2. The influence of nuclein, nucleic acid and of adenin on the uric acid elimination was studied. It was observed that all these substances caused an increase in the uric acid elimination.

3. The fate of thymin ingested with the food was investigated. The greater part of the ingested thymin was recovered from the urine.

4. An attempt was made to find thymin in the urine of the same dog after feeding on nuclein and on nucleic acid. The endeavor was not successful.

5. The influence of a diet containing a small proportion of protein but abundant in calories was studied. It was noticed that this diet occasioned an increase in the uric acid output.

6. The influence of fasting on the uric acid output was observed. It was noted in the course of the fast that the uric acid elimination was above the normal.

15 (158)

On the fractionation of agglutinins and antitoxin.

By **R. B. GIBSON** and **K. R. COLLINS.**

[*From the Research Laboratory of the Department of Health, of New York City.*]

E. P. Pick in 1901 associated a number of anti-substances individually with the one or the other of the two serum globulin fractions of the Hofmeister classification. In the pseudoglobulin [3.4 to 4.6 sat. $(NH_4)_2SO_4$ solution[1]] group of antibodies he placed the diphtheria and tetanus antitoxins and the typhoid agglutinin of horse serum; the lower or euglobulin fraction (2.9 to 3.4 sat.) comprises diphtheria and tetanus antitoxin and cholera lysin in the goat, typhoid agglutinin in the goat, rabbit and guinea pig, and finally cholera agglutinin in the horse and goat. It becomes possible, according to Pick, to separate the individual specifically reacting anti-substances by fractioning appropriate mixtures of sera. Such a possibility suggested the application of this method to the further study of certain anti-bodies, especially of the relation of specific and group agglutinins developed by immunization against a single strain of organism. Preliminary experiments in the course of our inves-

[1] The degrees of saturation, as here expressed, indicate a concentration equivalent to a content in 10 c.c. of solution of 3.4 c.c. and 4.6 c.c. of saturated ammonium sulphate solution respectively.

tigation indicated the unreliability of Pick's differentiation, and attention was accordingly directed to the actual possibility and practicability of distinguishing between anti-bodies by fractionation of the globulin. The availability of poly-agglutinative sera for the work gave a chance for making numerous and extended observations of the distribution of these anti-bodies in the fractions.

It was found repeatedly in experiments with rabbit and goat sera that the agglutinins for the dysentery group of organisms (Flexner Manila and Shiga), typhoid, coli and cholera, were not confined to either the pseudoglobulin or the washed [with 3.4 sat. $(NH_4)_2SO_4$ solution] euglobulin fractions; they were either split by the fractioning, the major portion occurring in the pseudoglobulin, or almost the entire amount of the agglutinating substances recovered were in this higher fraction in the original quantitative proportion to one another. With anti-dysentery horse serum, the dysentery (Shiga and Flexner) and coli agglutinins were fairly quantitatively though not qualitatively split between the pseudo- and euglobulin fractions, the latter containing the lesser amount. With an anti-cholera and anti-typhoid horse serum, the pseudoglobulin (two experiments) and also the filtrates from two additional 3.6 and 3.8 saturation precipitations contained the bulk of the agglutinins. In subsequent experiments with sera from other bleedings as well as with the sera used above, the typhoid agglutinin was divided between the two fractions with a somewhat larger proportion occurring in the pseudoglobulin.

It is apparently difficult to control all the conditions under which experiments of this type are made; absolutely constant results at times cannot be obtained on successive repetition of the work.

The results of exhaustion experiments on the two globulin fractions were the same as those that would be obtained in the use of the native serum, and failed to give any reason for believing that we were dealing with a separation of group and specific agglutinins through fractioning.

Precipitation of anti-diphtheria goat serum showed that about half the antitoxin remained in the pseudoglobulin; practically none was found in the euglobulin while the 3.4 saturated $(NH_4)_2SO_4$ solution washings contained the balance.

The results of the work thus far accomplished have demon-

strated the untrustworthiness of any such differentiation of the anti-bodies as those contained in the euglobulin and those of the pseudoglobulin. No evidence has been adduced from our experiments to show that the agglutinins developed in the rabbit, goat and horse can be classed as belonging to either globulin, or that these anti-bodies can be separated from one another by ammonium sulphate fractioning of polyagglutinative sera.

16 (159)

Further observations of the effects of ions on the activity of enzymes.

By **WILLIAM N. BERG** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons.*]

Previous communications from this laboratory[1] have made it evident that peptolysis of *fibrin* is unequal in rate and extent in acid solutions of equipercentage, equinormal (isohydric), equimolecular, or equidissociated (isohydrionic) concentration. The same may be said of tryptolysis of the same protein in a series of bases of analogous concentrations.

We have found that the sequence of zymolysis, both in rate and extent in a given group of acid or basic solutions, varies considerably with the nature of the protein. This fact makes it impossible accurately to formulate statements regarding various phases of peptolysis or tryptolysis without specifying the particular protein involved in the process; it also renders doubtful various general conclusions of common acceptance pertaining to digestion that have been derived, in one research or another, from the use of a single protein. A study of the peptolysis of *many proteins*

[1] Gies: *American Journal of Physiology*, 1903, viii (Proceedings of the American Physiological Society, 1902, p. xxxiv); *the same journal*, 1903, ix (Proceedings of the same Society, 1903, p. xvii); Gies and collaborators: *Biochemical Researches*, 1903, i, pp. 61–63. Also Berg (communicated by Gies): *Science*, 1906, xxiii, p. 335 (Proceedings of the Section of Biological Chemistry of the American Chemical Society in affiliation with Section C (Chemistry) of the American Association for the Advancement of Science, 1905); *Proceedings* of the American Association for the Advancement of Science, 1906, p. 331.