DURATION OF PASSIVE IMMUNITY.

By A. T. GLENNY and BARBARA E. HOPKINS.

(*Wellcome Physiological Research Laboratories, Herne Hill, London, S.E.*)

(With 2 Charts.)

PART I.

In the course of some work on the interference of passive immunity with the production of active immunity against diphtheria by the injection of toxin-antitoxin mixtures, it was necessary to work out in detail the rate of disappearance of antitoxin from the blood of rabbits injected intravenously with antitoxin obtained from a horse. Much has been written upon the subject of the duration of passive immunity and the general phenomena are well known, but it appeared to be of interest to analyse experimentally in greater detail the curves relating to the rate of disappearance of heterologous antitoxin. The present paper deals with the rate of disappearance of antitoxin in normal rabbits, and also in rabbits sensitised by small doses of serum such as are present in 1 c.c. of toxin-antitoxin mixtures intended for human immunisation.

Table I records the antitoxic values of three normal rabbits immediately after, and on successive days after, the intravenous injection of 750 units of

Table I.

Showing the antitoxic value of the blood of three normal rabbits at different intervals of time after the intravenous injection of 0·5 *c.c. unconcentrated horse serum containing* 750 *units diphtheria antitoxin.*

Time interval	Antitoxic value in units per c.c.		
	Rabbit 41	Rabbit 49	Rabbit 50
15 minutes	11·0	7·5	6·5
1 day	5·5	3·5	4·0
2 days	4·25	2·3	3·5
3 ,,	3·1	1·75	2·25
4 ,,	2·8	1·25	1·9
5 ,,	2·25	0·95	1·5
6 ,,	1·25	0·95	1·1
7 ,,	0·9	0·7	0·7
8 ,,	—	0·45	0·4
9 ,,	0·22	0·12	0·08
10 ,,	—	0·03	0·045
11 ,,	0·08	0·015	Reinjected
12 ,,	—	<0·0005	—
13 ,,	0·0015	—	—
14 ,,	—	—	—
15 ,,	—	—	—
16 ,,	<0·0005	—	—

https://doi.org/10.1017/S0022172400031375 Published online by Cambridge University Press

diphtheria antitoxin contained in 0·5 c.c. of unconcentrated horse serum. This serum, which was employed in all experiments recorded in this paper, contained 2200 units per c.c. when originally obtained from a horse seven years previously. The antitoxic serum was injected without dilution into one ear of each rabbit and 15 minutes later blood was drawn from the other ear. On each occasion, 2 to 5 c.c. of blood was taken, and the serum from each bleeding titrated for its antitoxic value by the intracutaneous method of testing. In order to demonstrate the rate of loss of antitoxin, Table II has been prepared,

Table II.

Showing the percentage daily loss of antitoxic value of the blood of three normal rabbits at different intervals of time after the intravenous injection of 0·5 *c.c. unconcentrated horse serum containing* 750 *units diphtheria antitoxin.*

	Time interval		Rabbit 41		Rabbit 49		Rabbit 50		Average
Phase A	0–1	day	50·0		53·3		38·4		47·2 %
Phase B	1–2	days	22·7		34·3		12·5		
	2–3	,,	27·1		23·9		35·7		
	3–4	,,	9·6	Average	28·6	Average	15·5	Average	
	4–5	,,	19·6	25·2	24·0	22·8	21·0	24·6	24·2 %
	5–6	,,	44·4		0·0		26·6		
	6–7	,,	28·0		26·3		36·3		
	7–8	,,	—		35·7		42·8		
Phase C	8–9	,,	50·5		73·3		80·0		
	9–10	,,	—		75·0		43·7		58·6 %
	10–11	,,	39·6		50·0		—		
	11–12	,,	—		—		—		
	12–13	,,	56·7		—		—		

giving the percentage amount lost from each day to the next. A consideration of these figures shows that the percentage loss is greater during the first 24 hours than during any of the following 6–7 days, that during these days the rate of loss appears uniform, and again, that the loss becomes accelerated after the seventh or eighth day.

The three rabbits appear to agree closely in the rate of loss from the second to the seventh or eighth day; curves 1–3 (Chart I), in which the logarithms of the antitoxic values are plotted against the time, show how the figures form a curve composed of the following three phases:

(*a*) A phase of initial loss occurring within the first 24 hours; during this period one-half of the total of the antitoxin present 15 minutes after the injection is lost. The average loss in three rabbits was 47·2 per cent., the actual loss varying from 38·4 to 53·3 per cent.

(*b*) The phase of gradual constant percentage loss, lasting 6–7 days, during which a quarter of the total antitoxin present each day is lost by the next day. This phase lasted six days in one rabbit, and between six and seven days in the other two, the average daily loss of the three rabbits was 24·2 per cent. and the averages for the individual rabbits were 22·8, 24·6 and 25·2 per cent. respectively, with variations in individual readings from 0–44 per cent. The

curve of this phase in each individual rabbit will be seen to fit closely to a standard curve.

(*c*) The phase of accelerated loss, occurring after the seventh or eighth day, during which one-half to three-quarters of the total of antitoxin present each day is lost by the next day. This phase appears to vary with the individual more than the other two phases.

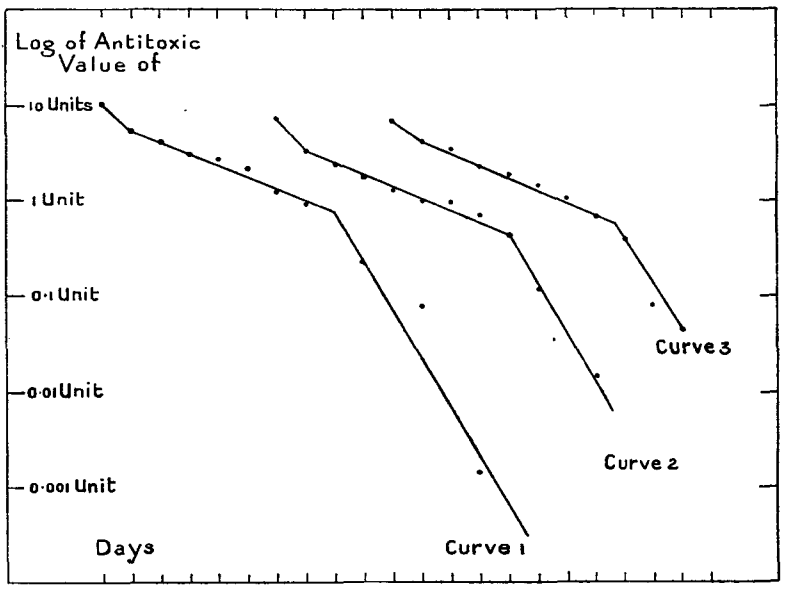

Chart I.

These three normal rabbits are chosen as giving typical curves of loss. Variations from this type occur, and it is hoped will shed further light on the meaning of these three phases. They will be dealt with in a later paper, but one rabbit (rabbit 42) may be referred to here as an instance. Its curve showed a fourth phase of slow loss resembling Phase B, and therefore probably due to natural elimination; this fourth phase succeeded to Phase C after the latter had continued for five or six days.

Tables III and IV and curves 4–10 (Chart II) record the results of the injection of antitoxin into rabbits that had been previously sensitised by the subcutaneous injection of small doses of horse serum. These rabbits had been used for testing the antigenic value of toxin-antitoxin mixtures and the majority had received either one or two subcutaneous injections of 1 or 5 c.c. of antigens containing about 1/100 of a c.c. of horse serum per c.c. of mixture. The same three phases are seen:

(*a*) The phase of initial loss shows again a 50 per cent. drop in 24 hours, individual rabbits varying from 43·7 to 57·9 per cent.

(*b*) The phase of gradual loss shows the same rate of progressive fall (average 24·2 per cent.) as in normal rabbits, but the duration of the

https://doi.org/10.1017/S0022172400031375 Published online by Cambridge University Press

phase is markedly curtailed (two days only in five rabbits and three days in two).

Table III.

Showing the antitoxic value of the blood of seven rabbits previously sensitised with small doses of horse serum, at different intervals of time after the intravenous injection of 0·5 *c.c. unconcentrated horse serum containing* 750 *units diphtheria antitoxin.*

	Rabbit						
	13	21	30	33	34	35	38
No. of sensitising doses	5	1	3	2	1	2	2
Volume of horse serum in each sensitising dose, c.c.	0·064	0·064	0·012	0·012	0·013	0·013	0·013
Interval in months after last injection	9	11	6	6	7	5	3
15 mins. after injection of 750 units	Antitoxic value in units per c.c.						
	5·0	5·75	6·0	8·0	7·5	7·0	9·5
1 day	—	2·75	3·25	4·5	3·5	3·75	4·0
2 days	2·5	2·5	2·75	3·5	2·5	2·75	3·3
3 „	1·6	1·9	2·25	2·25	1·8	2·1	2·75
4 „	0·005	0·25	1·5	0·03	1·3	0·014	0·08
5 „			0·11		0·06		

Table IV.

Showing the percentage daily loss of antitoxic value of the blood of seven rabbits previously sensitised with small doses of horse serum, at different intervals of time after the intravenous injection of 0·5 *c.c. unconcentrated horse serum containing* 750 *units diphtheria antitoxin.*

Time interval	Rabbit							Average
	13	21	30	33	34	35	38	
Phase A 0–1 day	—	52·2	45·8	43·7	53·3	46·4	57·9	49·9 %
Phase B 1–2 days	—	9·1	15·4	22·2	28·6	26·7	17·5	24·2 %
2–3 „	36·0	24·0	18·2	35·7	28·0	23·6	16·7	
3–4 „			33·3		27·7			
Phase C 3–4 „	99·7	86·8		98·6		99·3	97·1	94·0 %
4–5 „	—	—	81·2	—	95·4	—	—	

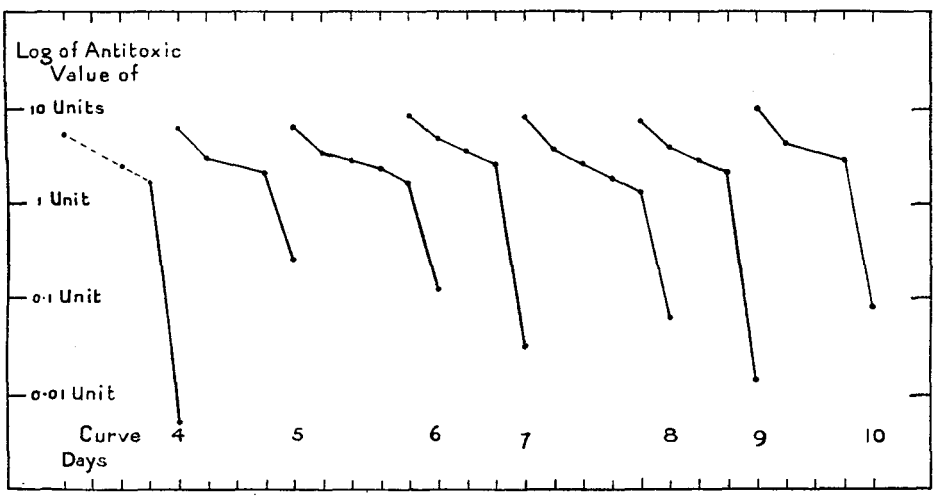

Chart II.

The similarity of this part of the curve in normal and sensitive animals is significant in supporting the idea that Phase B is due to natural elimination. It cannot, however, be much stressed, as there are not enough points on the curves representing sensitised animals to show quite conclusively the exact slope of Phase B or the exact point at which Phase C succeeds Phase B.

(*c*) The phase of accelerated loss occurring after the third or fourth day in the sensitised animals compared with seventh or eighth day in normal rabbits, shows a far greater rate of loss.

A theoretical explanation of the three phases is not yet forthcoming, but the following suggestions are put forward:

(*a*) The phase of initial loss may be due to the presence of precipitin; this is borne out by further work which shows that rabbits well immunised ("sensitised") to horse serum, that is to say, rabbits which would be expected to contain a greater amount of precipitin, lose a greater amount of antitoxin in the first 24 hours.

On the other hand, if this explanation is adopted the following points must be accepted: firstly, the existence in rabbits of normal (though possibly not specific) precipitin to horse serum, and secondly, that rabbits sensitised by small doses possess no greater amount of precipitin than do normal rabbits. This would be the case if any excess precipitin formed by these slightly sensitised rabbits were soon lost.

That this is possible is indicated by the normal rabbit, 42, already referred to, in which the precipitin formed was apparently insufficient to eliminate all the precipitinogen. This rabbit showed also a smaller drop at Phase A than normally occurs, which may account for its smaller production of precipitin after stimulation. There are several indications, to be dealt with in a later paper, that some relationship exists between the degree of initial loss and the rate and extent of the stimulated loss. This suggests that the amount of precipitin normally present is indicative of the degree of active immunity, and thus to some extent of the rate and magnitude of precipitin formation after stimulation.

Another possible explanation is that the initial loss is due to saturation of or adsorption to the tissues.

(*b*) The phase of gradual loss would appear to correspond to elimination of foreign protein by some natural method of metabolism, possibly excretion by the kidneys.

(*c*) The phase of accelerated loss is almost certainly due to the production of precipitin as the response to the injection of horse serum.

Alexander (1921) gives curves of precipitin content in rabbits which show rapid formation of the precipitin on the seventh and eighth days after the intravenous injection of 0·5 c.c. of normal horse serum, thus coinciding with the beginning of Phase C.

The idea that the accelerated loss is due to precipitin formation also agrees

with the work of Longcope and Rackemann (1918), and of Mackenzie and Leake (1921).

These authors (who are dealing with the disappearance from the blood in man of horse serum as a whole, and not the loss of antitoxin) show that there is a sudden loss of antigen, corresponding to a rapid production of precipitin. That the formation of precipitin and the rapid loss of antigen occur sooner in patients who have had previous doses of serum, and that the curve of loss is steeper, is suggested by one case that is quoted.

The comparison between the time of appearance and rapidity of Phase C in normal and sensitised animals suggests that precipitin formation follows the general laws of anti-body formation in that the latent period is shorter in immunised (here "sensitised") animals than in normal animals, and the anti-body is produced more rapidly and in greater amount. The secondary stimulus induces a far greater response than the primary.

Longcope and Rackemann have shown that there is some connection between the formation of precipitin, loss of precipitinogen and serum sickness. They have given curves showing that in the majority of cases, shortly after the onset of serum sickness, and for a little time after it terminates, precipitin and precipitinogen are both present, but that a few days after the symptoms have subsided all precipitinogen has disappeared. This means that by this time all antitoxin is also lost. The importance of this clinically lies in the fact that such patients as develop serum sickness quickly, that is to say, patients sensitised by previous doses of serum and those naturally somewhat hypersensitive, can avail themselves of the antitoxin injected for a short time only. The rare immediate reactors may possibly absorb very little, or destroy it all before it even reaches the blood. In a subsequent part of the paper we shall record the result obtained with one rabbit, which had previously received three large sensitising doses, and showed only 1/25,000 of the total antitoxin (injected subcutaneously) in its blood.

It is probable that the duration of the three phases differs in man and in rabbits. This would seem to be implied from the results given in the paper by Longcope and Rackemann already referred to, but as they were testing whole horse serum and not the antitoxin-containing fraction only, it cannot be assumed as certain. It is hoped that it may eventually be possible to show how these results obtained with rabbits are applicable to man.

CONCLUSION.

1. The course of elimination of passive immunity in rabbits injected intravenously with diphtheria antitoxin obtained from a horse, consists of three phases:

(*a*) an initial loss of 50 per cent. occurring within the first 24 hours;

(*b*) a gradual constant percentage loss of approximately 25 per cent. from day to day, lasting 6–7 days;

(*c*) a rapidly accelerated loss of 50 per cent. or more per day after the seventh or eighth day.

2. In rabbits sensitised with small doses of horse serum the same three phases are seen, but

(*a*) while the initial loss remains the same,

(*b*) the gradual constant percentage loss is of the same magnitude, but lasts only two or three days;

(*c*) the rapidly accelerated loss occurs after the third or fourth day and over 90 per cent. of the antitoxin present is lost within 24 hours.

3. Possible explanations of the three phases are:

(*a*) initial loss due either to precipitin to horse serum normally present in rabbits or to saturation of or adsorption to the tissues;

(*b*) a phase independent of precipitin action;

(*c*) accelerated loss due to formation of precipitin commencing seven or eight days after injection of horse serum in normal rabbits and three or four days after injection in sensitised rabbits.

REFERENCES.

ALEXANDER, H. L. (1921). Precipitin Response in the Blood of Rabbits following Subarachnoid Injections of Horse Serum. *Journ. Exper. Med.* XXXIII. 471.

LONGCOPE, W. T. and RACKEMANN, F. M. (1918). The Relation of Circulating Antibodies to Serum Disease. *Ibid.* XXVII. 341.

MACKENZIE, G. M. AND LEAKE, W. H. (1921). Relation of Antibody and Antigen to Serum Disease Susceptibility. *Ibid.* XXXIII. 601.