Our thanks are due to Armour and Co., of Chicago, for a gift of crystalline chymotrypsin, and to the Agricultural Research Council for financial support.

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Beta- and Gamma-Globulin Tetanus Antitoxin of the Hyperimmune Horse

PAST work has indicated that fractionation of diphtheria antitoxic horse serum yields fractions of which the dilution ratio, and hence the firmness of combination, may show considerable variations. There was a definite correlation between the *in vitro*/*in vivo* ratio and the dilution ratio[1]. Kekwick and Record[2] examined electrophoretically pure gamma- and beta-globulin from hyperimmune horse sera, and found that both fractions had antitoxic activity and that they differed in their $L+/L_f$ ratios and in their speed of flocculation. It was of interest to investigate whether this was a general phenomenon, or one confined to the diphtheria toxin–antitoxin system, by studying the relation between tetanus toxin and horse tetanus antitoxic sera.

Qualitative differences between tetanus antitoxic sera have been observed by Hartley (quoted in ref. 3) and Petrie[4]. Dilution tests[5] on two sera and two fractions from one of them indicated a similarity to the diphtheria toxin–antitoxin system, and Kekwick[6] demonstrated that both gamma- and beta-globulin were antitoxic.

Gamma- and beta-globulin were obtained by fractionation with sodium sulphate followed by electrophoretic separation[2,6] from sera of horses hyperimmunized intramuscularly with tetanus toxoid. Flocculation always gave multiple zones. The flocculation zone due to tetanus toxin was determined by preparing floccules near each of the indicating mixtures, using proportions providing excess of toxin or antitoxin. The floccules were washed and then added to a suitably diluted solution of antitoxin. Toxin–antitoxin floccules absorb considerable quantities of antitoxin and may be distinguished in this way from floccules derived from flocculation zones involving other antigens[7]. L_f and K_f were determined at 49° C. in phosphate buffer, $I = 0{\cdot}2$, pH 8.

The extent to which toxin–antitoxin dissociates on dilution was tested in two ways: (1) the amount of an antitoxin was found that would neutralize an $L+/5$ dose of toxin and was compared with the amount required to neutralize an $L+/250$ dose of toxin[5]; (2) the amounts required to neutralize the test dose of toxin in the mouse and in the guinea pig were compared. (M/G is ratio of $L+$units per ml. in the two cases.[4])

Antitoxin	K_f	$\frac{L+\text{units per ml.}}{L_f}$		$\frac{M}{G} \times 100$	$\frac{L+/250}{L+/5} \times 500$
		a	*b*		
'Keeble' serum	145′(1)	1·5	2·2	87	68
'Keeble' gamma-globulin	69′(2)	2·6	4·4	60	55
'Keeble' beta-globulin	165′(1)	1·2	1·3	106	95

(1) 6 units/ml. of flocculation mixture.
(2) 4·2 units/ml. of flocculation mixture.
(*a*) Determined from $L+/5$ measurements.
(*b*) Determined from $L+/250$ measurements.

It may be concluded that, in flocculating, tetanus–toxin–antitoxin systems resemble diphtheria toxin–antitoxin mixtures. The gamma-globulin combines more avidly than the beta-globulin with the toxin; it dissociates *less* on dilution.

This work will be presented in detail elsewhere. Our thanks are due to Dr. R. A. Kekwick for suggesting this investigation and for his encouragement.

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Connective Tissue Permeability and the Mode of Action of Hyaluronidase

THE following observations would seem to throw fresh light upon the question of the mode of action of hyaluronidase on connective tissue permeability.

A sheet of fresh fascia from the flank of a mouse was fixed as an occluding membrane across the end of a glass tube of some 0·5 cm. internal diameter: rubber rings were found to be the best method of sealing the membrane to the tube. Particular care was taken not to damage the membrane during dissection or to stretch it unduly afterwards.

Fascial membranes prepared in this way were always found to be permeable to physiological saline, and at a pressure of 12 cm. water the rate of flow through them was of the order of 60 μl. per min. This rate was almost immediately increased by some ten times when hyaluronidase (Benger's 'Hylase', 10 units per ml.) was added to the saline perfusate. The same result was obtained with a saline extract of mouse testis.

This effect of hyaluronidase was counteracted by dilute solutions of certain substances of large molecular size. Thus when the following substances, at the w/v per cent concentration indicated, were perfused, the original state of permeability was restored within an hour: starch, 0·005 per cent; agar, 0·01 per cent; gum arabic and citrus pectin, 0·05 per cent; leaf gelatine, 0·1 per cent; chondroitin sulphate and dried serum, 0·2 per cent. Mouse serum at a dilution of 1/40 and human synovial fluid at 1/100 were similarly effective.

In the case of synovial fluid, and to some extent with serum and pectin, the slowing of flow was partly due to viscosity, since it took place rapidly and normal flow was restored almost at once when saline was substituted. But with starch, agar and gum arabic the slowing was gradual, regular and also permanent, inasmuch as no quickening of flow took place when saline was again perfused.