A second specimen of *Pomatoceros* was fixed three days after it had been removed from its tube; it had not begun to form a new tube. Alkaline phosphatase was again found in the same four sites as in the other specimen, and also in the following places (Fig. 2):

(5) The borders of small irregular cavities (*C*) situated in the thick epithelium on the ventral surface of the thorax under the ventral collar fold. This is the tissue which probably secretes the calcareous tube, and alkaline phosphatase is perhaps concerned in this process, as it seems to be in the calcification of bone and in the formation of the calcareous shells of molluscs[5].

(6) The epithelium of the dorsal and ventral lips of the mouth.

(7) The nuclei of the endothelium in the walls of the blood vessels in some of the pinnules of the branchial crown. Bourne[5] has found the enzyme in endothelial nuclei of vertebrate capillaries.

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[1] Faulkner, G. H., *J. Linn. Soc. (Zool.)*, **37**, 109 (1930). This includes references to Malaquin's work.
[2] McIntosh, W. C., "British Marine Annelids", **4** (Ray Society, London, 1922–23).
[3] Fox, H. Munro, *Proc. Roy. Soc.*, B, **112**, 479 (1933).
[4] Fox, H. Munro, *Proc. Roy. Soc.*, B, **125**, 554 (1938).
[5] Bourne, G., *Quart. J. Exp. Physiol.*, **32**, 1 (1944).
[6] Faouzi, H., *J. Mar. Biol. Assoc. U.K.*, **17**, 379 (1930–31).
[7] Danielli, J. F., *J. Exp. Biol.*, **22**, 110 (1946).
[8] Thomas, J. G., "Pomatoceros, Sabella and Amphitrite" (Liverpool, 1940).
[9] Bradfield, J. R. G., *Nature*, **157**, 876 (1946).
[10] Liebmann, E., *J. Morph.*, **70**, 151 (1942).

PLANT VIRUS PROTEINS AND ANTIBODY–ANTIGEN REACTIONS

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PROTEIN mixtures such as those found in blood have been widely used in the study of antibody–antigen reactions. It is difficult to prepare pure samples of single proteins from such naturally occurring mixtures. On the other hand, preparations of certain plant virus proteins, derived from a single infectious unit, can be isolated in quantities sufficient for experimental work in a state which fulfils all the usual criteria of homogeneity. As they are also good antigens, they would appear to be especially suitable objects for the study of antibody–antigen reactions.

The method used by Boyd[1] has been applied to the study of the turnip yellow mosaic virus antisera produced in rabbits. Successive doses of 0·01, 0·1, 1·0, 10·0, and 100·0 mgm. of turnip yellow mosaic virus[2] (prepared by the method of Markham and Smith[3]) were given intravenously to a single rabbit. Bleedings were taken two weeks after each injection, followed by a rest period of a few days before the next injection.

The antiserum produced by the 0·01 mgm. injection was of low titre with marked suppression of precipitation in the region of antiserum excess. This antiserum would be of the *H* type according to Boyd's classification. With increasing dosage of antigen the antiserum produced gave progressively higher titres,

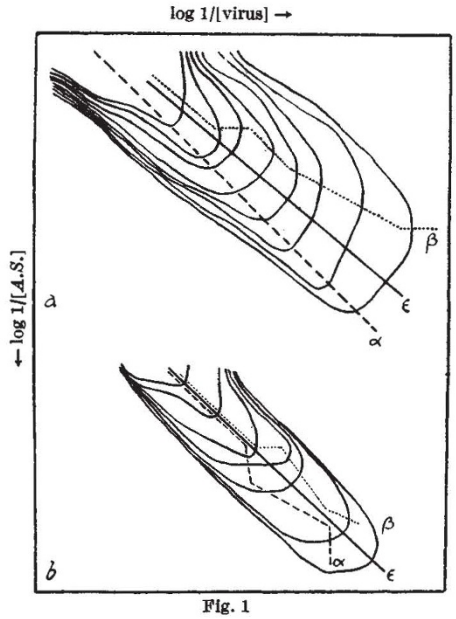

Fig. 1

and the type of precipitating system produced tended progressively towards Boyd's *R* type, with less-marked inhibition in the region of antiserum excess.

Fig. 1*a* shows the precipitating system for the antiserum produced after the 0·01 mgm. plus 0·1 mgm. injections. Fig. 2 shows the system for the antiserum from the same rabbit after the full course of injections.

If this antiserum obtained after the full course of injections is diluted with normal serum to make its titre approximate to one of the earlier antisera obtained, then the type of system produced approximates fairly closely to that produced by the earlier bleeding. For example, the antiserum obtained after the full course of injections (with a titre of 1 : 8192) was diluted to 1 : 256 with normal serum and a precipitation diagram prepared (Fig. 1*b*). This antiserum precipitated to a titre of 1 : 64, and there was fairly marked inhibition in the region of antiserum excess similar in amount to that found for the antiserum of Fig. 1*a* with a titre of 1 : 128.

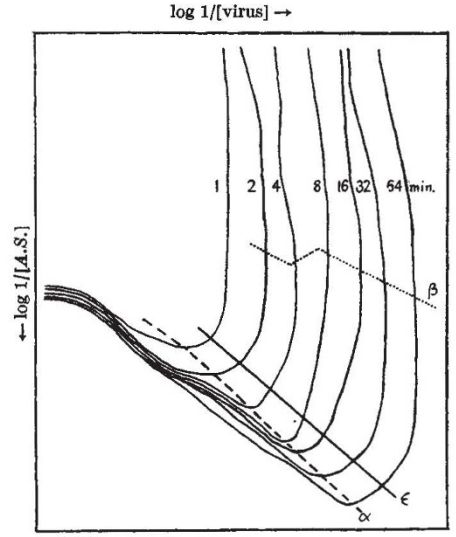

Fig. 2

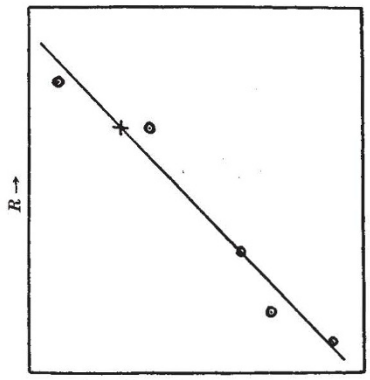

Fig. 3

The lowest concentration of virus which gives a precipitate is in general independent of the antiserum used, at antiserum dilutions where there is no inhibition by antiserum excess. The maximal inhibition by antiserum excess occurs with antiserum at 1 : 1. The ratio between the lowest concentration of virus giving precipitation with antiserum at 1 : 1 to the true virus end-point concentration gives a value for the amount of inhibition by excess antiserum which can be used to compare various antisera.

In Fig. 3 the ratio

$$R = \frac{[V] \text{ for antiserum at } 1:1}{[V] \text{ at real virus end point}},$$

where $[V]$ is end-point concentration of virus in mgm./ml., is plotted against the titre of the antiserum for the five sera used.

The point marked with a cross in Fig. 3 shows the position of the antiserum of Fig. 1*b*.

The inhibition in the region of antiserum excess shown in Fig. 1*b* can be due only to the presence of the non-specific constituents of the normal serum. On this account and in view of the close correlation between titre and amount of inhibition in the region of antiserum excess, it is suggested that the phenomenon of inhibition in the region of antiserum excess, the type of isochrones produced in this region, and the occurrence and position of the β-optimum, can be largely accounted for by some non-specific effect of normal serum constituents, at least in the system under investigation. Any theoretical consideration of the mechanism of this reaction, in the region of antiserum excess, will have to take this fact into account.

Rod-shaped plant viruses give a flocculent *H*-type precipitate whereas the spherical plant viruses give a dense granular *O*-type (Bawden[4]). Advantage has been taken of this fact in an investigation of the type of antiserum produced by a protein mixture. A rabbit was given a single intravenous injection of 1 ml. containing 1 mgm. of turnip yellow mosaic virus plus 1 mgm. of tobacco mosaic virus. An antiserum reacting with both viruses was obtained after two weeks. The same rabbit was given a second dose of 16 mgm. of each virus and a second antiserum obtained, of higher titre. These two antisera were tested against each virus separately, and against a mixture of known amounts of these.

That the two systems were precipitating independently was indicated by the following facts: (1) The position of the two α-optima for the two systems precipitating together was that which could be predicted from the two systems precipitating singly. (2) No significant decrease in titre of the antiserum for one virus was obtained after absorption with the other. (3) In the case of the two systems precipitating together, where the times for precipitation of the two systems were widely different in a single tube, the faster precipitate settled in the tube before the second precipitate formed, so that the latter formed a layer on top of the first.

In tubes where both systems precipitated about the same time, particularly in the higher concentrations, the gross floccules which were observed were homogeneous, and of indeterminate type. In view of the above, it is considered that this may possibly be due to mechanical entanglement of the smaller aggregates.

It is considered that, for the system studied, the antibodies produced in the rabbit are specific for the individual proteins of the mixture injected, and that in the *in vitro* reaction the two systems precipitate independently, except under conditions where the times for precipitation of the two systems are approximately the same, especially with high concentrations of the reagents.

A full account of these and other results will appear elsewhere. I wish to record my thanks to Dr. Roy Markham for his helpful encouragement and criticism.

[1] Boyd, W. C., *J. Exp. Med.*, **74**, 369 (1941).
[2] Markham, R., and Smith, K. M., *Nature*, **157**, 300 (1946).
[3] Markham, R., and Smith, K. M., *Parasitology* (1948, in the press).
[4] Bawden, F. C., "Plant Viruses and Virus Diseases", *Chron. Bot.* (1943).

THE DISORDERING OF β-BRASS BY COLD WORK

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THE order–disorder transformation in β-brass has received much attention in recent years. However, it has not yet been found possible to obtain the disordered state at room temperature by quenching. Furthermore, an attempt to produce the disordered state by cold work[1] has not been successful, because the maximum amount of deformation which could be given to the pure β-brass was only 9·5 per cent reduction in area by drawing.

Since crystals of β-brass can be very heavily deformed when embedded in crystals of the more ductile α-phase[2], the use of a duplex brass provides a more likely way of producing the disordered state in β-brass. The X-ray method of showing the existence of the super-lattice in the case of β-brass presents great experimental difficulties; consequently we have used the electrical resistivity of the alloy to indicate its state of order. If the order is destroyed by cold work, an increase in resistivity is to be expected, by analogy with experiments which have been made with other alloys[3].

Lengths of duplex brass wire containing 41·3 per cent zinc were annealed for 30 minutes at 800° C., then furnace-cooled to room temperature over a period of 12 hours. This treatment resulted in a coarse structure in which the α- and β-phases were present in approximately equal proportions. The wires were then cold drawn to various reductions in area up to 95·5 per cent, and the electrical resistivities were measured on a Kelvin double bridge within two hours of drawing.