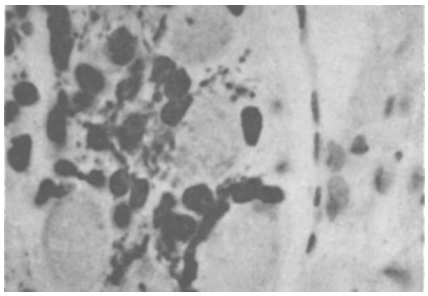

FIG. 4.
Sympathetic ganglion of the rabbit. Substrate: lauroyl-choline. Nuclei of satellite cells and granular material stained.

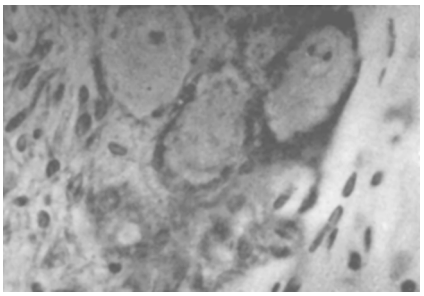

FIG. 5.
Another section of the same tissue block. Substrate: palmitoyl-choline. Nerve fibers around ganglion cells stained.

with palmitoylcholine it appears to be distributed in a uniform way mainly in nerve fibers. The most striking example is the sympathetic ganglia in which lauroylcholine produces an intense staining of the nuclei of the satellite cells (Fig. 4) while palmitoylcholine outlines the nerve fibers, the satellite cells being left entirely unstained (Fig. 5). This difference may indicate the existence of 2 (or more) different enzymes.

3. There is a species preference for certain substrates. Dog and mouse tissues give much more intense pictures with lauroyl- and myristoylcholine than with palmitoylcholine while for human and pigeon tissues the reverse holds true. Rat and guinea pig tissues fail to hydrolyze any of the substrates at a rate sufficiently high to give clearcut results.

Summary. Some choline esterases hydrolyze higher choline esters readily and they also survive to a demonstrable extent the procedures of histological fixation and embedding. It is possible to demonstrate them histochemically by incubating slides with the substrate in the presence of a cobalt salt; at the sites of choline esterase activity insoluble cobalt-soap will precipitate which can be transformed into black CoS. The histochemical distribution and properties of choline esterase are described.

16486

Characteristics of the Protein Groups Associated with the Hypotensive Activity of Trypsin.*

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In view of the action of trypsin and parenteral trypsin-like proteases and their inhibitors in certain types of shock,[1-5] blood coagulation[6-12] and other fields it is desirable to char-

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† The author is grateful to Miss Marianne Daries and Mr. Richard Moore for technical assistance.

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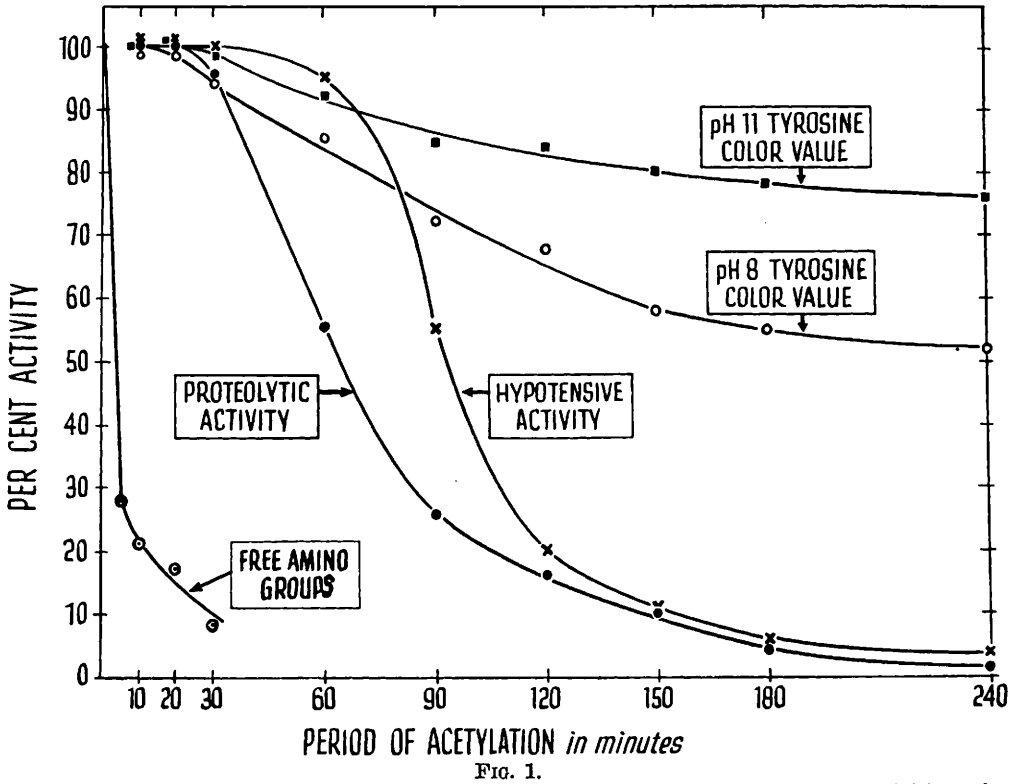

FIG. 1.

Tyrosine color value, free amino groups, and proteolytic and hypotensive activities of acetylated trypsin.

acterize the protein groups associated with the hypotensive effect of intravenously administered trypsin and to determine whether the hypotensive activity is due to its proteolytic activity. Findings relative to these points are given in the present report.

Methods. Hypotensive activity was determined in the usual manner of recording the carotid blood pressure in dogs. The per cent of normal activity was calculated as 100 times the reciprocal of the weight of the treated enzyme in mg per kg body weight required to cause a fall in blood pressure approximating the duration and degree of fall resulting from the injection of 1 mg of untreated trypsin per kg before and after the injection of the treated material. Injections were made into the femoral vein.

Proteolytic activity was estimated by determining the relative amount of a given preparation required to accomplish the same degree of digestion as a standard amount of untreated trypsin required for 50% hydrolysis of casein at pH 7 and 38°C in 1 hour.

Acetylation was carried out with ketene produced by pyrolysis of acetone. The pH during acetylation was maintained between 6 and 7 by the addition of sodium hydroxide. Iodination was carried out in 0.8 M phosphate

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buffer at pH 7.4 avoiding a large excess of free iodine at any time. Amino nitrogen was estimated by the method of Van Slyke[13] and the tyrosine-tryptophane color values were determined by the pH 8 and the pH 11 methods of Herriott.[14] Difco Laboratories crude trypsin, 1:250, was used.

Results. That free amino groups are not essential to the proteolytic or hypotensive activity of trypsin may be observed following the acetylation of the enzyme. After 30 minutes treatment with ketene the amino nitrogen was observed to decrease to 8% of its original value yet the proteolytic and hypotensive activities were essentially unchanged. This is shown in Fig. 1. As further indicated in this figure, following more prolonged acetylation essentially complete loss of hypotensive and proteolytic properties is observed with a decrease in tyrosine-tryptophane color value to about one-half of its original level.

It is seen that following treatment of the acetylated enzyme with alkali at pH 11, the tyrosine-tryptophane color values are only partially restored. With more prolonged treatment for as long as 4 hours at pH 9, 10, or 11, no further restoration of color value was observed. In repeated observations, in comparison with non-acetylated controls subjected to the same variations in pH, no significant restoration of hypotensive activity was observed as the result of alkaline hydrolysis of the acetylated enzyme.

It was originally thought that partial recovery of proteolytic activity follows hydrolysis at pH 11. However, since this is not observed at pH 9.5 or 10 it is believed that in the original observations there may have been greater loss of activity in the controls due to pH alone than there was in the acetylated preparations. Therefore, in view of the lack of recovery following this mild alkaline treatment it appears that the phenolic hydroxyl groups are not essential to either the hypotensive or the proteolytic activity of trypsin. That the amino and phenolic groups of trypsin are not essential for proteolytic activity is in agreement with the recent findings of Fraenkel-Conrat and associates.[15]

After treating trypsin with 1% formaldehyde at pH 11 for 1 hour the average values of dialysed and lyophilized preparations for proteolytic and hypotensive activities were respectively 52% and 14% of the corresponding values of the control preparations which were maintained at the same pH without formaldehyde.

Greater loss of hypotensive than proteolytic activity as the result of iodination has been described.[16] However, it may be further noted that after reacting with 2.0×10^{-3} m Eq of iodine per mg, about half of the saturating amount of iodine, which is sufficient to reduce the hypotensive effect to 2 to 5% of its original level, the tyrosine-tryptophane color value of trypsin may be 95% of that of the untreated material.

From the foregoing it would appear that trypsin shock as well as proteolytic activity is associated with groups which acetylate with ketene but the resulting acetyl linkages do not hydrolyze in dilute alkaline solution. There is a rapid loss of hypotensive effect upon treating with formaldehyde at pH 11. Iodination, which greatly decreases the hypotensive effect, is associated with only a slight decline in tyrosine-tryptophane color value.

Indole, guanidyl, and amide groups react rapidly with formaldehyde under the conditions employed.[18,19] However, of these 3 the indole groups iodinate with retention of considerable reducing power. Anson[20] has pointed out that tryptophane treated in 0.01 N acetic acid with 3 Eq of iodine per mole has the same color value as untreated tryptophane. Under

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TABLE I.
Influence of Bean Trypsin Inhibitors on the Hypotensive Activity of Trypsin.

Dog No.	Materials injected	Amount		Hypotensive effect	
		Inhibitor mg/kg	Trypsin mg/kg	Fall in blood pressure mm	Duration min.
1	Navy bean inhibitor alone	12,200	0	0	0
1	Soy bean inhibitor fraction* alone	42	0	12	1
2	Trypsin alone	0	2.5	44	9
2	Navy bean inhibitor incubated with trypsin at 25° 11 hrs	2.5	2.5	38	10
3	Trypsin alone	1	0	34	2.2
3	Soy bean inhibitor fraction* incubated with trypsin at 25° 10 min	1	1	36	2.0
4	Trypsin alone	0	15	60	45
4	Navy bean inhibitor inj. immediately before trypsin	12,000	15	57	30

* First alcohol-insoluble soy bean trypsin inhibitor (crude).[25]

the conditions of iodination followed in the present work, treatment with 2 Eq and 4 Eq of iodine per mole yielded color values equivalent to 77 and 83% of the original value respectively. It is of interest to note that reaction with formaldehyde which, like iodination, decreases proteolytic activity less than the hypotensive effect reduces the color value only 10 to 20%.

Herriott[14] has found that treatment of tryptophane or glycyl tryptophane with ketene results in a considerable loss in tyrosine-tryptophane color value with the ratio of the pH 8 to pH 11 values ranging from 0.6 to 0.73 for glycyl tryptophane and from 1.0 to 1.2 for free tryptophane. It is noted that this ratio for acetylated trypsin is within the limits given for glycyl tryptophane. A marked decrease in the tyrosine-tryptophane color value upon acetylating free tryptophane and lack of hydrolysis in alkaline solution was also observed under the conditions of acetylation and alkaline treatment used in the present work.

Therefore, while all of the characteristics of the groups associated with the hypotensive effect of trypsin are not limited to a single protein group, the single free amino acid which appears to best satisfy the requirements so far defined is tryptophane. However, it cannot be said with certainty that the properties of the indole structure are the same in a protein molecule as they are in the free amino acid. In a footnote Brand and Kassell[21] have given the tryptophane content of trypsin as 4.6%. Our values for the commercial trypsin used have been somewhat less.

In earlier reports it was pointed out that the hypotensive activity of iodinated trypsin does not parallel its proteolytic[16] or thromboplastic[22] activity. That proteolytic and hypotensive activities are not always decreased equally is further indicated by work with the trypsin inhibitors of beans. The preparation and differentiation of the inhibitors used and their inhibition of proteolysis have been reported.[16,23-25] However, as shown in Table I, the same inhibitors failed to have significant influence on the hypotensive effect of trypsin in dogs. It is observed that injection of inhibitors, with or without previous incubation with the enzyme prior to injection, does not significantly alter the hypotensive effect of the trypsin. Furthermore, the established slow rate of recovery of blood pressure in dogs following the injection of 5 to 15 mg of trypsin per kg was not altered by the injection of 10 times as much inhibitor during the recovery period. However, it should be added that the

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navy bean trypsin inhibitor does protect rabbits against the acute lethal effect of trypsin. The latter is to be described elsewhere.

Summary. Free amino and phenolic groups do not appear to be essential to the proteolytic or the hypotensive activity of trypsin. Characteristics of the protein groups associated with the hypotensive activity of trypsin are given. These correspond with the properties of free tryptophane although participation of other groups is not excluded. Changes in the proteolytic and hypotensive activities of trypsin are not always parallel.

16487 P

On the Relative Efficacy of Emetine in Intestinal and Hepatic Amebiasis.

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Although emetine has been used therapeutically in amebiasis for more than 35 years, the tissue distribution and concentration of this alkaloid have not been reported. Nevertheless, certain limitations of the drug's efficacy are well known. Emetine is considered curative in amebic hepatitis and even in amebic abscess. On the other hand, relapses of amebic dysentery or the recurrence of cysts in the stool are frequently seen after emetine therapy despite the ability of the drug to clear up the acute symptoms.[1]

There are various theories as to why emetine is ineffective in amebic dysentery and curative in amebic liver disease. One widely accepted theory is based on observations that, *in vitro,* emetine is capable of destroying the trophozoites but not the cysts of *Endameba histolytica.* Cysts are as a rule not found in tissues; their origin in the intestinal lumen is in trophozoites leaving the tissues and encysting. According to this theory, emetine is ineffective because both trophozoites and cysts occur in amebic dysentery. On the other hand, since only trophozoites are present in amebic liver disease this type of amebiasis is readily eradicated by emetine. Craig believes that emetine fails to eradicate all the trophozoites from the intestine, leaving some to start the disease process again.[1] No mention is made as to why the drug is capable of destroying the trophozoites in the liver.

TABLE I.
Liver and Intestinal Concentration of Emetine After an Intramuscular Injection of 6 mg per kg. Concentrations are averages of 3 rabbits in each group.

Rabbit group	Time after inj.	Emetine conc. (mg/kg) Liver	Intestine
1	1 hr	24.9	5.5
2	12 "	41.6	1.7
3	3 days	19.2	1.7
4	4 "	15.4	1.2
5	6 "	10.3	0
5	13 "	2.7	0
7	20 "	2.1	0
8	28 "	1.0	0

The role of blood and tissue levels of chemotherapeutic agents as a factor in effectiveness of therapy has assumed great importance in recent years. The following findings are presented to show that the tissue levels of emetine may explain the divergent results obtained with the drug in amebiasis of the liver and of the intestine.

Method. Each of 24 albino rabbits weighing about 2.5 kg were injected intramuscularly with 6 mg per kg of emetine hydrochloride in aqueous solution. They were then sacrificed in groups of 3 at intervals of from 1 hour to

* Present address: Medical Division, Food and Drug Administration, Washington, D.C.

1 Craig, C. F., *Etiology, Diagnosis, and Treatment of Amebiasis,* Williams & Wilkins Co., Baltimore, 1944.