PROTEOSE INTOXICATIONS AND INJURY OF BODY PROTEIN.

III. Toxic Protein Catabolism and Its Influence upon the Non-Protein Nitrogen Partition of the Blood.

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This paper contains experimental data which supplement the published experiments of Whipple, Cooke, and Stearns (1, 2) who showed the profound influence of proteose injections upon the elimination of nitrogen in the urine. A single injection of toxic proteose in a fasting dog will cause a great rise in the base-line level of nitrogen elimination —often an increase of 4 to 6 gm. above normal, lasting many days. This, of course, indicates a great destruction of body protein following a single intravenous toxic injection—an acceleration of tissue autolysis lasting over 2 to 7 days with a maximum nitrogen elimination occurring during the second 24 hour period. The toxic proteose causes a disturbance of tissue equilibrium which is not restored to normal for several days following a severe intoxication. It has been suggested that the toxic proteose may so injure cell protoplasm that the resultant cell autolysis may form other toxic split products capable of further injury to the body—a true vicious circle of intoxication (1). For this reason it seemed desirable to study the blood by all available methods to determine the nitrogenous products of increased tissue autolysis *in vivo*.

The term "proteose intoxication" is used in a liberal sense in this communication. It is generally admitted that the chemistry of the proteoses is at best unsatisfactory. There are few proteoses which can be accepted as chemically pure by a critical chemist and very few indeed that will meet the requirements for pure proteoses demanded

by Gibson (3). It must be admitted that even the pure proteos preparations may be mixtures of several proteoses. It is claimed that these pure proteoses are relatively inert, but we believe it is safer to say that the complicated methods used to purify the proteoses will completely denature these several proteoses. Repeated precipitation of the proteoses used in our experiments by means of alcohol will completely remove all toxicity, yet this does not mean a removal of toxic impurities, because a collection of all the alcoholic filtrates will likewise show no toxic substance. The whole question of isolation of pure proteoses is much like that of the isolation of pure ferments. The process of isolation destroys the toxicity of the proteose and the activity of the ferment.

The "proteose solutions" employed in our experiments are prepared from the material obtained from human or animal intestinal obstruction or closed intestinal loops by means of alcoholic precipitation, solution of the precipitate in water, and removal of albumin by heat in a dilute acid solution (1). A second alcoholic precipitation may be used but this will destroy some of the original toxicity. The fluid is slightly opalescent or clear broth-like with a faint amber color. In the concentration used, the solution usually contains about 5 mg. of dried substance per cubic centimeter. We hope to report in the near future on a chemical study of this material. We realize that this preparation may contain one or more primary proteoses and perhaps some β-nucleoprotein and nucleohistone.

The important fact remains that the proteose solution contains a substance or substances which are not present in the normal intestine but are abundant in the obstructed intestine (human or animal). It is highly probable at least that this "proteose" is concerned in the intoxication of intestinal obstruction which is perhaps as typical an example as may be found of true non-specific intoxication. All infections have an important non-specific intoxication factor which may not be very unlike this non-specific intoxication of intestinal obstruction. We believe that information concerning the non-specific intoxication of intestinal obstruction will be of value for a proper comprehension of the non-specific fraction of the general intoxication present in bacterial infections.

Methods.

Urea was determined as described by Van Slyke and Cullen (4).

The amino nitrogen, peptide nitrogen, and total non-protein nitrogen were determined as follows:[1] 15 cc. of blood were treated for 15 to 30 minutes with 1 cc. of a 10 per cent solution of Squibb's urease. The proteins were then precipitated by diluting to 150 cc. with 2.5 per cent trichloroacetic acid. The filtrate was received in a measuring cylinder and the volume noted. When the drainage had practically stopped, the filtrate was transferred to a beaker and boiled 15 minutes to decompose the trichloroacetic acid. A few drops of saturated potassium carbonate solution were added to render the solution alkaline to phenolphthalein. The solution was then concentrated at 20–30 mm. pressure to remove ammonia and reduce the volume to a few cubic centimeters. It was finally transferred from the distilling flask to a 15 cc. measuring flask.

1 cc. duplicates were used for determination of total nitrogen by the micro-Kjeldahl technique of Folin and Farmer (5), the ammonia being titrated with 0.02 N acid and alkali. 2 cc. portions of the solution were used for amino nitrogen determination (6). For peptide nitrogen a 5 cc. portion was mixed with 5 cc. of concentrated hydrochloric acid and heated 24 hours at 100°C. to hydrolyze peptides. The greater part of the free hydrochloric acid was removed by concentration nearly to dryness under diminished pressure in a 50 cc. distilling flask. The residue was taken up with about 20 cc. of water, rendered alkaline to phenolphthalein with a few drops of saturated potassium carbonate solution, and concentrated again nearly to dryness to remove ammonia. The residue was brought to 5 cc. volume and 2 cc. portions were used for amino nitrogen determination, the value determined being that of the amino nitrogen plus the peptide nitrogen, which is converted into amino nitrogen by the acid hydrolysis.

EXPERIMENTAL OBSERVATIONS.

Dog 18–12 (Table I).—Mongrel, adult male. This dog was injected intravenously under ether anesthesia with a proteose solution prepared from material obtained from closed loops of the small intestine of the dog. A large dose, 260 cc., was given slowly and was associated with considerable fall in blood pressure. Vomiting and diarrhea appeared during the 1st hour after injection. During the 2nd hour the diarrhea became blood-tinged. Respiration was slow and deep; vomiting at intervals; pulse weak. After this the condition of the dog showed little change. The temperature showed slight rise with a fall shortly before death. 5.25 hours after injection animal was moribund; given ether and killed.

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[1] The methods will be discussed in more detail in *The Journal of Biological Chemistry*. The present outline is, however, sufficient to permit repetition of the experiments.

Autopsy.—Typical of acute proteose intoxication described previously (7). Blood clots slowly. The spleen and liver are intensely engorged and deep purple in color. The duodenum, jejunum, and ileum show intense engorgement of the mucosa, which is velvety and deep purplish red. There is much fluid in the intestine.

TABLE I.

Dog 18–12. Acute Intoxication. Proteose Injection.

Time after injection.	Total non-protein nitrogen.	Non-protein nitrogen per 100 cc. of blood as.				Remarks.
		Urea.	Non-urea.	Amino nitrogen.	Amino nitrogen plus peptide nitrogen.	
hrs.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Before.	45.5	10.8	34.7	11.0	18.8	Weight 26.3 lbs.
After.	47.9	11.5	36.4	12.2	22.4	Immediately after injection.
2.25	53.5	15.7	37.8	12.3	—	
3.75	62.6	20.7	41.9	13.2	25.8	
5.25	61.8	22.9	38.9	13.7	24.6	Autopsy typical.

Dog 18–19 (Table II).—Fox-terrier, adult female. Under ether anesthesia the proteose solution was given intravenously, 110 cc. in amount. The proteose was prepared from a human case of intestinal obstruction, material being removed at operation. Injection caused a moderate fall in blood pressure. Vomiting began within 30 minutes. After 1 hour a little semifluid feces was passed. The temperature became subnormal; pulse very weak. 5 hours after injection dog is prostrated; pulse barely palpable. 6 hours after injection dog in severe shock but would live perhaps 1 or 2 hours longer. Ether anesthesia; killed.

Autopsy.—Performed at once; picture identical with that described for Dog 18–12. Lesions are typical of an acute proteose intoxication.

Dog 18–25 (Table II).—Mongrel, young adult male. Under ether anesthesia a proteose solution, 175 cc., was injected intravenously. This had no effect upon the blood pressure. Vomiting began in 30 minutes and the temperature, which had risen slightly, fell gradually until death. The clinical picture is similar to that of the dogs described above. After 3.5 hours dog moribund and was killed.

Autopsy.—Performed at once; picture typical of proteose intoxication, as described for Dog 18–12.

Dog 18–28 (Table II).—Mongrel, young adult male. Under ether anesthesia a proteose solution, 150 cc., was injected intravenously. This proteose was obtained from a case of human intestinal obstruction. There was considerable fall of blood pressure during and after the injection. A progressive fall in temperature developed during the course of the intoxication. Vomiting began 20 minutes

after injection and diarrhea within an hour. The clinical picture is identicalwith that described above. After 4.1 hours the dog is in severe shock but might live 1 hour longer. Ether anesthesia; killed.

Autopsy.—Performed at once; findings are typical of acute proteose intoxication, as described for Dog 18–12.

TABLE II.

Acute Intoxication. Proteose Injection.

Time after injection.	Total non-protein nitrogen.	Non-protein nitrogen per 100 cc. of blood as.				Remarks.
		Urea.	Non-urea.	Amino nitrogen.	Amino nitrogen plus peptide nitrogen.	
			Dog 18–19.			
hrs.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Before.	33.0	9.0	24.0	9.9	16.4	Weight 13.4 lbs.
After.	37.1	10.1	27.0	10.8	18.7	
6.0	49.9	22.9	27.0	10.8	18.7	Killed.
			Dog 18–25.			
Before.	—	—	—	—	—	Weight 22.5 lbs.
3.5	37.2	11.5	25.7	11.1	20.1	Killed.
			Dog 18–28.			
Before.	—	—	—	—	—	Weight 17.3 lbs.
4.1	59.5	19.6	39.9	12.1	20.3	Killed.

Dog 18–21 (*Table III*).—Mongrel collie, adult female. Ether anesthesia. Laparotomy with section of small intestine 12 inches below the duodenojejunal junction. The sectioned ends turned in and united to produce complete obstruction. During the first 2 days after the operation the dog showed no clinical evidence of intoxication; no vomiting. On 4th day vomiting of bile-stained fluid began. There was slight fall in temperature. 5th day, vomiting continued. Dog is quite sick. 5 p.m. Ether anesthesia; killed.

Autopsy.—Performed at once. The peritoneal cavity contains 350 cc. of pale, thin, yellow, turbid fluid. Peritoneal surfaces show acute inflammation. A peritonitis resulted from necrosis of the intestine at site of obstruction with a slight escape of intestinal contents. The peritonitis is probably of short duration. Abdominal viscera negative except for cloudy swelling. Small intestine above obstruction shows definite engorgement of mucous membrane. There is little fluid in the intestine. Blood collected in dry oxalate shows little if any blood concentration.

Dog 18–50 (*Table III*).—Small bulldog, male; weight 12 pounds. Ether anesthesia, laparotomy, and production of complete obstruction in the middle of the small intestine. Obstruction produced by section of intestine and inversion of cut ends. Vomiting began on the 2nd day after operation. There is little intoxication until the 6th day; on the 7th day after operation dog is intoxicated, vomiting gray, foul smelling fluid. Pulse is weak. Ether anesthesia; killed.

Autopsy.—Performed at once. Wound is clean. Peritoneal surfaces smooth and moist. The small intestine above the obstruction is dilated and thickened.

TABLE III.

Intoxication of Intestinal Obstruction.

Time after operation.	Total non-protein nitrogen.	Non-protein nitrogen per 100 cc. of blood as.				Remarks.
		Urea.	Non-urea.	Amino nitrogen.	Amino nitrogen plus peptide nitrogen.	
Dog 18–21.						
days	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
2	—	7.9	—	—	—	Obstruction in midjejunum.
3	—	6.5	—	—	—	
5	—	55.2	—	—	—	10.30 a.m.
5	79.5	52.8	26.7	10.8	22.6	5.10 p.m. Killed.
Dog 18–50.						
7	137.2	97.9	39.3	9.6	17.0	Obstruction in low jejunum.
Dog 18–41.						
2	87.1	55.0	32.1	12.7	16.8	Obstruction in low jejunum.
Dog 17–222.						
25	39.2	16.3	22.9	4.3	7.9	Intestinal loop of ileum.

Other organs negative. The jejunum contained the usual amount of creamy-grayish, semifluid material which contains large amounts of toxic proteose.

Dog 18–41 (*Table III*).—Fox-terrier, adult male; weight 22 pounds. October 4, 1917. Ether anesthesia and extirpation of head of pancreas. Both arms of the pancreas isolated and left intact in the peritoneum. Dog recovered perfectly following this operation. November 6. Dog in good condition; weight 19 pounds. Ether anesthesia and simple obstruction in middle of small intestine. Day following operation animal is sick. Temperature normal. 48 hours after operation dog is severely shocked with subnormal temperature; would probably die within a few hours. Ether anesthesia; killed.

Autopsy.—Performed at once. Peritoneal cavity clear. The pancreas shows a good deal of induration and atrophy but the parenchyma is present in considerable amounts. The obstructed intestine contains about 450 cc. of creamy, brown, thick fluid.

Dog 17–222 (Table III).—Mongrel collie, female; weight 20.5 pounds. Ether anesthesia with isolation of a closed loop of the ileum beginning just above the ileocecal valve measuring 100 cm. in length. Ileum united around the loop by enteroenterostomy. Dog recovered well after operation. During the 2nd week occasional attacks of vomiting. There was evidence of chronic intoxication with gradual loss in weight. 25 days after operation weight was 17 pounds. Dog is not acutely intoxicated. Ether anesthesia; killed.

Autopsy.—Performed at once. Peritoneum is clean. The isolated loop contains 900 cc. of thick, creamy, brown fluid. The intestinal walls are hypertrophied and thickened. There is no ulceration of the mucous membrane. The rest of the gastrointestinal tract is negative. Other findings have no significance.

TABLE IV.

Normal Dog after Meat Feeding.

Time after meat feeding.	Total non-protein nitrogen.	Non-protein nitrogen per 100 cc. of blood as.				Remarks.
		Urea.	Non-urea	Amino nitrogen.	Amino nitrogen plus peptide nitrogen.	
hrs.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
0	29.7	9.8	19.9	10.1	15.8	Weight 14.1 kg. Fed 290 gm. of cooked ground beef heart.
0.5	30.0	10.3	19.7	10.1	14.5	
1.5	38.3	15.3	23.0	10.7	15.5	
3.0	46.9	22.4	24.5	9.9	14.8	
5.0	51.7	28.4	23.3	9.6	15.1	
8.0	50.7	29.5	21.2	10.4	15.9	

DISCUSSION.

The results show that intoxication by injected proteose causes an immediate and rapid increase in autolysis of body protein. The effect is so marked that the increase in blood urea is comparable with that accompanying the digestion of a heavy feeding of meat. The other non-protein nitrogenous constituents are slightly increased, due almost entirely to increases in free amino acids (NH_2) and peptides (NH). The entire picture of the non-protein blood nitrogen is indistinguishable from that following a heavy protein meal (Table IV).

The same remarks apply to the intoxication following intestinal obstruction. The urea is enormously increased over the usual fasting value, indicating a protein catabolism so rapid that the kidneys fail to keep pace with it. The non-protein nitrogenous products of the blood other than urea are not appreciably altered.

There is no evidence to indicate that the intoxication results from the tissue autolysis. The reverse appears to be the case, because in no instance does the urea concentration reach a toxic level, while the other nitrogenous products are not increased at all beyond usual limits. Also, in Dog 18–25, Table II, death occurred before autolysis had gone far enough to raise even the urea beyond that observed in fasting.

On the other hand, the results present a good example of accelerated protein catabolism and tissue autolysis caused by the action of a toxin, uncomplicated by the presence of parasites, by abnormally high temperature, or by any other apparent factors save the toxin itself.

The fact that the peptide nitrogen of the blood is not increased to an abnormal degree by the intoxication does not exclude the possibility that among the products of the induced autolysis there may be toxic proteoses which add their effect to that of the injected or absorbed proteose. The amounts of such proteoses required to intoxicate are too little to increase measurably the peptide nitrogen of the blood.

SUMMARY.

The acute intoxication following an injection of a toxic proteose is usually associated with a large increase (40 per cent or more) in the non-protein nitrogen of the blood. This increase is found chiefly in the blood urea nitrogen, but the amino and peptide nitrogens also may show small increases. The changes observed in the blood non-protein nitrogen are identical with those which follow the feeding of large amounts of meat (8).

These facts indicate that the proteose intoxication causes an abnormally rapid autodigestion of tissue proteins, but that the nitrogenous end-products are, in chief part at least, the same that result from normal catabolism of food proteins. There is no evidence that the

autolytic products play any part in causing the intoxication. The possibility of such a part and a resultant vicious circle is not excluded, but from the available facts the autolysis appears more as a result rather than cause of the intoxication.

It appears possible that in disease or intoxication tissue catabolism may be enormously accelerated and yet yield the end-products of normal protein metabolism.

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