THE RÔLE OF ANHYDREMIA AND THE NATURE OF THE TOXIN IN INTESTINAL OBSTRUCTION.

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The reader is referred to an article by Ellis[1] for the most recent comprehensive review of the literature on experimental intestinal obstruction. The following report includes work on the water loss, chemistry of the blood, and nature of the toxin produced in the obstructed small intestine of dogs.

Anhydremia.

That anhydremia plays a leading rôle in the causation of symptoms and that it may even be the cause of death in intestinal obstruction was maintained by Hartwell and Hoguet[2] and supported by the work of McLean and Andries[3] and by that of Bacon, Anslow, and Eppler.[4] Draper[5] found an average dehydration of 10 per cent, but this also occurred when the dogs were fasted or subjected to pilocarpine and then without symptoms comparable with those seen in intestinal obstruction. Therefore, he concludes that water loss plays an unimportant part in intestinal obstruction. Draper's work was confirmed by Dragstedt, Moorhead, and Burcky,[6] who observed fatal obstruction in dogs without much vomiting or other loss of fluid.

During the past 2 years we have produced obstruction of the small intestine in a large number of dogs.[7] Changes in water content of

* Research fellow of the National Research Council.

[1] Ellis, J. W., *Ann. Surg.*, 1922, lxxv, 429.

[2] Hartwell, J. A., and Hoguet, J. P., *J. Am. Med. Assn.*, 1912, lix, 82.

[3] McLean, A., and Andries, R. C., *J. Am. Med. Assn.*, 1912, lix, 1614.

[4] Bacon, D. K., Anslow, R. E., and Eppler, H. H., *Arch. Surg.*, 1921, iii, 641.

[5] Draper, J. W., *J. Am. Med. Assn.*, 1914, lxiii, 1079.

[6] Dragstedt, L. R., Moorhead, J. J., and Burcky, F. W., *J. Exp. Med.*, 1917, xxv, 421.

[7] All operations were performed under ether anesthesia.

TABLE I.

Water Content of Blood and Tissues before and after Intestinal Obstruction.

Dog No.	Blood.				Spleen.		Muscle.		Liver.	Weight.		Vomiting.	Remarks.
	Systemic.		Portal.										
	Before.	After.	Before.	After.	Before.	After.	Before.	After.	After.	Before.	After.		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*kg.*	*kg.*		
1	77.4	74.6	78.2	73.1	76.3	76.3	72.2	66.3	72.7			+	Inversion of both ends of duodenum. Length of life 1 day. No peritonitis. Killed.
2	74.9	72.2	75.0		74.8	75.6	71.5	68.7	71.5	14.25		++++	Inversion of both ends of duodenum. Length of life 1 day. No peritonitis. Killed.
3	77.4	74.8	77.5		77.8	77.0	63.3	63.3	71.2	16.0		++++	Inversion of both ends of duodenum. Length of life 1 day. No peritonitis. Killed.
4	77.4	76.0	77.1	75.5	77.8	76.4	72.5	70.2	73.3	19.0	17.75	++++	Inversion of both ends through upper jejunum. No peritonitis. Lived 1 day.
5	73.9	73.4	72.7	73.9	77.2	75.5	71.9	72.1	72.2	19.6	18.9	+++	Inversion of both ends of terminal ileum. Lived 2 days.
6	77.1	76.9	76.4	77.0	76.2	76.3	69.8	68.3		22.9	22.3	0	" " " " " lower " " 3 " No peritonitis. No vomiting.
7	74.0	73.8	74.1	73.3	76.4	76.3	71.2	72.4		18.0	17.2		Clamping of terminal ileum. Lived 2 days. No peritonitis.
8	75.5	75.1	75.0	75.0	76.1	75.3	72.4	73.1	71.6	17.6	16.7	++++	Wire around terminal ileum. Duration of life 3 days.
9	78.9	79.3	78.5		75.2	77.3	71.8	71.8	71.9	12.2			" " " " " " " 3 " No peritonitis.
10	77.1	77.1	77.5	76.6	76.7	76.8	73.8	73.6	72.5	16.3	15.7		Wire about terminal duodenum. Anterior gastrojejunostomy. Beginning diffuse peritonitis.

the blood and tissues, and changes in blood chemistry were determined in about thirty animals. The data of ten fairly representative experiments are presented in Table I. The specimens obtained after obstruction were removed shortly before the death of the animal.

The figures in Table I show that a moderate loss of water may occur, especially in high obstruction, but it is an inconstant phenomenon apparently not related to the severity of the symptoms. In fact we have observed most severe symptoms without any apparent desiccation whatsoever.

The water determinations were made by quickly removing blood or tissues, hashing the latter, weighing in stoppered bottles, and drying to constant weight over phosphorus pentoxide in a high vacuum at room temperature. This procedure usually required 3 days.

Chemistry of the Blood (Table II).

Urea and Non-Protein Nitrogen.—An increase occurred in all instances but with few exceptions the elevation was slight and apparently proportional to the severity of the symptoms. The rise in non-protein nitrogen was almost entirely due to the increase in urea.

The increased non-protein nitrogen in intestinal obstruction has been recorded by a number of investigators. Tileston and Comfort[8] observed it in man; G. H. Whipple and collaborators[9] and Bacon, Anslow, and Eppler[4] noted an increase in the non-protein nitrogen in the blood of dogs. McQuarrie and Whipple[10] maintained that the increase of non-protein nitrogen is partly due to disturbed renal function, while Rabinowitch[11] observed good phthalein elimination and therefore believes that increased protein destruction is the cause and he is supported by Louria[12] who also determined the phthalein elimination in human intestinal obstruction.

Chlorides.—A marked decrease of blood chlorides occurred in nearly all our instances and this appeared to be related to the frequency of vomiting.

[8] Tileston, W., and Comfort, C. W., *Arch. Int. Med.*, 1914, xiv, 620.

[9] Cooke, J. V., Rodenbaugh, F. H., and Whipple, G. H., *J. Exp. Med.*, 1916, xxiii, 717.

[10] McQuarrie, I., and Whipple, G. H., *J. Exp. Med.*, 1919, xxix, 397.

[11] Rabinowitch, M., *Canad. Med. Assn. J.*, 1921, xi, 163.

[12] Louria, H. W., *Arch. Int. Med.*, 1921, xxvii, 620.

The decrease in chlorides is comparable with that observed by Macallum and associates[13] and by Hastings, Murray, and Murray[14] in experimental pyloric obstruction. Haden and Orr[15] conclude from their experiments on canine and human intestinal obstruction that renal insufficiency may be excluded as causative of the high non-protein nitrogen in the blood and that the lowering of blood chlorides is not to be accounted for by the loss of hydrochloric acid through vomiting.

TABLE II.

Chemistry of the Blood before and after Intestinal Obstruction.

Dog No.	Amount per 100 cc. of blood.													
	Urea nitrogen.				Non-protein nitrogen.				Chlorides.		Fibrin.		Calcium.	
	Systemic.		Portal.		Systemic.		Portal.		Systemic.		Systemic.		Systemic.	
	Before	After.	Before.	After.	Before.	After.	Before.	After.	Before.	After.	Before.	After.	Before.	After.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg*
1	14.0	45.3				93.2		96.2	560	454				
2	14.0	21.0	15.0	21.8	34.1		34.4		546	385				
3	17.5	33.0	17.1		35.4		34.6		539	385				
4	15.4	19.7	16.2	23.9	33.1	43.3	35.0	48.3	596	552				
5	11.2	19.1	11.9	21.0	33.0	38.0	30.3	37.1	586	378				
6	13.5	16.8	14.8	18.5	34.2	44.4	35.0	44.4	506	506				
7	13.0		14.9		38.0	49.2	38.0	50.2	532	379				
8	19.6	42.0	18.6	44.3	37.6	59.7	39.3	66.3	541	419				
9	14.0	25.3	14.0		34.9	46.1	34.9	46.1	581	394				
10	9.3	115.6	9.3	116.2	30.0	156.2	28.6	156.2	512	369				
11		39.0				73.1					530	719	9.1	9.7
12		29.8				61.2					450	579	9.9	9.9
13		170.0				231.0						1,468		11.1

Other Blood Constituents.—Fibrin estimated in three instances was found to be increased. The increase in fibrin is hard to explain in view of the delayed coagulation time in experimental obstruction observed by Whipple, Stone, and Bernheim[16] and others. The calcium content of the serum was unchanged. Cholesterol and sugar were determined a few times but no significant change was noted.

[13] Macallum, W. G., Lintz, J., Vermilye, H. N., Leggett, T. H., and Boas, E., *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 1.

[14] Hastings, A. B., Murray, C. D., and Murray, H. A., Jr., *J. Biol. Chem.*, 1921, xlvi, 223.

[15] Haden, R. L., and Orr, T. G., *J. Exp. Med.*, 1923, xxxvii, 365.

[16] Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1913, xvii, 286.

The Toxin.

Roger and Garnier[17] obtained a toxic substance from the contents of the obstructed small intestine of rabbits by precipitation with alcohol and extraction of the precipitate with water. G. H. Whipple and assistants[18] proceeded in a similar manner but further purified the material by precipitation with ammonium sulfate and showed that the toxin was resistant to heat, soluble in water, insoluble in alcohol, undigested by proteolytic ferments, non-dialyzable, and precipitated by half saturation with ammonium sulfate. Owing to its resistance to the action of trypsin they thought that the toxin was a heteroproteose. Gerard[19] suggested that the toxin might be a peptamine containing histamine because when hydrolyzed with 20 per cent hydrochloric acid it retained its toxic properties and yielded a fraction which gave the diazo reaction.

The present work includes an investigation of the properties and analyses of the toxic material, attempts at fractionation, and a study of the individual fractions. Owing to the difficulty of obtaining the substance (about 1 gm. of purified material was obtained from the obstructed intestine of fourteen dogs) it has only been possible to carry out a limited number of experiments. The method employed was that described by Ellis[1] with additional steps to further purification.

The mucous membrane and content of the obstructed intestine were boiled in 200 to 300 cc. of water, then filtered through gauze and cotton, and treated with 5 volumes of 95 per cent alcohol. The precipitate dissolved in water was boiled with 1 gm. of magnesium sulfate, then filtered through paper, and reprecipitated with 5 volumes of alcohol. The material from fourteen dogs obtained in this manner was dissolved in a small amount of distilled water and dialyzed in a collodion sac against distilled water for 5 days. Thymol was used as a preservative. The neutral mixture, now free from magnesium sulfate, was centrifuged and the supernatant fluid strongly acidified with acetic acid boiled for a few minutes, cooled, and again centrifuged. The clear fluid was again precipitated with 5 volumes of alcohol. The next morning the solid was removed and dried over phosphorus pentoxide in vacuum.

The dry substance weighed 1 gm. and possessed the following properties. 71 mg. dissolved in water and injected intravenously killed

[17] Roger, H., and Garnier, M., *Rev. méd.*, 1906, xxvi, 953.

[18] Whipple, G. H., Rodenbaugh, F. H., and Kilgore, A. R., *J. Exp. Med.*, 1916, xxiii, 123.

[19] Gerard, R. W., *J. Biol. Chem.*, 1922, lii, 111.

a dog weighing $5\frac{1}{2}$ kilos in $2\frac{3}{4}$ hours. Death was preceded by retching, vomiting, wide respiratory excursions of the abdominal type, bloody diarrhea, and tenesmus. At autopsy there was hemorrhage into the mucous membrane of the entire small intestine but this was most marked and confluent in the duodenum. Punctate and non-confluent hemorrhages were also present in the colon. The toxic substance is soluble in water with slight opalescence which disappears on adding a trace of alkali. Precipitation results when a large excess of acetic acid is added. It gives a strong Molisch reaction, also Millon, diazo, weak biuret, and Hopkins-Cole tests.

Analysis.—The substance was dried in vacuum over sulfuric acid at 100°C. 0.1002 gm. gave 0.1708 gm. of carbon dioxide and 0.062 gm. of water. 0.0958 gm. required 8.25 cc. of 0.1 N acid when analyzed according to Kjeldahl's method. 0.0479 gm. was dissolved in 5 cc. of water. 2 cc. of this solution yielded 0.270 cc. of nitrogen gas at 763.4 mm. and 25° when analyzed according to Van Slyke's method for amino nitrogen.

Found. Carbon 46.48 per cent.
Hydrogen 6.92 " "
Nitrogen 12.05 " "
Amino nitrogen before hydrolysis 0.69 per cent.
" " after " 8.00 " "
Ash 7.58 per cent.

Hydrolysis.—0.554 gm. of dry substance was hydrolyzed by boiling with 20 per cent hydrochloric acid under reflux for 36 hours. The acid was removed by evaporation *in vacuo* and the residue was dissolved in 50 cc. of slightly alkaline water. 2 cc. of this solution were analyzed for amino nitrogen according to Van Slyke's method and gave 3.25 cc. of nitrogen gas at 760.4 mm. and 28°. Before hydrolysis 5.7 per cent of the nitrogen was in the amino form; after hydrolysis 66 per cent was present as amino nitrogen. The hydrolysate gave a strong reaction for phosphate with ammonium molybdate and nitric acid but no reaction for sulfates. 8 cc. of the solution were precipitated with phosphotungstic acid according to the method of Hanke and Koessler.[20] The small precipitate which formed on standing was freed from phosphotungstic acid with barium hydroxide; the filtrate

[20] Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1920, xliii, 543.

was evaporated, alkalinized with sodium hydroxide, and extracted with amyl alcohol. The extract failed to give the diazo reaction for histamine.

Toxicity of the Dialysate.—The fluid obtained during the first 2 days of dialysis of the toxin was evaporated to a small volume and injected intravenously without toxic effects.

Toxicity of the Alcoholic Filtrate.—The alcoholic filtrate obtained after precipitation of the intestinal contents of three obstructed dogs was evaporated in vacuum, taken up in water, filtered, sterilized, and injected into the vein of a dog without toxic effect.

Toxicity of the Hydrolysate.—25 cc. of a solution of hydrolyzed toxin (equivalent to 0.277 gm. of dry substance) were injected into the vein of a 5 kilo dog without toxic effect.

Presence of Histamine in the Alcoholic Liquors.—The alcoholic filtrate obtained from precipitation of the contents of the intestine of twelve obstructed dogs was evaporated to 1,500 cc., acidified with dilute hydrochloric acid, filtered, and treated as follows:

Precipitation with basic lead acetate, filtration, and removal of the excess of lead with hydrogen sulfide, evaporation of the clear filtrate to 500 cc., and precipitation with phosphotungstic acid according to Hanke and Koessler's method. The phosphotungstate was decomposed with barium hydroxide, the barium phosphotungstate removed by filtration, and the filtrate freed from barium with sulfuric acid. The clear solution was evaporated to 400 cc. and precipitated with silver nitrate and barium hydroxide according to the Kossel and Kutscher process. At first sufficient barium hydroxide was added to produce a neutral reaction and the resulting precipitate was removed; then an excess of silver nitrate and barium hydroxide was added and the precipitate treated with sulfuric acid and hydrogen sulfide in the usual manner. The filtrate was evaporated to 10 cc., made alkaline with 3 gm. of sodium hydroxide, and extracted with amyl alcohol. The alkaline solution gave a strong diazo reaction, thus indicating the presence of considerable histidine. The alcoholic solution was extracted with 100 cc. of 0.1 N sulfuric acid. The acid liquid was neutralized with barium hydroxide and filtered. The clear barium-free filtrate was concentrated to 4.5 cc. 1.5 cc. of this solution injected intravenously into a guinea pig killed by asphyxia in 3 minutes. 0.5 cc. killed a large guinea pig by asphyxia in 5 minutes. Autopsy showed marked distension of the lungs.

The histamine content of the fluid was determined colorimetrically by von Fürth and Hryntschak's[21] method. It was found that each

[21] von Fürth, O., and Hryntschak, T., *Biochem. Z.*, 1914, lxiv, 172.

cubic centimeter contained 0.82 mg., or about 4 mg. in the total amount of fluid.

Test for the Presence of Toxin in the Liver of Obstructed Dogs.—A watery extract of the livers of two obstructed dogs was precipitated with 5 volumes of alcohol, the precipitate was dissolved in water and injected intravenously into a dog without producing signs or symptoms indicative of toxicity.

Test to Determine Whether the Pancreas Is Necessary for the Formation of Toxin.—Toxin prepared from the obstructed intestine of a depancreatized dog produced death when injected intravenously into another dog.

Fractionation Experiments.—2.5 gm. of purified material, prepared as already described from the obstructed intestine of nineteen dogs, were dissolved in 150 cc. of water. The turbid solution, which changed to a colloidal suspension on adding 17 cc. of glacial acetic acid, was dialyzed in collodion sacs against distilled water for 24 hours. The dialysate when evaporated to a small volume gave a positive test for phosphate but negative biuret, Fehling, Millon, and diazo reactions, and was not toxic when injected intravenously into a dog.

The material in the collodion sacs was now further dialyzed against three 4 liter portions of distilled water; it consisted of an upper clear layer, a middle layer containing suspended matter, and a thick sediment. The upper layer was filtered and precipitated with 5 volumes of 95 per cent alcohol (fraction 1). The remainder of the material in the dialyzing tubes was centrifuged, and the supernatant fluid filtered and precipitated with 5 volumes of alcohol (fraction 2). The sediment in the centrifuge tube was stirred with water and centrifuged for 2 hours without obtaining good sedimentation; the supernatant fluid was then poured off and precipitated with alcohol (fraction 3). The final residue in the centrifuge tube constitutes fraction 4.

Fraction 1 gave a slight biuret and Millon reaction and a strong Molisch and pentose reaction (Bial). On adding the diazo reagent no reaction was obtained at first but on long standing a brown color gradually developed. When evaporated with dilute nitric acid a yellow residue remained which became orange-red on adding sodium hydroxide and bluish purple when heated with this reagent, thus

indicating the presence of purines. Dried to constant weight in vacuum over phosphorus pentoxide at 100°, the substance analyzed as follows: 0.0496 gm. required 2.7 cc. of 0.1 N acid according to Kjeldahl's method. 0.0576 gm. was dissolved in 5 cc. of water; 2 cc. of this solution gave 0.25 cc. of nitrogen gas at 755 mm. and 21° when analyzed for amino nitrogen according to Van Slyke's method. Another 2 cc. portion of the solution was hydrolyzed by boiling with 25 per cent hydrochloric acid for 25 hours, neutralized, and diluted to 8 cc. 2 cc. of the dilution gave 0.53 cc. of nitrogen at 24° and 749 mm. of mercury.

0.0079 gm. of substance was dissolved in 3 cc. of 0.1 N sodium hydroxide. 1 cc. of this solution was mixed with 1 cc. of N sulfuric acid and hydrolyzed in a Folin sugar tube by immersion in boiling water for 4 hours.[22] The solution was neutralized and its reducing power determined according to the method of Folin and Wu.[23]

Found. Nitrogen 7.63 per cent.
Amino nitrogen before hydrolysis 0.61 per cent.
" " after " 5.05 " "
Reducing power expressed as glucose 28.8 per cent.

0.100 gm. of this fraction injected intravenously killed a dog weighing 6.4 kilos in 3½ hours. Autopsy showed characteristic hemorrhages in the mucosa of the small intestine.

Fraction 2 gave all the reactions obtained with the first fraction and analyzed as follows: 0.0647 gm. analyzed according to Kjeldahl's method required 5.04 cc. of 0.1 N acid. 0.0057 gm. was dissolved in 3 cc. of 0.1 N sodium hydroxide. 1 cc. of this solution was hydrolyzed by heating with 1 cc. of N sulfuric acid and the reducing power determined according to Folin and Wu.

Found. Nitrogen 10.91 per cent.
Reducing power after 3 hours hydrolysis 18.4 per cent.
" " " 4 " " 18.7 " "

0.110 gm. was heated for 4 hours with 10 cc. of 0.5 N sulfuric acid with reflux condensation, then neutralized and injected into a dog

[22] Previous experiments with yeast nucleic acid had shown that this period of hydrolysis yielded the maximum amount of reducing substance.

[23] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

weighing 3.1 kilos without any toxic manifestations. 0.100 gm. heated for 4 hours as above but with 10 cc. of 0.5 N sodium hydroxide was neutralized and injected intravenously into a dog weighing 2.8 kilos without any toxic symptoms.

Fraction 3 gave tests identical with those obtained with fraction 1, except that the biuret test was stronger. When dissolved in water it formed a turbid suspension which cleared on the addition of alkali but became cloudy again when acidified. This fraction, as well as all the others, was soluble in an excess of mineral acid. Dried in vacuum at 100° over phosphorus pentoxide it analyzed as follows: 0.0462 gm. gave 0.0046 gm. of $Mg_2P_2O_7$ after fusion with sodium hydroxide and sodium nitrate. 0.0931 gm. gave 0.1598 gm. of CO_2, 0.0506 gm. of H_2O, and 0.0061 gm. of ash when burnt in the combustion tube. 0.1127 gm. required 11.91 cc. of 0.1 N acid when analyzed according to Kjeldahl's method. 0.0606 gm. was dissolved in 5 cc. of water containing a trace of alkali. 2 cc. of this solution (0.02424 gm. of substance) gave 0.28 cc. of nitrogen at 20° and 762 mm. when analyzed according to the method of Van Slyke for amino nitrogen. Another 2 cc. portion (0.02424 gm.) was hydrolyzed with boiling 20 per cent hydrochloric acid for 62 hours, then neutralized, and made up to 8 cc. 2 cc. of this solution (0.00606 gm.) gave 0.87 cc. of nitrogen at 22° and 750 mm. 0.010 gm. was dissolved in 4 cc. of 0.1 N sodium hydroxide. 1 cc. of this solution (0.0025 gm.) was heated for 4 hours with 1 cc. of N sulfuric acid and its reducing power determined according to the Folin and Wu method.

Found.	Carbon	46.80 per cent.
	Hydrogen	6.08 " "
	Nitrogen	14.79 " "
	Phosphorus	2.77 " "
	Ash	6.55 " "
	" calculated from phosphorus content 6.34 per cent.	
	Amino nitrogen before hydrolysis 0.658 per cent.	
	" " after " 7.98 " "	
	Reducing power expressed as glucose 11.2 per cent.	

0.025 gm. was injected intravenously into a small dog weighing 3.6 kilos and produced toxic effects including vomiting and bloody diarrheal stools. The dog recovered after 5 days.

Fraction 4 was washed with alcohol and dried. It contained 12.04 per cent of nitrogen and gave the same tests as the previous fractions. It was not further investigated.

DISCUSSION.

The data regarding the toxin appear to support most of the conclusions of G. H. Whipple and collaborators; but the greater solubility of the substance in alkali and strong mineral acids than in weak acids, its non-coagulability by heat, its chemical reactions and analyses including the presence of phosphoric acid and pentose characterize it as a nucleoprotein. The toxic material does not contain histamine free or combined as suggested by Gerard. Though a small amount of histamine is present in the content of the obstructed bowel it appears insufficient to contribute to its toxic nature.

The more soluble fractions of the toxin contain less nitrogen and more carbohydrate relatively speaking and may therefore be cleavage products derived from the original material by hydrolytic removal of the purines or pyrimidines, thus leaving substances richer in pentose and poorer in nitrogen.

SUMMARY.

1. Data are presented which show that anhydremia does not play a leading rôle in the causation of symptoms in intestinal obstruction.

2. Chemical changes in the blood corresponding with those noted by previous investigators were observed.

3. Additional data regarding the chemistry of the toxin found in the obstructed intestine of dogs were obtained.