Studies on Tissue Protease

IV. The *in vivo* Activation of Protease System and Gluconeogenesis In Liver

(Directed by Asst. Prof. M. Hayakawa)

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In the preceding paper¹⁾ it was demonstrated that epinephrine produced remarkable augmentation of proteolytic enzyme system on liver, and it was suggested thereby that such phenomenon might relate to gluconeogenesis which should be carried into effect immediately after consumption of glycogen deposit in liver. However, the relation to gluconeogenesis of adrenal cortex has been suggested by Long²⁾ and the protein catabolic effect of adrenal cortical substances is now rather common knowledge. On the other hand epinephrine is known as an effective activator for the pituitary-adrenal system. Regarding these matters further studies were made on *in vivo* activation of the liver protease system.

EXPERIMENTAL

The method was the same as the one employed in the preceding paper²⁾.

Results

Coinciding with preceding observations, epinephrine delivery through the systemic vein results in acceleration of heterolytic and autolytic proteolysis of liver and causes augmentation of tryptase as well as that of catheptic protease (Tables I, II & III, Animal, No. 73). In this case the animal was killed four hours after epinephrine delivery, so its effect is shown to be able to continue at least four hours. Blood cell counts of the animal before and after the delivery are shown in Table IV. Eosinophil cell and lymphocyte counts fall apparently four hours after the delivery, i.e., response to the latter of its pituitary-adrenal system appears to be well preserved.

To determine whether or not epinephrine exerts its effect on liver protease system via such endocrine system, further experiments were carried

TABLE I
Epinephrine Effect on Tryptic Proteolysis of Rabbit
Liver at pH 7.8

(Increases of acidity in 4 cc. digestion mixture (cc. of 0.1 N NaOH))

Substrate*	Time in hrs.	Animal (Source of enzyme solution)							Normal control†
		No. 73†	adrenalectomized			glucose administered			
			No. 61	No. 72	No. 74	No. 68	No. 70	No. 71	
Casein (a)	24	0.25	0.35	0.35	0.35	0.15	0.15	0.15	0.15
	72	0.55	0.6	0.7	0.7	0.35	0.35	0.3	0.25
	120	0.75	0.8	0.85	0.8	0.35	0.4	—	0.35
Casein (b)	24	0.3	0.35	0.4	0.4	0.3	0.3	0.3	0.3
	72	1.2	1.25	1.2	1.1	0.6	0.75	0.65	0.55
	120	1.45	1.55	1.5	1.5	0.8	0.8	—	0.7
Casein (c)	24	0.45	0.55	0.5	0.45	0.4	0.35	0.3	0.35
	72	0.9	0.95	0.9	0.8	0.55	0.65	0.65	0.5
	120	1.2	1.3	1.35	—	0.75	0.8	—	0.7
Casein (d)	24	0.7	0.75	0.7	0.1	0.55	0.6	0.6	0.5
	72	1.4	1.5	1.55	1.4	0.95	0.95	1.05	0.9
	120	1.75	1.85	1.85	—	1.05	1.1	—	1.1
Gelatin (a)	24	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1
	72	0.25	0.3	0.25	0.3	0.2	0.2	0.15	0.2
	120	0.35	0.35	0.35	0.35	0.3	0.25	0.2	0.3
Gelatin (b)	24	0.3	0.3	0.35	0.3	0.3	0.25	0.15	0.25
	72	0.6	0.65	0.65	0.65	0.4	0.4	0.35	0.4
	120	0.75	0.8	0.85	0.85	0.5	0.5	0.55	0.6
Pepton (a)	24	0.7	0.9	0.9	0.95	0.75	0.65	0.6	0.7
	72	1.4	1.6	1.7	1.7	0.9	0.8	0.8	1.0
	120	1.5	1.6	1.7	1.7	0.95	0.95	0.95	1.1

Epinephrine doses: 2.0 mg. intravenously.

Animal: No. 73, 3.0 kg.; No. 61, 3.2 kg.; No. 72, 3.2 kg.; No. 74, 3.4 kg.;
No. 68, 2.3 kg.; No. 70, 3.0 kg.; No. 71, 3.3 kg.

* (a) without albumin or manganese activation; (b) with dialyzed serum albumin solution; (c) with manganese activation ($M/10\text{-MnSO}_4$ solution); (d) with combined activation with dialyzed serum albumin and manganese sulfate solutions.

† Killed 4 hours after the epinephrine delivery.

‡ Average of five animals (Nos. 35, 36, 45, 46 and 47).

out on animals adrenalectomized. On such animals, too, epinephrine causes augmentation of liver protease system, and such effect is independent of pituitary-adrenal cortical system (Tables I, II & III, Animals: Nos. 61, 72 & 74). Epinephrine itself exerts, however, no influence upon liver proteolysis in vitro when it is added directly with liver homogenates (Table V). Therefore its effect in vivo is indirect.

If such augmentation could be related directly to gluconeogenesis in

TABLE II
Epinephrine Effect on Catheptic Proteolysis of Rabbit Liver
at pH 4.5

(Increases of acidity in 4 cc. digestion mixture; cc. of 0.1 N NaOH)

Substrate*	Time in hrs.	Animal (Source of enzyme solution)							Normal control†
		No. 73†	adrenalectomized			administered glucose			
			No. 61	No. 72	No. 74	No. 68	No. 70	No. 71	
Casein (a)	24	0.5	0.6	0.6	0.65	0.35	0.3	0.35	0.25
	72	1.05	1.05	1.05	0.95	0.4	0.4	0.45	0.35
	120	1.2	1.2	1.2	1.25	0.55	0.5	0.55	0.45
Casein (b)	24	0.9	0.95	0.9	—	0.55	0.5	—	0.45
	72	1.55	1.3	—	—	0.7	—	—	0.6
	120	1.8	1.7	—	—	0.8	—	—	0.75
Gelatin (a)	24	0.65	0.7	0.75	0.65	0.35	0.3	0.25	0.2
	72	0.75	0.8	0.85	0.8	0.45	0.35	0.3	0.35
	120	0.9	0.9	0.9	0.9	0.6	0.55	0.6	0.45
Gelatin (b)	24	0.9	0.9	—	—	0.55	—	—	0.5
	72	1.2	1.25	—	—	0.85	—	—	0.7
	120	1.25	1.25	—	—	0.85	—	—	0.7
Pepton (a)	24	0.55	0.6	0.5	0.5	0.4	0.3	0.35	0.35
	72	0.7	0.7	0.7	0.7	0.45	0.4	0.5	0.55
	120	0.9	0.95	0.9	0.9	0.55	0.6	0.6	0.65
Pepton (b)	24	0.75	0.85	—	—	0.55	—	—	0.5
	72	1.0	1.2	—	—	0.7	—	—	0.75
	120	1.1	1.3	—	—	0.8	—	—	0.8

Epinephrine dose: 2.0 mg. intravenously.

Animal, body weight (kg.): No. 73, 3.0; No. 61, 3.2; No. 72, 3.2; No. 74, 3.4; No. 68, 2.3; No. 70, 3.0; No. 71, 3.3.

* (a) without cystein, (b) with cystein activation.

† Killed 4 hours after the epinephrine delivery.

‡ Average of three animals (Nos. 45, 46 and 47).

liver it would be expected that the effect of epinephrine would be abolished under some conditions. Hyperglycemic state, for example, may account for such a condition, because there should be no need for gluconeogenesis and so none for protease augmentation. Therefore the effect of epinephrine was examined on three animals injected with 20 cc. of 10%-glucose solution and kept beforehand in a hyperglycemic state (urine sugar). After epinephrine delivery blood sugar rose further of the three animals and fell in one animal (Table VI), and activity of liver protease system was, as was expected, by no means accelerated (Tables I, II & III, Animals, Nos. 68, 70 & 71). Effect on liver protease system of epinephrine was thus abolished by prior administration of glucose solution.

TABLE III
Epinephrine Effect on Catheptic Proteolysis of Rabbit Liver
(Increases of acidity in cc. of 0.1 N NaOH for 1 g. liver.)

pH of Autolysis	Time in hrs.	Autolytic proteolysis of the liver of animal							Normal control†
		No. 73*	adrenalectomized			administered with glucose			
			No. 61	No. 72	No. 74	No. 68	No. 70	No. 71	
4.5	24	1.9	2.2	2.2	2.15	1.5	1.4	1.55	1.5
	72	2.7	2.8	2.85	2.95	2.15	2.1	2.1	2.1
	120	3.0	3.25	3.3	3.45	2.45	2.4	2.5	2.5
7.8	24	0.9	1.05	0.95	0.95	0.4	0.45	0.4	0.4
	72	1.1	1.25	1.25	1.25	0.7	0.6	0.6	0.55
	120	1.35	1.5	1.45	1.65	0.9	0.9	0.85	0.85

Epinephrine dose: 2.0 mg. intravenously.

* Killed 4 hours after the epinephrine delivery.

† Average of three animals (Nos. 45, 46 and 47).

TABLE IV
Blood Picture of Animal No. 73 before and after the
Epinephrine Delivery*

	Before	After
Eosinophil† cell count	250	75
Leucocyte count	15,000	22,000
Lymphocyte count	5,150	3,630
(%)	(52) %	(17.5) %
Neutrophil cell count	4,050	17,380
(%)	(40.5) %	(79.0) %

* Animal No. 73, 3.0 kg. delivered with 2.0 mg. epinephrine through the system vein and killed four hours after the delivery.

† According to Dunger's method.

Prior administration of 10 cc. of 10%-alanin solution which was thought to be a effective precursor of glucose was also shown to prevent similarly augmentation of tryptase or retard that of catheptase, and epinephrine exerts no remarkable effect on tryptic proteolysis of the liver of animal treated with it a short time after a meal (Table VII).

Therefore, the above assumption that enzyme augmentation may relate to gluconeogenesis appears to be founded in these facts observed.

Epinephrine is, on the other hand, known also to accelerate glycolysis of muscle, therefore, its effect on muscle proteolysis was examined.

TABLE V
Influence of Epinephrine upon Liver Proteolysis*
in vitro

(Increases of Acidity in 4 cc. digestion mixture; cc. of 0.1 N NaOH)

Substrate	Time in hrs.	0.1%-epinephrine, cc.			
		0	0.5	1.0	2.0
2% Casein	24	0.15	0.15	0.15	0.15
	72	0.25	0.3	0.25	0.25
	120	0.35	0.35	0.3	0.35
2% Pepton	24	0.6	0.6	0.65	0.6
	72	0.75	0.7	0.75	0.7
	120	0.9	0.95	0.85	0.9

* Normal liver (animal No. 63, 3.2 kg.).

20 cc. substrate-buffer (pH 7.8) solution + 2 cc. enzyme solution (liver homogenate 1 : glycerin water 3) + 0–2.0 cc. epinephrine solution + 2.0–0 cc. buffer solution

Epinephrine treatment for enzyme solution :

2 cc. original enzyme solution was added with 0–2.0 cc. 0.1%-epinephrine solution and 2.0–0 cc. buffer solution and incubated at 37°C for forty minutes (final volume being made 4 cc. respectively).

TABLE VI
Change of Blood Sugar (mg/dl) after Glucose
Administration and Epinephrine Delivery

Animal No.	Before	After glucose	After epinephrine
68	114	460	480
70	121	509	380
71	129	509	543

Muscle tryptase is, in native state, low in activity as was so when previously observed³). In the case of epinephrine or insulin delivery no change in tryptase or ereptase activity was observed (Table VIII).

DISCUSSION

Although catabolic effect of adrenal cortical substance is well established, relationship between this and gluconeogenesis or details of biological mechanism of the latter has not yet been elucidated.

From the results presented above epinephrine is evidenced to cause, independent of the adrenal cortex, augmentation of the liver protease system and this should be interpreted as indicating increase in the rate of protein metabolism which should directly relate to gluconeogenesis ad-

TABLE VII

Influence of Prior Administration of Alanin etc. upon
Epinephrine Effect on Rabbit Liver Proteolysis

(Increases of acidity in 4 cc. digestion mixture (cc. of 0.1 N NaOH)).

Animal No.	Time in hrs.	pH 7.8				pH 4.5		
		Casein (a)*	Casein (b)†	Gelatin	Pepton	Casein	Gelatin	Pepton
86‡	24	0.15	—	0.1	0.45	—	—	—
	72	0.2	—	0.15	0.95	—	—	—
	120	0.3	—	0.15	1.0	—	—	—
106§	24	0.1	0.2	0.1	0.45	0.3	0.35	0.25
	72	0.2	0.3	0.15	0.75	0.45	0.6	0.5
	120	0.2	0.5	0.15	0.75	0.65	0.7	0.55
Normal//	24	0.15	0.3	0.1	0.7	0.25	0.2	0.35
	72	0.25	0.55	0.2	1.0	0.35	0.35	0.55
	120	0.35	0.7	0.3	1.1	0.45	0.45	0.65

* Without albumin.

† With dialyzed serum albumin solution.

‡ Epinephrine delivery short time after meal.

§ Prior administration of 1 g. alanin.

// Cf. Tables I and II.

TABLE VIII

Influence of Epinephrine upon Tryptic Proteolysis of
Striped Muscle of Rabbit

(Increases of acidity in 4 cc. digestion mixture (cc. of 0.1 N NaOH)).

Animal No. (body weight, kg.)	Time in hrs.	Hydrolysis at pH 7.8 of			Remarks
		Casein	Gelatin	Pepton	
64 (3.2)	24	0.2	0.05	0.25	normal control
	72	0.3	0.1	0.45	
	120	0.35	0.25	0.6	
67 (3.0)	24	0.1	0.05	0.2	0.5 mg. epinephrine intravenously
	72	0.25	0.1	0.45	
	120	0.4	0.2	0.6	
68 (2.3)	24	0.1	0.05	0.3	glucose (10 g.) administration prior to epinephrin delivery (2 mg.)
	72	0.25	0.15	0.45	
	120	0.35	0.2	0.55	
69 (3.3)	24	0.15	0.05	0.3	Insulin delivery (200 units)
	72	0.2	0.15	0.55	
	120	0.3	0.15	0.6	

vancing therein. From such point of view it is concluded that gluconeogenesis in the liver does not require the presence of adrenal cortex. Selye⁴⁾

is of the opinion that the corticoid aids rather the utilization of protein catabolites for gluconeogenesis or protein re-synthesis. And it was concluded by Ingle⁵⁾ that the negative nitrogen balance after traumatic injury is not caused specially by the increase in secretion of the cortical hormones.

Referring to catabolic effect of adrenal cortex, Bondy⁶⁾ showed that it did not require the presence of the liver. That epinephrine causes augmentation of the liver protease system independent of adrenal cortex is, therefore, compatible with Bondy's results. According to Selye⁴⁾ the breakdown of body protein, except those of lymphocytes, takes place independent of the adrenal cortex. White and Dougherty⁷⁾ showed on fasting mice that a decrease in nitrogen content was seen in livers of adrenalectomized animals as well as in those not operated on. These observations show the possibility that protein metabolism of the liver can progress regardless of adrenal cortex and that it is controlled by a mechanism other than that of metabolism of the other body protein. From the results obtained, epinephrine, namely adrenal medulla or sympathetic nerve, is concluded to be a factor which controls the metabolism of liver protein.

But it is very interesting that glucose or alanin is antagonistic to the effect of epinephrine, as was so in the experiments conducted by Engel⁸⁾ on the effect of adrenal cortical extract. Bondy⁶⁾ showed such effect of glucose to be carried out in the periphery to the liver, but detail of mechanism of antagonistic effect against epinephrine of glucose or alanin is open to question. These substances are, at least, said to spare the liver protein in occasion of "emergency reaction".

Engle⁸⁾ considered the effect of adrenal cortex on protein metabolism was secondary to the changes in the carbohydrate/fat metabolism. From the above the effect of epinephrine should be indirect upon the protease system itself, and the augmentation of liver protease system, namely, the increase in the rate of protein metabolism, should also be considered to be brought into effect secondary to glycogenolysis and corresponding with gluconeogenesis directly. It is suggested that gluconeogenesis occurs first with consumption with liver protein. The protein catabolites which could come from other tissue proteins under adrenal cortical influence may serve for the re-synthesis of liver protein or directly for gluconeogenesis. The role of adrenal cortex for gluconeogenesis may be explained as supplying amino acid to the liver and or sparing liver protein.

SUMMARY

1. Epinephrine delivery in vivo causes augmentation of the liver protease system on animals fasted for twenty four hours. But no change in protease activities is seen in vitro when epinephrine is directly added to

liver homogenates.

2. Such phenomenon is observed in the absence of adrenal cortex but not observable on animals treated with epinephrine a short time after a meal.

3. Prior administration of glucose or alanin prevents epinephrine effect, i.e., no augmentation of protease system is seen.

4. Tryptic muscle proteolysis remains unaffected after epinephrine treatment.

5. The biological mechanism of gluconeogenesis is briefly discussed with enzyme augmentation phenomenon.

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References

- 1) Ito, Tohoku J. Exp. Med., 1955, **61**, 389.
 - 2) Long, Katzin & Fry, Endocrinology, 1940, **26**, 309.
 - 3) Hayakawa & Ito, Tohoku J. Exp. Med., 1954, **60**, 353, 361.
 - 4) Selye, Textbook of Endocrinology. 2nd Ed., Montreal, 1949, 843.
 - 5) Ingle *et al.*, Amer. J. Physiol., 1947, **149**, 510.
 - 6) Bondy, Endocrinology, 1949, **45**, 605.
 - 7) White & Dougherty, *ibid.*, 1947, **41**, 230.
 - 8) Engel, Schiller & Pentz, *ibid.*, 1949, **44**, 458.
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