

clamp. Then both halves of the clamp are united and clamped. I believe that when we deal with small blood vessels it is easier to hook the walls on the pins than to turn them back like a cuff as is done in Crile's cannula. When the clamp is closed, both blood vessels are connected with the endothelial surfaces.

I have performed several operations on dogs, uniting the femoral or cervical vein of one dog to the femoral artery of another. The transfusion was kept up for over half an hour, until the donor was practically exsanguinated. There was no clotting, leakage, or any other defect in the clamp.

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The further separation of antitoxin from its associated proteins in horse serum.

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The literature concerning means of purification of anti-bodies and their chemical characteristics has been thoroughly reviewed by Gibson,¹ Ledingham,² Banzhaf and Gibson,³ and Brieger and Kraus.⁴

Stark⁵ was the first to report that by heating for one hour at 56° C., ovalbumin could be converted into a body, which, because of its precipitation and solution reactions, and also its composition, was obviously a globulin. Later Noll⁶ showed the same to be true of albumin in rabbit, dog and horse serum.

My experiments were to ascertain the resulting conditions after heating antitoxic horse serum, citrated plasma, and Gibson's concentrated and partially purified antitoxic globulin solution.

An antitoxic serum by the Gibson method¹ gave the following: An elimination of 23 per cent. protein and an increase of antitoxic units per gram protein of 30 per cent. over the native serum. A

¹ *Journal of Biolog. Chem.*, 1, p. 161, 1906.

² *Journal of Hyg.*, vii, p. 65, 1907.

³ *Journal of Biolog. Chem.*, iii, p. 253, 1907.

⁴ *Berl. klin. Woch.*, xliv, p. 946, 1907.

⁵ *Zeitschr. f. Biol.*, xl, p. 494, 1900 (new series, vol. 22).

⁶ *Hofmeister's Beiträge*, iv, p. 563, 1904.

series of the same antitoxic serum was heated for from 6 to 72 hours in closed containers, at a temperature of 57° C. After cooling to room temperature, the series was saturated with sodium chloride and brought up to a dilution of 1 : 10 with saturated sodium chloride solution. Twelve hours later the resulting precipitations were filtered off. Potency tests on these filtrates showed a loss of 5 per cent. after heating 6 hours and an increasing loss up to 22 per cent. after heating 72 hours. The protein converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. for the 6-hour period, increasing up to 48 per cent. for the 72-hour period. The increase of antitoxic units, per gram protein, was 35 per cent. after 6 hours heating, increasing up to 53 per cent. after 48 hours.

Owing to the larger per cent. destruction of antitoxin at the 72-hour heating than the per cent. increase on conversion, the potency per gram protein dropped to 52 per cent. increase over the native serum. On separating the remaining unconverted albumin from this series, the increase of antitoxic units, per gram protein, was 60 per cent. after 6 hours heating, increasing to 78 per cent. after 48 hours. The 72-hour heating showed an increase of 73 per cent. over the native serum.

Citrated plasma under the same conditions gave practically the same results. Gibson's antitoxic globulin solution (blood alkalinity) containing only that globulin soluble in saturated sodium chloride solution was heated under the same conditions. The potency loss was 5 per cent. for the 6-hour period, and an increasing loss up to 23 per cent. for the 72-hour period. The soluble globulin converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. after 6 hours' heating, increasing to 47 per cent. after 72 hours. The increase of antitoxic units, per gram of protein, was 37 per cent. after 6 hours' heating, increasing to 54 per cent. for the 24 hours. Here again the 72-hour heating period caused a larger per cent. destruction of antitoxin than per cent. globulin converted into an insoluble condition (in saturated sodium chloride solution), dropping to an increase of 46 per cent., per gram protein, over Gibson's antitoxin globulin solution. This work which is being carried out further is practically and scientifically important, and may throw some light on the chemical characteristics and the nature of antitoxins.