

of the non-supplemented control group. Nine or more individuals were used in each group.

The uric acid of the whole blood was determined at 35 to 40 days of age. The average value for the carotene group was 3.7 mg. per 100 cc. of blood, whereas that for the xanthophyll group was 7.2 mg., and for the chlorophyll group 5.8 mg. The birds which subsisted on the unsupplemented white corn ration showed an average value of 9.8 mg., while those receiving the same ration but with the white corn replaced by yellow corn had a uric acid value of 3.3 mg., which is considered normal. Our observations with a number of young chicks show the normal uric acid value to be 4.00 mg. per 100 cc. of whole blood or less. In earlier work a high uric acid value was noted in birds deprived of food. It is not known whether the high values noted in vitamin A deficient chicks are due indirectly to the deficiency which is accompanied by a reduced intake of food or whether the vitamin A deficiency is directly responsible.

The results confirm those of Karrer, Euler and Rydbom with regard to carotene and xanthophyll. In addition chlorophyll has been shown to lack provitamin A properties.

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Quantitative Aspect of the Hypothetical Incorporation of Injected Antigen in Resulting Antibody. II. Experimental.

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The authors¹ have previously examined, from an arithmetical point of view, the Buchnerian hypothesis that antibody is a conjugate of body globulin and injected antigen. These speculations led us to undertake the following experimental investigation based upon the reactivity of artificial compound proteins in which the hapten chosen to confer the new specificity contains integrally an inorganic atom (As) for which a direct and very delicate chemical test is available. Two compounds, ovomucoid-diazo-arsanilic acid and casein-diazo-arsanilic acid, were prepared. The serum of a rabbit immunized against the first reacted with the second, showing the presence of an arsanilic-acid-specific antibody. The optimal precipitation ratio by volumes of antiserum to casein-diazo-arsanilic acid

¹ Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1931, **21**, 113.

was found to be about 150. Such a mixture is considered neutral—at least neither reagent is present in any large excess. It seems reasonable to suppose that within this zone, antibody and antigen enter respectively into the precipitate in the proportion of one molecule to one molecule, or one molecule to a small number of molecules. If it is assumed that the antibody is a globulin conjugate of the antigen, and contains a similar amount of arsenic (as would necessarily be true if there were no breaking up of the injected antigen), it can be shown from the amounts of arsenic and casein found in the casein-diazo-arsanilic acid solution, and the above ratio, that 10 cc. of antiserum should have contained about 20 mmg. (20×10^{-6} g.) of arsenic, an amount easily detectable. None was found. This would seem to cast doubt on the Buchnerian hypothesis.

In a recent paper Heidelberger and Kendall² similarly come to the conclusion that the injected antigen in one of their experiments must have split into at least two specifically reactive fragments, if the Buchnerian hypothesis be true.

However, from the amounts of arsenic and casein in the casein-diazo-arsanilic acid solution it is possible to arrive at a new estimate, namely, the number of diazo (haptens) groups in combination with one molecule of protein. For casein it was found to be 110, which in view of the roughness of the data may be considered a fair approximation to the theoretical value of 160. The questions now arise—with how many of these groups (haptens) must antibody react in order to produce precipitation, and how much is the injected antigen supposed to break up *in vivo* before entering into the specifically reactive hypothetical antibody-conjugate? The answers seem to be within reach of further experimental inquiry in which the authors are now engaged.

We are indebted to Mr. Saul Kamens for assistance in this work.

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Experiments on Gill Reduction in Neotonous *Triturus viridescens*.

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In certain regions neotonous individuals of *Triturus viridescens* are found in the same ponds with metamorphosed ones. Their gills

² Heidelberger, M., and Kendall, F. E., *Science*, 1930, **72**, 252.