minutes, being fatal in some cases.

At lowered pH levels, such as pH 5, the iodination of trypsin is considerably retarded.

The amount of iodine taken up at this pH in a period of time sufficient to iodinate the enzyme extensively at pH 7 does not cause appreciable lowering of proteolytic activity or hypotensive effect. Likewise, the pH influences the rate of iodination of dilute solutions of histidine and tyrosine. Under conditions similar to those maintained for the iodination of trypsin, the time required for 1×10^{-4} millimoles of tyrosine to decolorize 2 equivalents of iodine per mole increases from 1 minute at pH 7 to 480 minutes at pH 5. The comparable values for histidine under the same conditions were found to be 17 minutes at pH 7 and more than 3000 minutes at pH 5. This suggests further consideration of these groups in the iodination of trypsin, with possible emphasis on tyrosine.

Iodination of a substrate rather than the enzyme, under similar conditions, decreases the proteolytic activity of untreated trypsin. Thus, the digestion of casein which has reacted with 2×10^{-3} milliequivalents of iodine per mg may be limited to about 45% of that of untreated casein. Furthermore, it has been observed that the iodination of an active protein substrate such as insulin serves to increase its resistance to trypsin. If the iodination is limited so that the iodinated hormone retains about one-half (52%) of its original activity it tends to resist digestion and further inactivation (to 44% of the original activity) by trypsin under conditions which markedly reduce the original activity of noniodinated insulin to 3%.

Summary. Iodination serves to decrease greatly the hypotensive effect of intravenously-administered trypsin. Proteolytic activity is decreased to a lesser extent. Iodination of a substrate under similar conditions decreases the ease with which it is digested by untreated trypsin.

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Production of Ketosis in Alloxan Diabetic Rats with Nicotinic Acid.

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There is much evidence to support the belief that niacin is an important constituent of the enzyme systems of the body[1,2] although the reported effects of this vitamin on tissue metabolism and its co-activity with other vitamins lack clarity in certain instances.

Nicotinic acid appears to play a minor role in fat metabolism, resulting in an increase in body fat;[3] in a reduction of the lipotropic effect of choline;[4] and in an increased fatty infiltration of the liver in rats.[5]

The role of niacin in carbohydrate metabolism has been established largely by observations made on glycemic changes in the intact animal. Whether the administration of this vitamin produces hyperglycemia,[7-9]

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