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The non-protein nitrogen of blood: 1. The removal of the protein. 2. The estimation of creatine.

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In a previous publication,[1] the author described a modification of the method of Folin and Denis[2] for the determination of non-protein nitrogen in blood. Trichloracetic acid was used to precipitate the protein and the trace that remained in the filtrate was removed by shaking with kaolin. It was shown that the nitrogen of an amino-acid mixture added to blood could be completely recovered by this method and that no nitrogen was split off from proteins by this treatment. Bock[3] recently described another method for obtaining protein-free filtrates from blood. He coagulated the protein by running the blood into boiling 0.01 *N* acetic acid, evaporated the filtrate to a small volume, precipitated most of the remaining protein with trichloracetic acid and removed the last traces with kaolin. It seemed that it should be possible to remove the protein from the filtrate from the coagulum by means of kaolin, directly, without the use of trichloracetic acid. This was found to be the case. Kaolin is added to the filtrate from the coagulum, shaking the mixture thoroughly, until the foam, which is at first voluminous and persistent, becomes scanty and comparatively evanescent. One drop of glacial acetic acid for each 100 c.c. of fluid is then added in order to agglutinate the kaolin and the mixture is then filtered. The filtrate is protein-free, so nearly as may be determined by the usual tests. It may be evaporated to small volume (less than one tenth that of the original blood) either at atmospheric or reduced pressure without foaming. Determinations of the nitrogen in such filtrates agree with those obtained by the trichloracetic acid-kaolin method. However, both methods are made inaccurate through the removal

[1] Greenwald, *Journal of Biological Chemistry*, 1915, 21, 61.

[2] O. Folin and W. Denis, Ibid., 1913, 11, 527.

[3] Bock, Ibid., 1917, 28, 357.

by the kaolin of nitrogenous substances other than protein. This is most marked with substances of a basic nature, including diamino-acids and ammonia (present as ammonium chloride), and is absolutely quantitative with creatinine, even with amounts several times as great as those in the filtrates from the blood coagula. In the presence of trichloracetic acid, absorption appears to be less marked. The omission of kaolin after the precipitation of the protein by means of trichloracetic acid apparently leads to more accurate figures for the total non-protein nitrogen of the blood. These are about 3 mg. per 100 c.c. of blood higher than those obtained with the use of kaolin. According to Folin and Denis,[1] the results obtained by their meta-phosphoric acid method agree very closely with those obtained by their methyl alcohol method. Since the latter has been shown to give too low results,[2] it would seem that meta-phosphoric acid, also, does not yield all the non-protein nitrogen to the filtrate. Gettler and Baker[3] used hydrochloric acid and mercuric chloride for the precipitation of protein and obtained higher values for the non-protein nitrogen of the blood than have other observers. Comparison of the two methods in this laboratory[4] failed, in the few experiments made, to reveal any marked difference in the results obtained by the use of hydrochloric acid and mercuric chloride and by the use of trichloracetic acid and kaolin. Gettler and Baker do not direct the use of sodium sulfide or other precipitants for the mercury before the distillation. Unless the mercury is removed or precipitated as sulfide, the results be as much as 50 per cent. too low.

Although creatinine, under the same conditions, is quantitatively removed by kaolin, *creatine is not absorbed at all*, either from dilute aqueous solution or when added to blood. This offers a method for the estimation of creatine in the blood. The filtrate from the kaolin is treated with a small volume of *N* hydrochloric acid and evaporated to approximately that volume. (In most of the experiments, a volume of filtrate equivalent to 50 c.c. of blood was evaporated to 5 or 10 c.c. after the addition

[1] Folin and W. Denis, Ibid., 1916, 26, 491.

[2] I. Greenwald, loc. cit.; Bock, loc. cit.

[3] A. O. Gettler and W. Baker, Ibid., 1916, 25, 210.

[4] I. Greenwald, loc. cit. (page 64) and unpublished expts.

of 10 c.c. of *N* hydrochloric acid.) This is then neutralized with sodium hydroxide and creatinine is determined in the usual manner. When only small amounts of blood are available, 1 c.c. of acid is used and, after evaporation and neutralization, the solution is diluted to 25 c.c. with saturated picric acid and the determination is made by Folin's micro-method. The amount of creatine thus found is about 4 mg. per 100 c.c. of blood. Added creatine is recovered quantitatively, showing that there is no conversion to creatinine in the process of coagulating the protein. No claim is made that the substance reacting with picric acid and sodium hydroxide under these conditions is creatinine derived from the creatine of the blood. However, it is apparently not formed by the condensation of glucose, urea and uric acid. A solution containing 350 mg. glucose, 125 mg. urea and 5 mg. uric acid was evaporated with 10 c.c. of *N* hydrochloric acid. There was an apparent creatine content of 0.1 mg. Smaller amounts of urea and glucose gave no perceptible amounts of chromogenic substance.

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Anaphylaxis in the dog.

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1. Dogs in severe anaphylactic shock have been bled to death, and the blood has been used to transfuse normal dogs. No symptoms of any kind have been produced. Hence the conclusion is drawn that the symptoms of anaphylaxis are not due to the presence of toxic substances in the blood.

2. The liver of sensitized dogs has been perfused with normal blood by means of anastomosis of the portal vein with the carotid of another dog. The blood, as it flows from the inferior cava, clots within a few minutes. If the antigen (horse serum) is injected into the connecting tubing, the blood in the cava soon becomes less coagulable, or quite incoagulable. The conclusion is drawn that the incoagulability of the blood is due, at least in part, to the reaction of the sensitized liver cells to the antigen.