THE PEPTOLYTIC POWER OF LIVER, SPLEEN, AND KIDNEYS IN POISONING BY PHOSPHORUS AND CHLOROFORM.

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In another communication it was shown that the feeding of sugar greatly increased the esterase content of the livers of both normal and phosphorus-poisoned dogs.[1] The present paper records the results of a study of the peptolytic power of the organs of the same animals used in the previously reported experiments.

Cohnheim[2] discovered in the press juice of the intestinal mucosa a ferment, which he called erepsin, capable of splitting peptone into amino-acids. Salkowski[3] appears to have been the first to demonstrate intracellular enzymes when he showed that liver and muscle underwent "autodigestion." Vernon[4] has made extensive studies of intracellular erepsin. Abderhalden and Teruuchi[5] suggested the term "ereptase" to distinguish intracellular erepsin from the extracellular ferment of the same name in the succus entericus. Vernon[6] found ereptase in the following organs, which are given in the order of the quantity present: intestinal mucous membrane (especially that of the duodenum), kidney, spleen, lung, pancreas, liver, submaxillary gland, thyroid, adrenal, heart, brain, ovary, skeletal muscle, and blood serum. Cohnheim and Pletnew[7] demonstrated that the peptolytic activity possessed by different organs and tissues is independent of the presence of traces of blood. Abderhalden[8] found peptolytic ferments in the kidney, lung, small intestine, and ovary, but little in skeletal muscle. Raubitschek,[9]

[1] Simonds, J. P., *J. Exp. Med.*, 1918, xxviii, 663.

[2] Cohnheim, O., *Z. physiol. Chem.*, 1901, xxxiii, 451; 1902, xxxv, 134.

[3] Salkowski, E., *Z. klin. Med.*, 1890, xvii, suppl., 77.

[4] Vernon, H. M., *J. Physiol.*, 1904, xxx, 330.

[5] Abderhalden, E., and Teruuchi, Y., *Z. physiol. Chem.*, 1906, xlix, 1.

[6] Vernon, *J. Physiol.*, 1905–06, xxxiii, 81.

[7] Cohnheim, O., and Pletnew, D., *Z. physiol. Chem.*, 1910, lxix, 108.

[8] Abderhalden, E., *Z. physiol. Chem.*, 1910, lxvi, 137.

[9] Raubitschek, E., *Z. exp. Path. u. Therap.*, 1907, iv, 675.

on the other hand, was unable to demonstrate the presence of ereptase in any tissue except the intestinal mucous membrane.

Vernon[10] believes that in growing animals the ereptic power of the tissues is closely related to their functional activity and functional capacity, since the tissues of embryos contain little or no ereptase. He found the tissues of non-hibernating hedgehogs more active than those of hibernating animals. Vernon found further that the ereptic activity of tissues is only moderately affected by diet. Cats fed on a mixed diet, including meat, had the most ereptase, while those fed on bread and milk had the least. Response to changes of diet was slow.

Comparatively little work has been done upon the effect of disease upon the ereptic power of the different organs and tissues. Vernon[10] found that guinea pigs of one-half their normal weight showed less than one-half the normal tissue ereptase. In man, the ereptic power of the kidneys diminished approximately in proportion to the severity of the nephritis. Colwell[11] found the tissues of patients who had died of malignant disease poorer in ereptase than those of normal persons. But Colwell and McCormac[12] observed that the tissues of cancerous mice were more strongly peptolytic than those of normal mice. This difference was accounted for by the greater emaciation and cachexia in human cancer patients. Jacque and Woodyatt,[13] and Hamburger[14] demonstrated erepsin in the gastric juice of patients with carcinoma of the stomach. They believed that this erepsin was liberated from disintegrated cancer cells. Ereptase has been demonstrated in press juice from carcinomas.[15]

Although numerous studies have been made of the autolysis of livers of animals poisoned with phosphorus and chloroform, little attention appears to have been paid to the ereptic power proper of these organs. There is good reason for believing that the autolytic changes in these livers may not be a correct index of their ereptic activity. The only two reports of studies of the ereptase of phosphorus-poisoned livers which I have been able to find in the literature are absolutely contradictory. Thus, Bergell and Lewin[16] claim that in phosphorus poisoning the ereptic ferment of the liver is destroyed. Abderhalden and Schittenhelm,[17] on the other hand, state that the press juice of the livers of dogs poisoned with phosphorus exert as great, if not greater, influence in splitting dipeptides as that of normal dogs.

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[10] Vernon, Intracellular enzymes, London, 1908, 40 ff.

[11] Colwell, H. A., *Arch. Middelsex Hosp.*, 1909, xv, 96.

[12] Colwell, H. A., and McCormac, H., *Arch. Middlesex Hosp.*, 1909, xv, 104.

[13] Jacque, J. L., and Woodyatt, R. T., *Arch. Int. Med.*, 1912, x, 560.

[14] Hamburger, W. W., *J. Am. Med. Assn.*, 1912, lix, 847.

[15] Abderhalden, E., and Medigreceanu, F., *Z. physiol. Chem.*, 1910, lxvi, 265.

[16] Bergell, P., and Lewin, K., *Z. exp. Path. u. Therap.*, 1906, iii, 425.

[17] Abderhalden, E., and Schittenhelm, A., *Z. physiol. Chem.*, 1906, xlix, 41.

Technique.

The technique used in the experiments reported here was suggested by the work of Vernon.[18] In a study of the ereptase content of different organs Vernon used glycerol extracts of 1 gm. of ground tissue in 2 cc. of glycerol. He used 0.25 cc. of the extract and found that the amount of enzyme present increased up to the 21st day and slowly decreased after the 25th day. He employed a colorimetric method based upon a modification of the biuret test to estimate the amount of peptone split into amino-acids. In my experiments larger amounts of tissue and glycerol and of the extract (5 gm. of tissue in 10 cc. of glycerol, weighed, not measured; and 1 cc. of diluted extract) were used in the belief that the percentage of error would be thus lessened. Instead of the colorimetric method, Sörensen's formaldehyde titration method was employed.[19]

The extracts used in these experiments are the same as those used in a study of esterase reported in another paper[1] in which the details of their preparation are given. The reaction of these glycerol extracts was always slightly acid. To neutralize 1 cc. of diluted liver extract, which was the amount employed in the tests, 0.10 to 0.12 cc. of 0.1 N sodium hydroxide was required; for 1 cc. of spleen extract, 0.07 to 0.08 cc.; and for 1 cc. of kidney extract, 0.05 to 0.06 cc.

As substrate, a 4 per cent solution of Witte's peptone, always from the same original container, was employed. The peptone was dissolved in distilled water by heat, cooled, and filtered. This slightly turbid filtrate was rendered neutral to phenolphthalein when it became quite clear. 9 cc. of the peptone solution were run into test-tubes from a burette, and 1 cc. each of extract and toluene was added. Each tube was shaken eighty times, and placed in the incubator. At the end of 24 and 48 hours and 6 days, duplicate (sometimes triplicate) tubes were shaken thoroughly and the contents filtered through paper until clear. Although clear when placed in the incubator, all the tubes, except the controls, when removed for examination showed a precipitate. 5 cc. of the filtrates were removed to Erlenmeyer flasks, diluted with 45 cc. of distilled water, and 5 cc. of

[18] Vernon, *J. Physiol.*, 1904–05, xxxii, 33.

[19] Sörensen, S. P. L., *Biochem. Z.*, 1908, vii, 45.

approximately neutral 40 per cent solution of formaldehyde and three drops of phenolphthalein solution added. After standing for a few minutes the contents of the flasks were titrated with 0.1 N sodium hydroxide. The following controls were made: (1) 1 cc. of organ extract in 9 cc. of distilled water under toluene to exclude increases in acidity due to changes in the extract itself. This was not found to occur. (2) 9 cc. of peptone solution with 1 cc. of distilled water to determine the amount of preformed amino-acids in the peptone solution. This was not found to increase upon standing in the incubator. (3) 5 cc. of the formaldehyde solution were diluted with 50 cc. of distilled water and titrated to determine the amount of unneutralized acid in the formaldehyde.

EXPERIMENTAL.

Inasmuch as the animals made the basis of the experiments reported here are the same that were used in a study of esterase,[1] in the report of which the protocols were given in some detail, it is only necessary to make the following statements concerning them. All the animals were killed by bleeding from the carotid artery under light ether anesthesia. As controls, dogs which had not been submitted to any experimental procedure were used. A series of animals was submitted to profound chloroform anesthesia for periods of 4 or more hours and killed 2 days later. The phosphorus-poisoned dogs were divided into two groups. Group I received subcutaneous injections of phosphorus in oil. Group II was given phosphorus in approximately similar proportions but with the administration of 150 to 300 gm. of sugar in solution daily by stomach tube for 3 days previous to, and 1 or 2 days after the injections of phosphorus. The "sugar-fed" animals were dogs which received 200 to 300 gm. of sugar daily for 5 or 6 days. The liver, spleen, and kidneys of each animal were removed immediately after death and the entire organ was ground fine in a meat chopper. The results of these experiments are shown in Table I.

TABLE I.

Ereptic Power of Glycerol Extracts of Liver, Spleen, and Kidney Expressed in Cubic Centimeters of 0.1 N Sodium Hydroxide Required to Neutralize the Amino-Acids.

Animals.	Preformed amino-acid in peptone.	Amino-acids due to cleavage by ereptase of extract.		
		Liver.	Spleen.	Kidney.
	cc.	*cc.*	*cc.*	*cc.*
Control 1	1.35	2.50		4.60
" 2	1.50	2.05	1.85	4.45
" 3	1.40	1.75	2.00	3.70
" 4	1.25	2.30	1.70	4.25
" 5	1.25	2.60		
Chloroform 1	1.35	2.05	2.10	4.45
" 2	1.30	2.15	2.20	4.50
Phosphorus Ia		1.80	1.45	2.70
" Ib	0.75(?)	1.35	1.80	3.35
" Ic	1.40	1.25	2.45	4.75
" Id	1.45	1.65	2.75	5.05
Phosphorus IIa		2.15	2.75	4.80
" IIb	1.40	1.45	1.90	4.20
" IIc	1.45	2.20	2.85	4.65
" IId	1.45	2.35		5.50
Sugar-fed 1	1.45	1.80	1.90	3.30
" 2	1.30	2.45	2.70	4.95
" 3	1.85	2.55	3.00	4.45

DISCUSSION.

The ereptase content of the liver, spleen, and kidneys of normal dogs is reasonably constant. The results of titrations are not so uniform as those in the study of esterase. But the formaldehyde titration method is admittedly not so easily carried out, and the end-point is not so sharp as that for the titration of acids liberated by the hydrolysis of esters. The percentage of error is therefore somewhat greater. It was only after making many titrations that sufficient experience was attained to make possible reasonably uniform

results. From Table I it appears that the ereptic power of the normal kidney is approximately double that of the normal liver; and that of the liver is, in most instances, equal to, or slightly greater than that of the spleen. The position of the spleen in Vernon's[6] list indicates that he found its ereptase content greater than that of the liver.

In a considerable series of tests it was found that the ereptic activity of extracts of liver is not materially influenced by the presence of bile in a dilution of 1:200. In this respect ereptase differs sharply from esterase.

Vernon[20] obtained the highest cleavage of peptone by ereptase in a slightly alkaline solution. This was not uniform for all organs, however, a fact which led him to conclude that there is a multiplicity of ereptases instead of a single peptolytic ferment common to all organs and tissues. In my own extracts the ereptic power showed little variation in media with reactions ranging from slightly alkaline to slightly acid. In this respect it is similar to the ereptic ferment found by Jobling and Strouse[21] in extracts of leucocytes. Table I is made up from titrations of filtrates of mixtures which were slightly acid. The substrate was neutral. The acidity of the mixture was therefore due to the minute amount of acid in 1 cc. of the diluted extract.

Chloroform poisoning does not appear to reduce materially the ereptic power of the organs studied. Poisoning by phosphorus, on the other hand, causes a considerable decrease in the ereptase content of the liver, but apparently has little effect upon that of the kidneys or spleen. Neither phosphorus nor chloroform caused any definite change in the esterolytic power of the liver. Thus there appears to be a difference in the enzymic activity after poisoning by these two substances. The histologic differences are well known. Also, Opie and Alford[22] observed that a carbohydrate diet offered a greater protection against poisoning by chloroform than against poisoning by phosphorus.

Feeding sugar in large amounts for 5 or 6 days does not increase the ereptase content of the livers of normal dogs. It does, however, appear to prevent the decrease of this ferment due to phosphorus

[20] Vernon, Intracellular enzymes, London, 1908, 15.

[21] Jobling, J. W., and Strouse, S., *J. Exp. Med.*, 1912, xvi, 269.

[22] Opie, E. L., and Alford, L. B., *J. Exp. Med.*, 1915, xxi, 1.

poisoning. In this respect also the behavior of ereptase differs from that of esterase. For it was found that the feeding of sugar increased the esterolytic power of both normal and phosphorus-poisoned dogs.

SUMMARY.

1. Glycerol extracts of liver, spleen, and kidney contain an ereptic ferment capable of splitting peptone into amino-acids.

2. Poisoning by phosphorus appears to reduce the ereptic power of the liver, and to a less extent that of the kidneys.

3. Poisoning by chloroform appears to have no appreciable effect upon the ereptase content of the liver, spleen, or kidneys.

4. Feeding of sugar to normal animals has little or no effect upon the ereptic power of the liver, spleen, or kidneys.

5. Feeding of sugar before and after poisoning with phosphorus appears to prevent the reduction of ereptic power of the liver.