THE EFFECT OF CONCENTRATION OF THE CONSTITUENTS ON A HÆMOLYTIC REACTION.¹

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THE chief object of the following observations was to study the effect of concentration of the corpuscle emulsion on the dosage of complement in an ordinary hæmolytic system.

The hæmolytic serum used was that of the rabbit immunised against ox corpuscles, and several samples of such a serum were employed. The complement was that of the guinea-pig; usually the serum of two animals was pooled and used when from one to two days old. Any natural immune-body present was always removed by treatment at 0° C. with ox corpuscles. The ox blood corpuscles were washed five times with saline, and were never more than two days old when used. The mixture of the elements was invariably made up to a volume of 3 c.c., and the routine incubation period was one and a half hours. All the elements in a mixture were mixed *seriatim*, *i.e.* corpuscles previously sensitised with immune-body were not employed. As a matter of fact it is immaterial whether this is done or not, except that the use of previously sensitised corpuscles rather hastens the reaction, especially when strong emulsions are used. Although on a series of daily estimates the dose of immune-body does not vary much, the precaution was taken of estimating it afresh daily.

The minimum hæmolytic dose of immune-body adopted was the usual one, namely, that dose which just gives complete hæmolysis with excess of complement. The minimum hæmolytic dose of complement was that obtained with the use of one minimum hæmolytic dose of immune-body.

EFFECT OF CONCENTRATION OF IMMUNE-BODY ON THE DOSE OF COMPLEMENT.

It is, of course, well known (1902¹) that an excess of immune-body over the minimum hæmolytic dose will lower the minimum

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TABLE I.

To estimate Minimum Hæmolytic Dose of Complement with $\left\{ \begin{array}{l} \text{A. 1 M.H.D. of I.-B.} \\ \text{B. 5} \quad \text{"} \quad \text{"} \\ \text{C. 10} \quad \text{"} \quad \text{"} \\ \text{D. 50} \quad \text{"} \quad \text{"} \end{array} \right.$
 1 c.c. 1 per cent. Ox Blood Corpuscles with

(In each case the tubes are made up to 3 c.c. with normal saline).

A.					
	Red Blood Corpuscles, 1 per Cent.	Immune-Body, 1 in 10.	Complement, 1 in 10.	Saline.	Result in 1½ hour.
1	1 c.c.	0.1 c.c.	0.7 c.c.	1.2 c.c.	C.
2	1 "	0.1 "	0.6 "	1.3 "	C.
3	1 "	0.1 "	0.5 "	1.4 "	C.
4	1 "	0.1 "	0.4 "	1.5 "	C.
5	1 "	0.1 "	0.3 "	1.6 "	C.
6	1 "	0.1 "	0.2 "	1.7 "	C.
7	1 "	0.1 "	0.1 "	1.8 "	++
8	1 "	0.1 "	0.08 "	1.82 "	+
9	1 "	0.1 "	0.05 "	1.85 "	0
10	1 "	0.1 "	0.02 "	1.88 "	0

B.					
	Red Blood Corpuscles, 1 per Cent.	Immune-Body, 1 in 10.	Complement, 1 in 10.	Saline.	Result in 1½ Hour.
1	1 c.c.	0.5 c.c.	0.7 c.c.	0.8 c.c.	C.
2	1 "	0.5 "	0.6 "	0.9 "	C.
3	1 "	0.5 "	0.5 "	1.0 "	C.
4	1 "	0.5 "	0.4 "	1.1 "	C.
5	1 "	0.5 "	0.3 "	1.2 "	C.
6	1 "	0.5 "	0.2 "	1.3 "	C.
7	1 "	0.5 "	0.1 "	1.4 "	C.
8	1 "	0.5 "	0.08 "	1.42 "	C.
9	1 "	0.5 "	0.05 "	1.45 "	C. (?)
10	1 "	0.5 "	0.02 "	1.48 "	0

C.					
	Red Blood Corpuscles, 1 per Cent.	Immune-Body, Neat.	Complement, 1 in 10.	Saline.	Result in 1½ Hour.
1	1 c.c.	0.1 c.c.	0.7 c.c.	1.2 c.c.	C.
2	1 "	0.1 "	0.6 "	1.3 "	C.
3	1 "	0.1 "	0.5 "	1.4 "	C.
4	1 "	0.1 "	0.4 "	1.5 "	C.
5	1 "	0.1 "	0.3 "	1.6 "	C.
6	1 "	0.1 "	0.2 "	1.7 "	C.
7	1 "	0.1 "	0.1 "	1.8 "	C.
8	1 "	0.1 "	0.08 "	1.82 "	C.
9	1 "	0.1 "	0.05 "	1.85 "	C. (?)
10	1 "	0.1 "	0.02 "	1.88 "	0

D.					
	Red Blood Corpuscles, 1 per Cent.	Immune-Body, Neat.	Complement, 1 in 10.	Saline.	Result in 1½ Hour.
1	1 c.c.	0.5 c.c.	0.7 c.c.	0.8 c.c.	C.
2	1 "	0.5 "	0.6 "	0.9 "	C.
3	1 "	0.5 "	0.5 "	1.0 "	C.
4	1 "	0.5 "	0.4 "	1.1 "	C.
5	1 "	0.5 "	0.3 "	1.2 "	C.
6	1 "	0.5 "	0.2 "	1.3 "	C.
7	1 "	0.5 "	0.1 "	1.4 "	C.
8	1 "	0.5 "	0.08 "	1.42 "	C.
9	1 "	0.5 "	0.05 "	1.45 "	C. (?)
10	1 "	0.5 "	0.02 "	1.48 "	0

hæmolytic dose of complement, or, in other words, will liberate more hæmolytic energy. Not only is this the case, but the velocity of the reaction is also increased.

As is often observed in such reactions, the effect of increasing the dose is not indefinite, a point being reached when a further increase of immune-body does not cause a further decrease in the minimal dose of complement. This is probably related to the fact that corpuscles are only capable of absorbing a certain amount of immune-body.

In this connection we note that with one sample of immune-body, of which corpuscles could absorb about one minimum hæmolytic dose, it was found that the effect of one and a half minimum hæmolytic doses and of five minimum hæmolytic doses in lowering the dose of complement was the same. Similarly with another immune-body which was taken up in about fourteen doses, while there was a progressive lowering of the dose of complement with the use of up to twelve doses, the effects of twelve doses and of thirty doses on the minimum hæmolytic dose of complement were the same.

In the cases examined the amount of immune-body taken up by red blood corpuscles appeared to be a function of the immune-body itself, and largely independent of the qualities of the complement and of the corpuscles. Thus in the cases of the two immune-bodies just mentioned, on several occasions they were treated with the same complement and the same corpuscles (the samples of these being, however, different at the different trials). It was found, as just stated, that the one immune-body was always absorbed by the corpuscles in only about one minimum hæmolytic dose, and the other invariably in about fourteen doses.

It may be said that no decrease in the minimum hæmolytic dose of immune-body occurs when excess of complement over the minimum hæmolytic dose is added.

EFFECT OF CONCENTRATION OF CORPUSCLES ON MINIMUM HÆMOLYTIC DOSE OF IMMUNE-BODY.

An example of the result of investigating this point is found in Table II.

It will be noted that, with the three concentrations used, the dose of immune-body is very closely proportional to the number of corpuscles present. Thus with 0·2 per cent. suspension, the minimum hæmolytic dose of immune-body is between 0·04 and 0·05 c.c. of 1–100 dilution. With 1 per cent. (*i.e.* five times the number of corpuscles) the dose is 0·2 of 1–100 dilution, or about five times that for 0·2 per cent. suspension. With 5 per cent. suspension the dose is 0·1 of a 1–10 dilution, *i.e.* again about five times the dose for a 1 per cent. suspension. Immune-body from four different animals was examined,

TABLE II.

To estimate Immune-Body for { A. 0.2 per cent Red Blood Corpuscles.
 B. 1.0 " " "
 C. 5.0 " " "

A.					
	Red Blood Corpuscles, 0.2 per Cent.	Immune-Body, 1 in 100.	Complement, 1 in 10.	Result.	
				30 Minutes.	90 Minutes.
1	1 c.c.	0.09 c.c.	1 c.c.	C.	C.
2	1 "	0.08 "	1 "	C.	C.
3	1 "	0.07 "	1 "	0	C.
4	1 "	0.06 "	1 "	0	C.
5	1 "	0.05 "	1 "	0	C.
6	1 "	0.04 "	1 "	0	
7	1 "	0.03 "	1 "	0	
8	1 "	0.02 "	1 "	0	
9	1 "	0.01 "	1 "	0	
10	1 "	0.00 "	1 "	0	0

B.					
	Red Blood Corpuscles, 1 per Cent.	Immune-Body, 1 in 100.	Complement, 1 in 10.	Result.	
				30 Minutes.	90 Minutes.
1	1 c.c.	0.9 c.c.	1 c.c.	C.	C.
2	1 "	0.8 "	1 "	C.	C.
3	1 "	0.7 "	1 "	C.	C.
4	1 "	0.6 "	1 "	C.	C.
5	1 "	0.5 "	1 "	C.	C.
6	1 "	0.4 "	1 "	C.	C.
7	1 "	0.3 "	1 "	C.	C.
8	1 "	0.2 "	1 "	0	0
9	1 "	0.1 "	1 "	0	0
10	1 "	0.0 "	1 "	0	0

C.					
	Red Blood Corpuscles, 5 per Cent.	Immune-Body, 1 in 10.	Complement, 1 in 10.	Result.	
				30 Minutes.	90 Minutes.
1	1 c.c.	0.9 c.c.	1 c.c.	C.	C.
2	1 "	0.8 "	1 "	C.	C.
3	1 "	0.7 "	1 "	C.	C.
4	1 "	0.6 "	1 "	C.	C.
5	1 "	0.5 "	1 "	C.	C.
6	1 "	0.4 "	1 "	C.	C.
7	1 "	0.3 "	1 "	C.	C.
8	1 "	0.2 "	1 "	C.	C.
9	1 "	0.1 "	1 "	+++	C.
10	1 "	0.5 "	1 "	0	+++

Thus by	{	A.	Minimum hæmolytic dose of immune-body for 1 c.c. 1 per cent. R.B.C. = 0.025			
Calculation		B.	"	"	"	= 0.020
in		C.	"	"	"	= 0.020

and in three this simple direct arithmetical ratio between minimum hæmolytic dose and strength of suspension was noted. The fourth immune-body was, however, extremely interesting, for here, instead of the ratio being direct, it was doubled,—that is to say, with the 1 per cent. suspension the minimum hæmolytic dose was ten times that for the 0·2 per cent. suspension instead of five, and that for the 5 per cent. emulsion was ten times that for the 1 per cent. This observation was repeatedly confirmed, and held good for a second bleeding of the animal, after it meantime had received several fresh injections of blood corpuscles.

EFFECT OF CONCENTRATION OF CORPUSCLES ON MINIMUM HÆMOLYTIC DOSE OF COMPLEMENT.

A large number of observations were made on this point with different samples of guinea-pig complement. The general result was in practically every case the same,—namely, that in concentrated emulsions of corpuscles what might be called the minimum dose of complement per corpuscles was less than in less concentrated emulsions. The emulsions of corpuscles used were usually 0·2 per cent., 1 per cent., and 5 per cent. An example of this is given in Table III.

There was in nearly every case observed a markedly increased hæmolytic power of complement in the 1 per cent. emulsion as compared with the 0·2 per cent. The increase was relatively less with the 5 per cent. Two experiments were performed in which the action of complement in a 50 per cent. emulsion (roughly the concentration obtaining in the circulating blood) was compared with that in a 1 per cent. and in a 5 per cent. emulsion, and, although there was still a tendency for the power of the complement to increase, the rate of increase was very small.

The general result of this part of the inquiry was to show that just as, keeping the concentration of the corpuscles constant, an increase in concentration of immune-body causes a lowering of the dose of complement necessary for complete hæmolysis, so an increase of concentration of corpuscles with a constant dose of immune-body per corpuscle causes a similar lowering of the dose of complement necessary for a similar complete hæmolysis.

EFFECT OF INCREASED CONCENTRATION OF IMMUNE-BODY PLUS INCREASED CONCENTRATION OF CORPUSCLES ON MINIMUM HÆMOLYTIC DOSE OF COMPLEMENT.

The experiments under this heading are summarised in Table IV.

TABLE III.

To estimate Minimum Hæmolytic Dose of Complement for { A. 1 c.c. 0.2 per cent.
B. 1 c.c. 1.0 " "
C. 1 c.c. 5.0 " "

A.					
	Red Blood Corpuscles, 0.2 per Cent.	Immune-Body, 1 in 100.	Complement, 1 in 10.	Result.	
				30 Minutes.	90 Minutes.
1	1 c.c.	0.04 c.c.	0.5 c.c.	0	++++
2	1 "	0.04 "	0.4 "	0	+++
3	1 "	0.04 "	0.3 "	0	+++
4	1 "	0.04 "	0.2 "	0	0
5	1 "	0.04 "	0.1 "	0	0
6	1 "	0.04 "	0.08 "	0	0
7	1 "	0.04 "	0.06 "	0	0
8	1 "	0.04 "	0.04 "	0	0
9	1 "	0.04 "	0.02 "	0	0
10	1 "	0.04 "	0.01 "	0	0

B.					
	Red Blood Corpuscles, 1 per Cent.	Immune-Body, 1 in 100.	Complement, 1 in 10.	Result.	
				30 Minutes.	90 Minutes.
1	1 c.c.	0.2 c.c.	0.9 c.c.	++++	C.
2	1 "	0.2 "	0.8 "	++++	C.
3	1 "	0.2 "	0.7 "	++++	C.
4	1 "	0.2 "	0.6 "	++++	C.
5	1 "	0.2 "	0.5 "	++++	C.
6	1 "	0.2 "	0.4 "	0	++++
7	1 "	0.2 "	0.3 "	0	+++
8	1 "	0.2 "	0.2 "	0	++
9	1 "	0.2 "	0.1 "	0	0
10	1 "	0.2 "	0.05 "	0	0

C.					
	Red Blood Corpuscles, 5 per Cent.	Immune-Body, 1 in 10.	Complement, 1 in 10.	Result.	
				30 Minutes.	90 Minutes.
1	1 c.c.	0.1 c.c.	0.9 c.c.	C.	C.
2	1 "	0.1 "	0.8 "	C.	C.
3	1 "	0.1 "	0.7 "	C.	C.
4	1 "	0.1 "	0.6 "	C.	C.
5	1 "	0.1 "	0.5 "	++++	++++
6	1 "	0.1 "	0.4 "	+++	+++
7	1 "	0.1 "	0.3 "	0	0
8	1 "	0.1 "	0.2 "	0	0
9	1 "	0.1 "	0.1 "	0	0
10	1 "	0.1 "	0.0 "	0	0

Thus by Calculation in		{ A. Minimum hæmolytic dose for 1 c.c. 1 per cent. 3.0 c.c. 1 in 10.			
		{ B. " " " 1 " 1 " 0.5 " "			
		{ C. " " " 1 " 1 " 0.12 " "			

TABLE IV.

M.H.D. of C. with 0.04 I.-B. 1 in 10	with 1 per cent. R.B.C. 1 c.c. = 0.4 c.c. 1 in 10.
" " 0.2 " (5 doses) " 1 " "	1 c.c. = 0.04 c.c. "
" " 0.4 " (10 doses) " 1 " "	1 c.c. = 0.04 c.c. "
" " 0.2 " (1 dose) " 5 " "	1 c.c. = 0.8 c.c. "
" " 1.0 " (5 doses) " 5 " "	1 c.c. = 0.2 c.c. "
" " 2.0 " (10 doses) " 5 " "	1 c.c. = 0.2 c.c. "
" " 0.4 " (1 dose) " 10 " "	1 c.c. = 1.5 c.c. "
" " 2.0 " (5 doses) " 10 " "	1 c.c. = 0.4 c.c. "
" " 4.0 " (10 doses) " 10 " "	1 c.c. = 0.4 c.c. "

Note that with multiple doses of immune-body (5 X and 10 X) the dose of complement per corpuscle is the same for the 10 X as the 5 X, and that the amount of complement required is proportional to the amount of corpuscles present.

In each case the doses of complement are, for the sake of comparison, calculated for a 1-10 concentration of complement. The general result is that *for multiple doses of immune-body* (i.e. for five minimum hæmolytic doses of immune-body and ten minimum hæmolytic doses of immune-body) the dose of complement per corpuscle is the same, and the amount of complement required is proportional to the number of corpuscles present.

REFERENCE.

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