REVERSIBLE NEUTRALIZATION BY CONGO RED OF THE ANTHRACIDAL POWER OF SERUM

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In earlier papers (Gordon, 1930) it was shown that congo red has an inactivating effect on serum complement, both haemolytic and bactericidal, and that this effect can be reversed by treating the serum and congo red mixture with charcoal, the charcoal removing the congo red and leaving the complement active again. A similar reversal of inactivation is obtained by using instead of the charcoal, heated serum (55° C. for 30 min.) or protein solutions. Later (Gordon, 1931), it was shown that congo red had an inactivating effect on the haemolysins of *Streptococcus haemolyticus* and *B. welchii*. The reversibility of this effect was not so easy to demonstrate as with complement. Charcoal had a destructive effect on the haemolysins and so could not be used. It was found, however, that when the concentration of congo red was just sufficient to neutralize the streptococcal haemolysin, the addition of cuprammonium artificial silk adsorbed the congo red and liberated the haemolysin. In the case of *B. welchii* this method of reversal was not suitable, as the artificial silk had a destructive effect on the haemolysin. Instead, reversibility was demonstrated by adding ox serum to the mixture of congo red and haemolysin. This brought about a redistribution of the congo red between the ox serum and the haemolysin and if the amount of congo red used had been only just sufficient to neutralize the haemolysin of *B. welchii*, then the haemolytic activity could again be demonstrated. Gordon and Robson (1933) showed that congo red interfered with the anaphylactic reaction tested both *in vivo* and *in vitro*, the guinea-pig uterus being used in the *in vitro* experiments, in which the inhibitory action of the dye was shown to be reversible. It was suggested that the congo red interfered with the entrance of antigen into the cell.

In the course of studies on the anthracidal action of rabbit serum it was thought of interest to study the effect of congo red on this property. It was found that a fresh congo red solution (prepared in distilled water) added to rabbit serum inactivated the anthracidal power. Three strains of *B. anthracis* were used, one from the National Collection of Type Cultures and the other two from cases of anthrax isolated in this laboratory. A light suspension from an 18-hour growth on agar of the organism was made in distilled water, and 0·05 c.c. of this suspension was added to the tubes of serum and to those of serum plus congo red. The tubes were subcultured to agar plates at once, and also at intervals of 4 and 8 hours. The plates were incubated at 37° C. and

readings taken after 24 hours. Table I shows that 0·2 c.c. of 1 per cent congo red solution added to 3 c.c. of rabbit serum is just about the neutralizing dose, the serum still showing some little anthracidal power after 8 hours. Other experiments not recorded in this table show that amounts of congo red below 0·2 c.c. for 3 c.c. serum have only a slight inhibiting effect on the anthracidal power. The reversibility of this reaction, i.e. the removal of congo red and restoration of anthracidal power was next attempted. Treating the congo red and serum mixture with charcoal followed by incubating and centrifuging yielded a fluid freed from most of its congo red, but without any anthracidal power. When however charcoal was added to *normal* serum (not treated with

Table I. *The inactivating effect of congo red on the anthracidal power of rabbit serum and its reversal by charcoal-treated serum*

Treated sera prepared as follows:

A. 3 c.c. rabbit serum + 0·2 c.c. 1 per cent congo red in distilled water.
B. 3 c.c. „ „ + 0·3 c.c. 1 „ „ „ „
C. 3 c.c. „ „ + 0·4 c.c. 1 „ „ „ „

Charcoal-treated serum: 0·5 g. Norit charcoal autoclaved in tube and 9 c.c. rabbit serum added; incubated at 37° C. for 24 hours, then centrifuged and the supernatant fluid used.

Mixture used	Subcultured		
	At once	After 4 hours	After 8 hours
2 c.c. A	+	+	+/2
1 c.c. A + 1 c.c. charcoal-treated serum	+	+/2	6
2 c.c. B	+	+ +	+ +
1 c.c. B + 1 c.c. charcoal-treated serum	+	+/2	11
2 c.c. C	+	+ +	+ +
1 c.c. C + 1 c.c. charcoal-treated serum	+	+	+/2
2 c.c. normal rabbit serum	+	−	−
1 c.c. normal rabbit serum + 1 c.c. charcoal-treated serum	+	−	−
1 c.c. normal rabbit serum	+	−	−
1 c.c. charcoal-treated rabbit serum	+	+ +	+ +

+ + = heavy growth; + = moderate growth; +/2 = scanty growth; − = no growth. The figures give the numbers of colonies where these could be counted.

congo red) it was found that the anthracidal power had been removed, showing that charcoal was of no value as an adsorbent for demonstrating the reversibility of the congo red effect. Artificial silk was next tried, but here again the amount necessary to remove the congo red had a destructive action on the anthracidin, as was proved by trials with normal untreated serum. A further attempt however proved to be successful. It would have been useless of course to make use of normal rabbit serum, or serum heated at 55° C. to remove the congo red (as with *B. welchii*), since both sera contain anthracidin and the reappearance of anthracidal power would have proved nothing. However, serum treated with charcoal is no longer anthracidal, and if such serum be added to serum inactivated by congo red, any restoration of anthracidal power can only be attributed to the removal of congo red from anthracidin originally inactivated by this dye. The table gives details of such an experiment. 2 c.c. of a serum and congo red mixture had no anthracidal power as tested against 0·05 c.c. of a

suspension of anthrax bacilli. If, however, to 1 c.c. of this mixture there was added 1 c.c. of serum previously treated with charcoal and this deprived of its anthracidal power, then the anthracidal power of the serum treated with congo red was restored. This experiment demonstrates the reversibility of yet another congo red inhibition, and as in the other cases referred to in this paper, the reversal appears to be due to a redistribution of the congo red between a new adsorbent introduced into the system and the bactericidin to an amount less than the inhibitory dose, provided that the original quantity of dye used was not too large.

In the absence of definite chemical knowledge of the structure of bactericidins and haemolysins it is not possible to offer an explanation of the mechanisms of these inhibitions by congo red. It may be stated however that at a *p*H such as that of serum congo red does not show great avidity for or firmness of combination with protein. The reaction might be described according to taste as a reversible adsorption similar to that of acetic acid by charcoal, or as the formation of a loose (or easily hydrolysable) compound. The similar behaviour of congo red towards complement and bactericidins might be taken as an indication of the presence in these substances of active chemical groups like those of proteins.

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