DURATION OF PASSIVE IMMUNITY.

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(*From the Wellcome Physiological Research Laboratories.*)

PART II.

(With Charts III—XII.)

(Continued from Vol. XXI, No. 2.)

In Part I of this paper we traced the rate of disappearance of diphtheria antitoxin obtained from a horse, when injected intravenously into normal rabbits and into rabbits previously sensitised by the subcutaneous injection of small volumes of horse serum. We described the curve of antitoxin content as falling into three phases: Phase A, a rapid loss of 50 per cent. during the first 24 hours, Phase B, a period of slow elimination lasting seven or eight days in normal rabbits during which about 25 per cent. of the amount present each day is lost by the next day, and Phase C, a period of accelerated loss apparently due to the formation of precipitin in response to the antigenic stimulus of the injection of horse serum. It would be expected *a priori* that if heterologous serum were injected into a number of normal animals, *e.g.* rabbits, all the phenomena which followed would be practically identical in the different animals. Early in our work it became obvious that considerable differences occurred in the response in different animals; these differences, particularly in regard to the duration of Phase B and the duration and intensity of Phase C, are set out in full detail in the tables in this paper and various explanations of the differences are considered. Observations on two atypical normal rabbits 42 and 61 are recorded in Table V. The rate of elimination of horse serum from rabbit 42, as indicated by antitoxic content, differs considerably from that of other normal rabbits 41, 49, and 50, recorded in Part I, Tables I and II. Reference to Table V and Curve 11 on Chart III shows that Phase C is succeeded by a fourth phase of slow elimination indicating apparently that rabbit 42 did not produce sufficient precipitin to eliminate all the precipitinogen injected. In contrast to this rabbit, rabbit 61 (Table V and Curve 12 on Chart III) responded earlier with a more rapid excretion probably due to a more rapid production of precipitin than did the normal rabbits previously recorded.

The duration of Phase B must be taken as the Latent Period between the injection of precipitinogen and the production of precipitin; the rapidity of accelerated loss during Phase C must be taken as an indication of the rapidity

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Table V.

Showing the differences between the responses of two normal rabbits to an 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 42	Antitoxic value in units per c.c. Rabbit 61	Percentage daily loss Rabbit 42		Percentage daily loss Rabbit 61	
15 minutes	6·5	10·5				
1 day	—	4·0	—		61·9	
2 days	3·0	3·0	—		25·0	Phase B Average 27·1
3 ,,	3·0	—	0·0		—	
4 ,,	1·8	1·5	40·0	Phase B Average 20·3	29·3	
5 ,,	1·5	0·8	16·7		46·7	
6 ,,	1·1	0·14	26·7		82·5	Phase C Average 77·5
7 ,,	0·9	0·02	18·2		85·7	
8 ,,	—	0·001	—		95·0	
9 ,,	0·22	0·0005	50·6			
10 ,,	0·08	—	63·7	Phase C Average 57·5		
11 ,,	0·035	—	56·2			
12 ,,	0·016	—	54·3			
13 ,,	0·006	—	62·5			
14 ,,	0·004	—	33·3			
15 ,,	—	—	—			
16 ,,	0·002	—	29·3			
17 ,,	—	—	—			
18 ,,	0·001	—	29·3	Phase D Average 21·5		
19 ,,	—	—	—			
20 ,,	0·001	—	0·0			
21 ,,	—	—	—			
22 ,,	—	—	—			
23 ,,	0·0006		15·6			
24 ,,	—					
25 ,,	—					
26 ,,	<0·0005					

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of formation of precipitin, *i.e.* of immunity response to the injection of horse serum. The difference between the normal rabbits so far recorded is suggestive, therefore, of differences in degree of natural immunity. Rabbit 61, apparently more highly immune naturally than the average rabbit, responds to the injection of an antigen by a more rapid and intense production of antibody, than do the majority of normal rabbits. Similarly rabbit 42 must be regarded as less highly immune naturally, because the same stimulus in this rabbit produced a smaller production of antibody than in other normal rabbits.

It was this conception that led us to suggest that the 50 per cent. loss during Phase A was due to natural precipitin. Later considerations have led

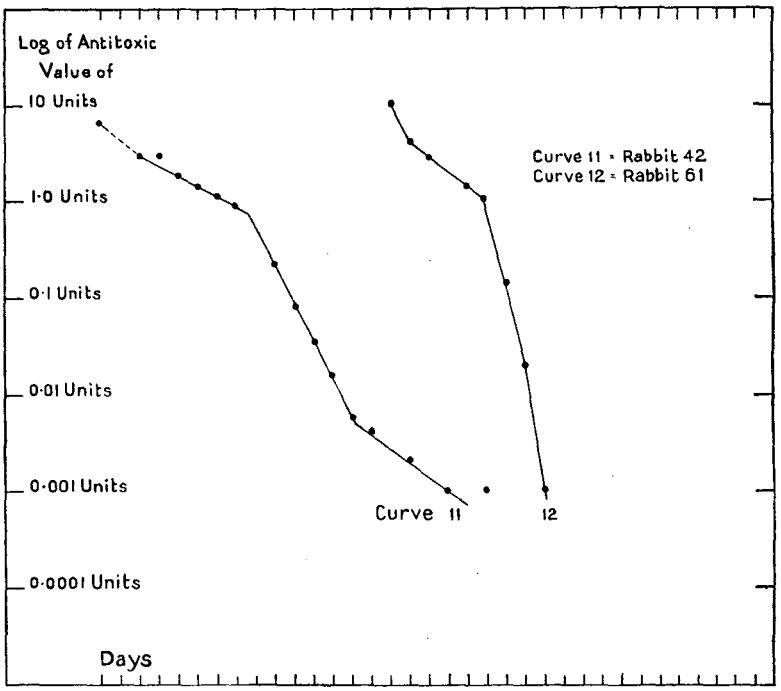

Chart III.

us to abandon this idea. At first the variation in percentage loss during Phase A was taken to be of marked significance. Although no bleeding was taken from rabbit 42 on the day following injection, yet the shape of the curve of antitoxic content for this rabbit suggests that the loss during Phase A was decidedly smaller than the average loss of 50 per cent. It therefore appeared that rabbits 42 and 61, regarded as the least and the most highly immune rabbits, exhibited the smallest and the greatest loss respectively during Phase A. Later considerations, however, have shown that the antitoxic value recorded for rabbit 42, 15 minutes after injection, was lower than the value observed in other rabbits of similar weight. The connection between the weight of the animal and the amount of antitoxin detectable in the serum 15 minutes after

an intravenous injection of a given amount of antitoxin will be considered in a later part. Again, rabbit 61 gave a value nearly 30 per cent. higher than the theoretical value for the 15 minutes' reading, but if the actual 24 hours' reading be compared with the theoretical 15 minutes' reading it shows a loss of 50·9 per cent., thus falling into line with other normal rabbits. It is probable, therefore, that these differences in percentage loss during Phase A are due to some unexplained error in testing and are of no significance. There remain, however, the apparent differences in natural immunity.

The five normal rabbits under review were again injected with horse serum containing diphtheria antitoxin, in order to determine whether the initial differences in immunity response continued. Rabbits 50 and 61 were re-injected 10 and 11 days respectively after the first injection; the results of re-injection are recorded in Table VI and Curves 13 and 14 on Chart IV. Rabbit 61 was not bled 15 minutes after injection, but the value taken has been calculated according to the weight of the animal. The results obtained failed to show any differences in immunity response to the second injection because the usual phases are here masked by the greater loss caused by excess precipitin still present or still being formed in response to the initial stimulus; rabbit 61, however, had eliminated all antitoxin earlier than rabbit 50.

Table VI.

Showing the differences between the responses of rabbits 50 *and* 61 *when re-injected after a short interval with* 0·5 *c.c. of unconcentrated horse serum containing* 750 *units diphtheria antitoxin.*

	Units of antitoxin remaining		Percentage daily loss	
Rabbit	50	61	50	61
No. of days between 1st and 2nd injection	10	11	—	—
Time interval				
15 minutes	8·0	(8·02)		
			65·6	59·4
1 day	2·75	3·25		
			54·6	38·5
2 days	1·25	2·0		
			82·4	60·0
3 „	0·22	0·8		
			86·4	96·2
4 „	0·03	0·03		
			85·0	95·0
5 „	0·0045	0·0015		
			66·7	
6 „	0·0015	—		
			20·0	
7 „	0·0012	?0·0005		
			50·0	
8 „	0·0006			

Rabbits 41, 42 and 49 received their second injection of antitoxic horse serum 7–10 weeks after their first injection. It will be seen from Table VII and Curves 15, 16 and 17 on Chart IV that rabbit 42 again shows a smaller

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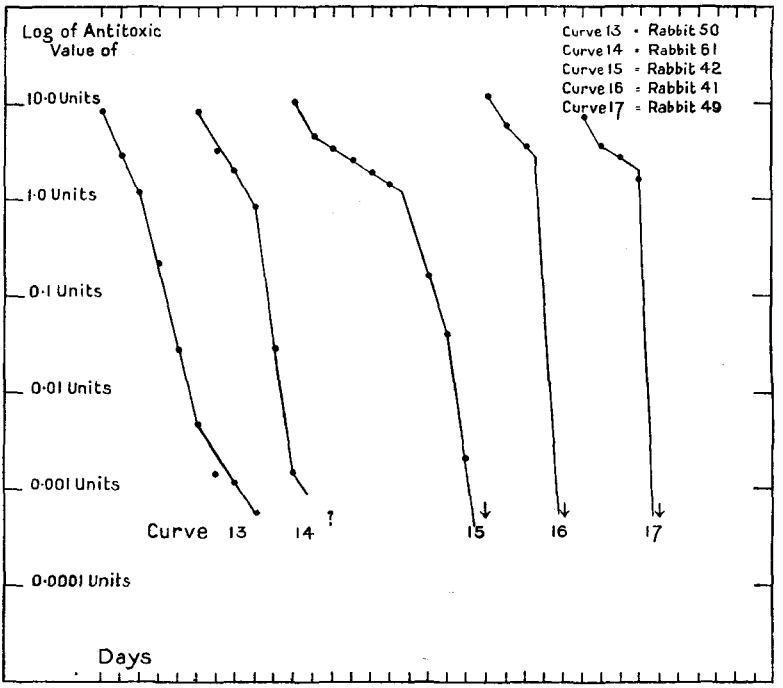

Chart IV.

Table VII.

Comparing responses of rabbits 41, 42, *and* 49 *when re-injected after a considerable space of time with* 0·5 *c.c. unconcentrated horse serum containing* 750 *units diphtheria antitoxin.*

	Units of antitoxin remaining			Percentage daily loss			
Rabbit	42	41	49	42	41	49	
Number of days between 1st and 2nd injection	72	47	62	—	—	—	
Time interval							
15 minutes	10·0	12·0	6·5				
				52·5	54·2	46·2	
1 day	4·75	5·5	3·5	31·5	31·8	21·4	
				Phase B Average 26·3	?	41·8	Phase B
2 days	3·25	3·75	2·75	23·1			
3 ,,	2·5	—	1·6	28·0	(*c.* 99·0) Phase C	(*c.* 99·0) Phase C	
4 ,,	1·8	<0·0005	<0·0005	22·2			
5 ,,	1·4	—	—	—			
6 ,,	—	—	—	(66·2)			
7 ,,	0·16	—	—	75·0			
8 ,,	0·04	—	—	Phase C Average 78·7			
9 ,,	0·002	—	—	95·0			
10 ,,	<0·0005	—	—	75·0			

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immunity response. Rabbits 41 and 49 were regarded as average normal rabbits according to their responses to the first injection of horse serum. The responses of these rabbits to a second injection agree closely with those obtained with sensitised rabbits in Part I (see Tables III and IV, Curves 4–10).

A comparison between Curves 12 and 15 shows that an untreated normal rabbit number 61, may exhibit the same degree of apparent immunity as

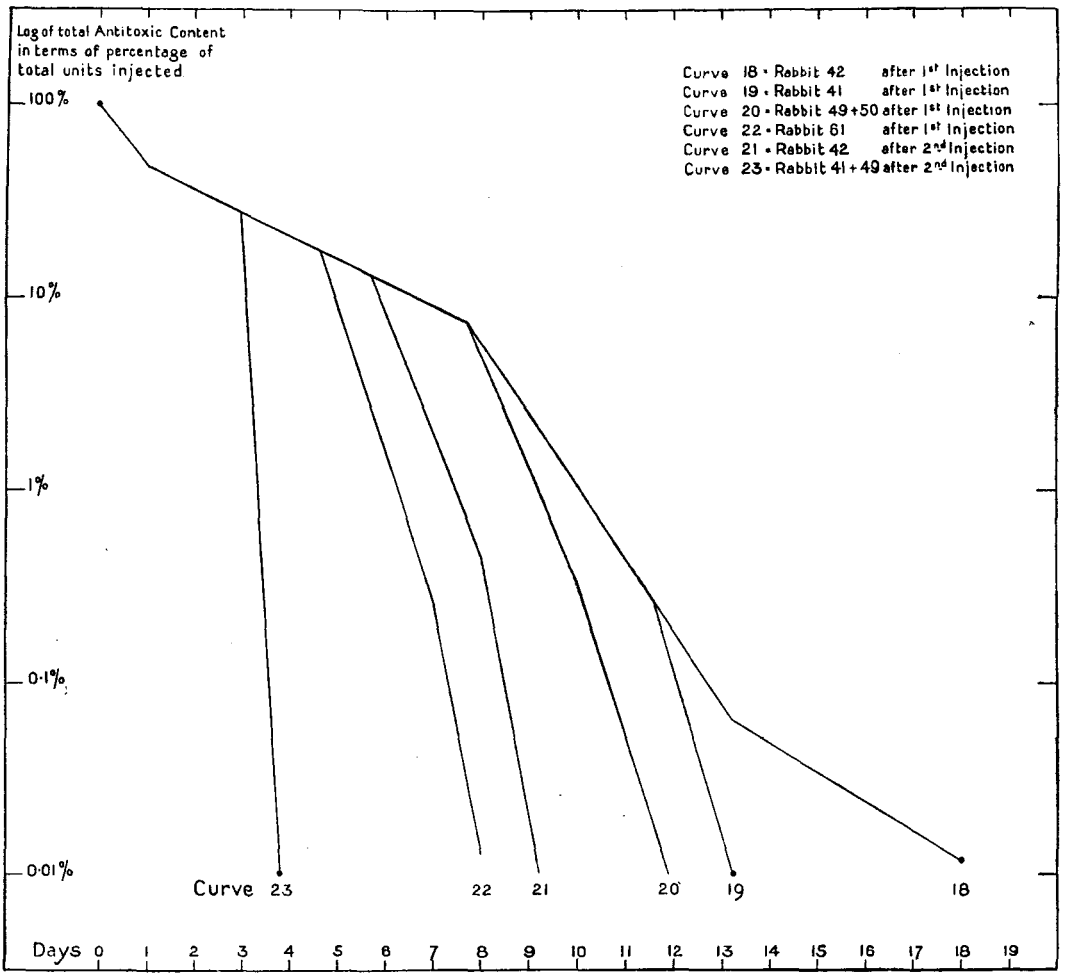

Chart V.

another rabbit, number 42, that had already received an initial sensitising injection.

Chart V has been compiled in order to show more clearly the points of similarity and difference between the various curves of these five rabbits. This chart differs from the previous ones in recording the total antitoxic content of each rabbit on different days rather than the antitoxic value per c.c.; this

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total content has been calculated by multiplying the antitoxic value by the available serum volume, taken as a definite fraction of the body weight. In this way the values for the different rabbits are comparable, and we have drawn to represent Phases A and B for all the rabbits a single standard curve from which the curve representing Phase C for each individual rabbit branches off. By taking the available serum volume as 44·2 c.c. per kilo. of body weight the average antitoxin content immediately after injection of the five normal rabbits recorded in Tables I and V and the three re-injected rabbits recorded in Table VII becomes equal to 750 units, the amount injected. It is not suggested that the course of elimination from each rabbit followed exactly the course depicted by the curves, but the general comparison of one rabbit with another is clearly indicated. Phase B ends, for most of the normal rabbits, between the seventh and the eighth day after injection, but differences occur in the slope of the curves representing Phase C. The general interpretation of this chart is that after a single injection of the same volume of horse serum into five normal rabbits the latent period before the formation of precipitin was 7–8 days in four rabbits, but the amount formed varied; the immunity response was least in rabbit 42 represented by Curve 18, more in rabbit 41 (Curve 19) and again more in rabbits 49 and 50 (Curve 20 represents rabbit 49 along its full length and rabbit 50 until re-injected). The fifth normal rabbit number 61 (Curve 22) showed a latent period of just under five days and the intensity of the immunity response, indicated by the steepness of the section of curve for Phase C, was greater than that of the other four rabbits. Upon again injecting rabbits 41 and 49, the curve of antitoxic content (curve 23) is moved well to the left showing a latent period of only three days followed by a rapid production of precipitin as indicated by the steepness of the section of curve representing Phase C. Rabbit 42 upon re-injection also showed an increased response (Curve 21). While the various curves following the first injection of horse serum into these rabbits show that normal rabbits differ in responsiveness to an injection of horse serum, the position of the curve for rabbit 61, standing as it does to the left of the curve following the second injection into rabbit 42, is strongly suggestive that this difference in responsiveness is a difference in degree of natural immunity.

If this view were correct and some normal rabbits became during life more immune to horse serum than others, young rabbits would probably show a lower average degree of immunity than older rabbits that have had more opportunity of acquiring an artificial immunity. The ages of the five normal rabbits were not recorded, but at the time of their first injection they each weighed between 1800 and 2050 grams.

A number of very young rabbits were injected intravenously with antitoxic horse serum and the curves of their antitoxic content traced. Tables VIII and IX and Chart VI give the results obtained with four rabbits from the same litter injected at various ages. These rabbits did not show a uniform progressive immunity to horse serum in accordance with their age at the time of injection.

Table VIII.

Showing the antitoxic value of the blood of four normal rabbits of the same litter, at different intervals of time, after the injection of 0·5 c.c. unconcentrated horse serum containing 750 units of diphtheria antitoxin.

Rabbit	76	79	86	93
Weight at time of injection	510	740	680	1020
Age at time of injection	7 wks.	11 wks.	13 wks.	16 wks.
Time interval	Antitoxin value in units per c.c.			
15 minutes	35·0	23·0	25·5	15·5
1 day	17·0	11·0	11·0	7·5
2 days	12·0	7·5	8·0	4·75
3 ,,	6·5	6·5	6·0	—
4 ,,	5·5	5·5	4·75	2·75
5 ,,	4·75	3·75	3·0	2·25
6 ,,	—	—	—	0·8
7 ,,	3·5	0·8	1·62	0·45
8 ,,	1·87	0·25	1·25	—
9 ,,	1·5	0·07	0·9	0·12
10 ,,	1·25	0·011	0·7	—
11 ,,	0·8	0·003	0·35	—
12 ,,	0·5	0·0014	0·22	0·015
13 ,,	—	—	—	0·008
14 ,,	—	?0·0006	0·14	0·005
15 ,,	0·14	?0·0005	0·11	0·0025
16 ,,	0·09	<0·0005	0·08	0·001
17 ,,	0·055	—	0·045	—
18 ,,	0·05	—	0·035	?0·001
19 ,,	0·035	—	0·022	0·0005
20 ,,	—	—	—	—
21 ,,	0·018	—	0·015	
22 ,,	0·012	—	0·011	
23 ,,	0·009	—	0·0055	
24 ,,	—	—	—	
25 ,,	0·0055	—	0·0045	
26 ,,	0·003	—	0·0040	
27 ,,	—	—	—	
28 ,,	0·0016	—	—	
29 ,,	—	—	—	
30 ,,	0·0010	—	0·0025	
31 ,,	0·0010	—	—	
32 ,,	0·0006	—	0·002	
33 ,,	0·0005	—	—	
34 ,,	—	—	—	
35 ,,	—	—	—	
36 ,,	—	—	—	
37 ,,	—	—	—	
38 ,,	—	—	?0·0015	
39 ,,	—	—	?0·0010	
40 ,,	—	—	—	
41 ,,	—	—	?0·0005	
42 ,,	—	—	—	

Two of the rabbits showed such a low degree of immunity that the existence of Phase C in Curves 24 and 26 is difficult to demonstrate. These two rabbits 76 and 86, aged 7 and 13 weeks and weighing 510 and 690 grams at the time of injection, retained some antitoxin for over four weeks; their curves, if plotted on the standard curve on Chart V would be well to the right of Curve 18. On the other hand, two others of the same litter, 79 and 93, aged 11 and 16 weeks and weighing 740 and 1020 grams, were far more responsive. Curves 25

Table IX.

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

Time interval	Rabbit 76		Rabbit 79		Rabbit 86		Rabbit 93	
0–1 day	51·4	Phase A	52·2	Phase A	56·8	Phase A	51·6	Phase A
1–2 days	29·4		31·8	Phase B	27·3		36·7	Phase B
2–3 „	45·8		13·3	Average	25·0		—	Average
3–4 „	15·4		15·4	23·1	20·8		23·9	26·3
4–5 „	13·6	Phase B	31·8		36·8	Phase B	18·2	
5–6 „	—	Average	—		—	Average	64·4	
6–7 „	14·2	25·2	53·8		26·5	26·2	43·7	
7–8 „	46·6		68·7		22·8		—	
8–9 „	19·8		72·0		28·0		48·4	
9–10 „	16·7		84·3	Phase C	22·2		—	Phase C
10–11 „	36·0		72·7	Average	50·0	Phase C	—	Average
11–12 „	37·5		53·3	67·5	37·1	Average	50·0	50·1
		Phase C				43·5		
12–13 „	—	Average	—		—		46·7	
13–14 „	—	36·5	?35·5		20·2		37·5	
14–15 „	34·6		—		21·4		50·0	
15–16	35·7		—		27·3		60·0	
16–17 „	38·9		—		43·7			
17–18 „	9·1		—		22·2			
18–19 „	30·0		—		37·1			
19–20 „	—		—		—			
20–21 „	28·3		—		17·4			
21–22 „	33·3		—		26·7	Phase D		
22–23 „	25·0	Phase D	—		50·0	Average		
23–24 „	—	Average	—		—	22·6		
24–25 „	21·8	26·7	—		9·5			
25–26 „	45·4		—		11·1			
26–27 „	—		—		—			
27–28 „	27·0		—		—			
28–29 „	—		—		—			
29–30 „	21·0		—		11·1			
30–31 „	—		—		—			
31–32 „	—		—		10·5			

and 27 showing the logs of the antitoxic values of these two rabbits exhibit irregular features when compared with standard curves on Chart V. The early appearance of Phase C would place these curves between that representing the first injection into rabbit 61, and that representing the second injection into rabbit 42. The slope of the Phase C section of the curve, however, is less steep than that of Curves 19 and 20. The latent period was short, but the rate of production of precipitin was slow.

If the differences of responses of the different rabbits tested are due to immunity acquired since birth, then these marked differences in young rabbits from the same litter would indicate that such immunity is not acquired at all consistently, and that marked differences will be seen between the responses of different young rabbits. Unless acquired immunity is lost as easily as it is gained; older rabbits should show more frequently a high degree of immunity.

Tables X and XI record the results of injecting three old rabbits and two young rabbits with antitoxic horse serum. These three old rabbits 83, 109,

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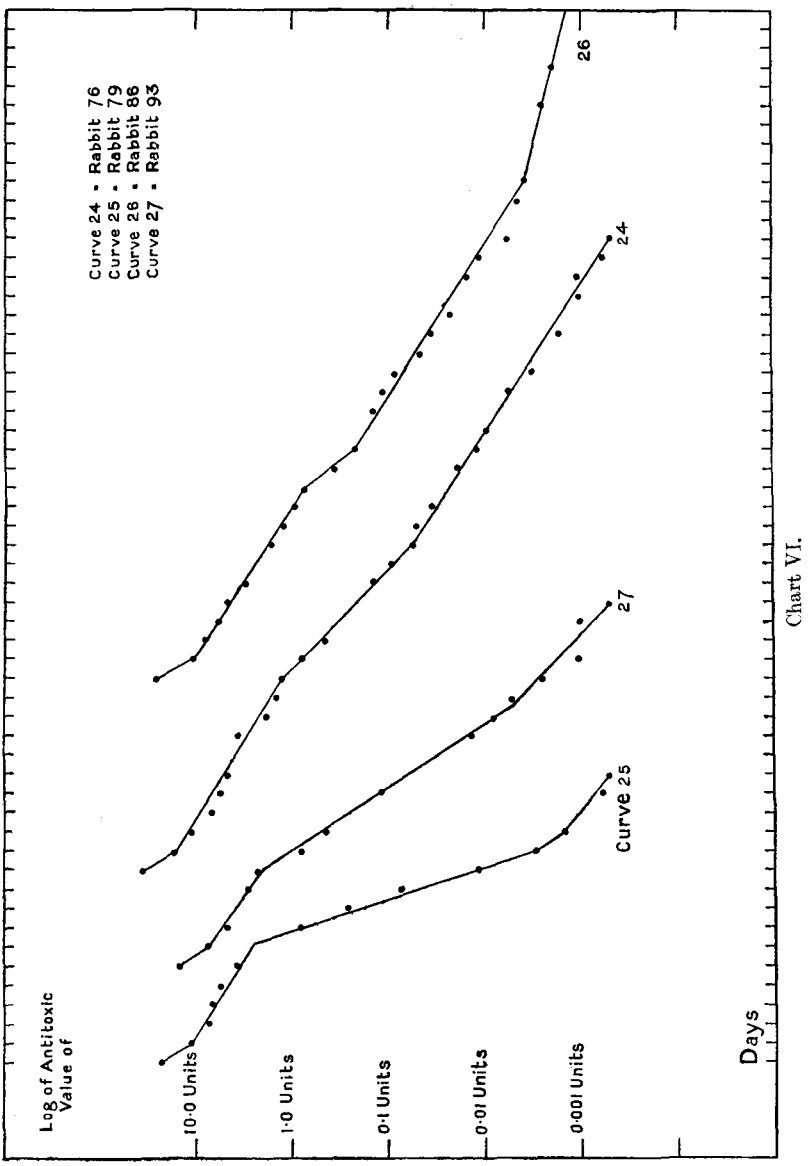
and 110 (Curves 28, 29, 30 on Chart VII) appeared highly immune and all antitoxin was lost in nine days or less.



The steepness of the slope of the portions of the curves representing Phase C is particularly noticeable. The formation of precipitin is obviously very rapid in old rabbits.

Of the two young rabbits, number 84 (Curves 31 and 31A) was only four weeks old and weighed only 400 grams when first injected; a moderate response

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Table X.

Showing the antitoxic content of three old and two young rabbits at different intervals of time, after the injection of 0·5 *c.c. unconcentrated horse serum containing* 750 *units of diphtheria antitoxin.*

Rabbit	83	109	110	84	94
Weight in grams at time of injection	3240	2840	2810	400	910
Age	Over 2 yrs.	—	—	4 wks.	7 wks.
Time interval	Antitoxic value in units per c.c.				
15 minutes	5·25	7·0	8·0	29·0	16·0
1 day	2·75	4·5	3·75	15·5	9·5
2 days	2·0	—	—	13·0	5·75
3 ,,	1·62	2·5	2·25	11·0	—
4 ,,	1·37	1·12	1·62	8·5	3·5
5 ,,	1·12	0·7	0·45	6·5	2·75
6 ,,	—	0·035	0·0015	—	0·12
7 ,,	0·005	0·001	<0·001	2·75	0·004
8 ,,	?0·0005	<0·001	—	2·25	0·002
9 ,,	—	—	—	1·25	0·0015
10 ,,	—	—	—	0·4	—
11 ,,	—	—	—	0·11	—
12 ,,	—	—	—	0·012	<0·0005
13 ,,	—	—	—	—	—
14 ,,	—	—	—	0·002	
15 ,,	—	—	—	0·0006	
16 ,,	—	—	—	<0·0005	

Table XI.

Showing the percentage daily loss in antitoxic value of the blood of three old and two young rabbits at different intervals of time, after the injection of 0·5 *c.c. unconcentrated horse serum containing* 750 *units of diphtheria antitoxin.*

Time interval	Rabbit 83		Rabbit 109		Rabbit 110		Rabbit 84		Rabbit 94	
0–1 day	47·6	Phase A	35·7	Phase A	53·1	Phase A	46·5	Phase A	40·6	Phase A
1–2 days	27·3	Phase B Average 20·0	—	Phase B Average	—	Phase B Average	16·1		39·4	Phase B Average 27·6
2–3 ,,	19·0		25·5		22·5		15·4		—	
3–4 ,,	15·4		55·2	Phase C Average 71·2	28·0	Phase C Average 85·4	22·7	Phase B Average 21·8	22·0	
4–5 ,,	18·2		37·5		72·2		23·5		21·4	
5–6 ,,	—	Phase C Average	95·0		99·6		—		95·6	Phase C Average 66·8
6–7 ,,	93·3		97·1				35·0		96·7	
7–8 ,,	—		—		—		18·2		50·0	
8–9 ,,	—		—		—		44·4		25·0	
9–10 ,,	—		—		—		68·0	Phase C Average 66·6		
10–11 ,,	—		—		—		72·5			
11–12 ,,	—		—		—		89·1			
12–13 ,,	—		—		—		—			
13–14 ,,	—		—		—		59·2			

resulted. This rabbit does not show the usual form of Phase B. Curve 31A has been plotted from the direct values of antitoxin content from the second to the seventh day and shows that the direct values rather than the logs of the values lie upon a straight line. A possible explanation is that although Phase B consists of a normal process of elimination of a foreign protein at a pace determined by the concentration of that protein in the blood yet there is a limit to the amount of protein that a rabbit can eliminate in a given time. Thus it is possible that so small a rabbit as 84 could eliminate in one day only

the amount of protein represented by a change in antitoxic value of 2 units per c.c. of blood.

Rabbit 94 (Curve 32), although only seven weeks old, showed a latent period of only five days, and must be regarded as highly immune for a young rabbit.

A number of other rabbits were injected with diphtheria antitoxin in an endeavour to determine further points of interest. All the rabbits previously recorded had been injected with 0·5 c.c. of unconcentrated horse serum. The effect of varying the amount of serum injected is shown in Tables XII, XIII, and Curves 31 and 34 on Chart VIII. Rabbit 71 received one-tenth the usual

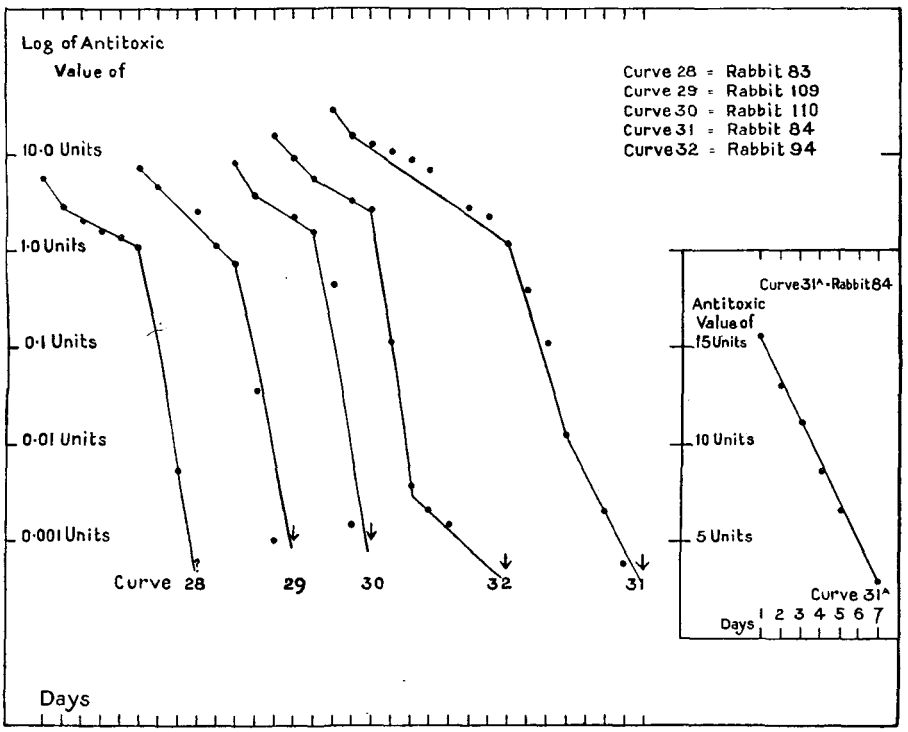

Chart VII.

amount of antitoxic horse serum, rabbit 72 received 0·5 c.c. diluted with nine times that amount of normal horse serum and rabbit 74 was injected with 10·0 c.c. of unconcentrated antitoxic horse serum representing twenty times the usual amount injected.

The three rabbits gave fairly typical curves; Phase A was exceptionally high in rabbit 71 which was injected with a small quantity of serum, and exceptionally low in rabbit 74 which was injected with twenty times the usual amount of serum. Rabbit 74 is of particular interest in that Phase A appears to extend over two days; the relatively high percentage loss for rabbit 72 between the first and the second day is also suggestive of a lengthened Phase A.

Table XII.

Showing the antitoxic content of the serum of normal rabbits at various intervals of time after intravenous injection of varying amounts of unconcentrated horse serum containing diphtheria antitoxin.

	Rabbit 71	Rabbit 72	Rabbit 74	Rabbit 75
Weight	1470	1590	2380	1190
Units of antitoxin injected	75	750	15,000	900
Volume	0·05 c.c.	5·0 c.c.*	10·0 c.c.	10·0 c.c.†
Time interval	Antitoxic value in units per c.c.			
15 minutes	1·325	9·5	160·0	17·0
1 day	0·55	4·5	90·0	8·5
2 days	0·45	3·25	52·0	6·0
3 ,,	0·3	2·75	45·0	5·0
4 ,,	0·22	2·25	35·0	—
5 ,,	0·15	1·325	22·0	2·25
6 ,,	0·11	0·7	—	1·75
7 ,,	0·09	0·25	6·5	0·8
8 ,,	0·07	0·14	1·62	0·22
9 ,,	0·03	0·08	0·14	0·04
10 ,,	0·011	0·033	0·009	0·005
11 ,,	0·0015	0·012	0·002	—
12 ,,	<0·0005	0·006	0·001	<0·0005
13 ,,	—	?0·0010	—	—
14 ,,	—	—	<0·0010	
15 ,,	—	—	—	—
16 ,,	—	—	—	—
17 ,,	—	—	—	—

* 0·5 c.c. diphtheria antitoxic serum diluted with 4·5 c.c.

† 10 c.c. of rabit serum (74) 24 hours after the injection of unconcentrated horse serum.

Table XIII.

Showing the percentage daily loss in antitoxic value of the blood, of four normal rabbits at different intervals of time, after the intravenous injection of different quantities of diphtheria antitoxin contained in unconcentrated horse serum.

Time interval	Rabbit 71		Rabbit 72		Rabbit 74		Rabbit 75	
0–1 day	58·5	Phase A	52·6	Phase A	43·7	Phase A	50·0	Phase A
1–2 days	18·2		27·7		42·2	Av. 42·9	29·4	
2–3 ,,	33·3		15·4	Phase B	13·4	Phase B	16·7	Phase B
3–4 ,,	26·7	Phase B	18·2	Average	22·2	Average	—	Average
4–5 ,,	31·8	Average	41·1	25·6	37·1	24·2	32·9	25·3
5–6 ,,	26·7	25·3	47·1		—		22·2	
6–7 ,,	18·2		64·3		45·6		54·3	
7–8 ,,	22·2		44·0	Phase C	75·0	Phase C	72·5	Phase C
8–9 ,,	57·1		42·8	Average	91·3	Average	81·8	Average
9–10 ,,	63·3	Phase C	58·7	56·7	93·5	72·2	72·9	72·9
10–11 ,,	86·4	Average	63·6		77·8		—	
11–12 ,,	>66·7	68·4	50·0		50·0		>68·4	
12–13 ,,	—		83·3					
13–14 ,,	—		—					

Phase B was longest for rabbit 71 and Phase C shortest in the same rabbit. If these differences are significant it would indicate that a longer latent period follows the injection of a smaller amount of antigen but the rate of production of precipitin is greater, relative to the amount of precipitinogen, in the animal

injected with the smallest quantity. The other rabbit 75 recorded in this table was injected with 10 c.c. of serum taken from rabbit 74, 24 hours after the injection of horse serum. This was done to determine whether antitoxin obtained from a horse undergoes any radical alteration after injection into a rabbit, more particularly at the end of Phase A. The curve exhibited by rabbit 75 is typical of the curve of excretion of antitoxin obtained from horses showing that no such alteration has occurred.

In the next group of rabbits recorded in Tables XIV and XV, and Curves 35 to 38 on Chart IX, the diphtheria antitoxin injected was contained in

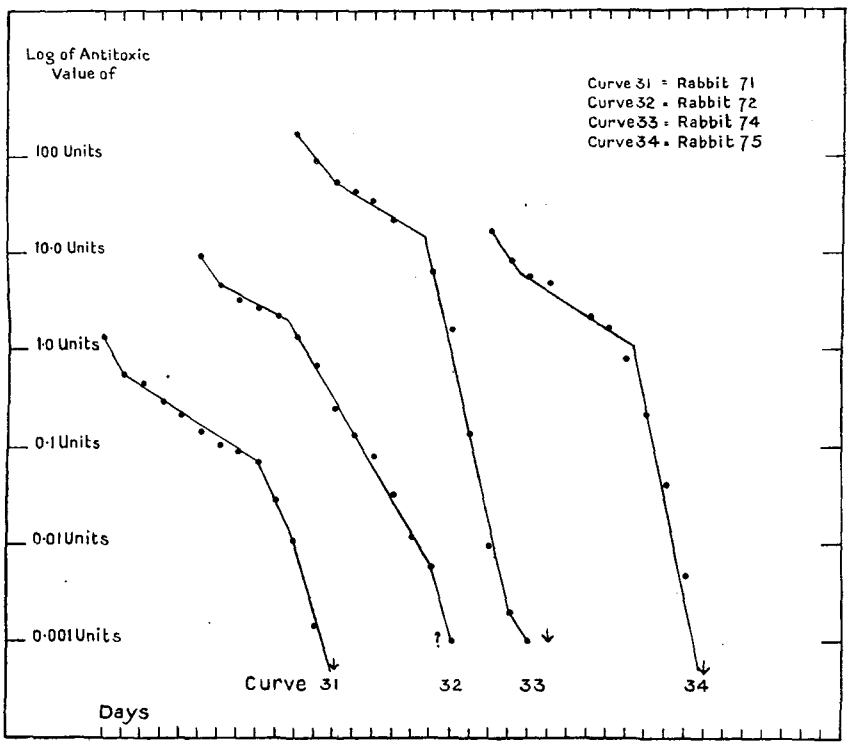

Chart VIII.

purified pseudo-globulin. This concentrated product contained about 50 per cent. more protein than the antitoxic horse serum previously used. No special precautions had been taken in the preparation of this product to render it entirely free from traces of euglobulin or of albumen. Only one rabbit, 73, was traced in detail. This rabbit received slightly more protein than the majority of rabbits already recorded and gave a typical curve. The other three rabbits were not tested until the sixth day. In all cases large quantities of protein were injected, 6 c.c. of pseudo-globulin containing from 7200 to 9000 units. The outstanding feature of the results obtained from these rabbits is the presence of Phase D. It must be remembered that we have no test for

Table XIV.

Showing the antitoxic content of normal rabbits at different intervals of time after the intravenous injection of diphtheria antitoxin contained in purified horse pseudo-globulin.

Rabbit	73	103	104	105
Weight	1390	1640	1930	1620
Units of antitoxin injected	1000	8400	9000	7200
Volume	0·63 c.c.	6 c.c.	6 c.c.	6 c.c.
Time interval	Antitoxic value in units per c.c.			
15 minutes	15·0	(114)*	(103)*	(100)*
1 day	6·25	—	—	—
2 days	5·5	—	—	—
3 ,,	4·75	—	—	—
4 ,,	3·5	—	—	—
5 ,,	—	—	—	—
6 ,,	1·62	13·0	12·0	7·5
7 ,,	—	10·0	6·5	3·75
8 ,,	0·11	5·75	3·25	2·25
9 ,,	0·0035	3·0	1·62	1·25
10 ,,	0·0001	0·55	0·62	0·55
11 ,,	<0·0005	0·055	0·3	0·17
12 ,,	—	—	—	—
13 ,,	—	0·003	0·075	0·08
14 ,,	—	0·0015	0·05	0·05
15 ,,	—	0·0015	0·015	0·015
16 ,,	—	0·0015	0·008	0·015
17 ,,	—	0·001	0·007	0·01
18 ,,	—	<0·001	0·006	0·008
19 ,,	—	—	—	—
20 ,,	—	—	0·003	0·0045
21 ,,	—	—	—	—
22 ,,	—	—	0·002	0·004
23 ,,	—	—	—	—
24 ,,	—	—	0·0015	0·003
25 ,,	—	—	—	—
26 ,,	—	—	—	—
27 ,,	—		0·0015	0·0015
28 ,,	—	—	—	—
29 ,,	—	—	0·001	<0·001
30 ,,	—	—	—	
31 ,,	—	—	<0·001	

* Values calculated according to weight of animal.

the total absence of antitoxin, but it is possible to say that the amount of antitoxin present is less than one thousandth of a unit per c.c. and in many of our earlier experiments we were able to detect with comparative certainty the presence or absence of one two-thousandth part of a unit per c.c. The shape of the curve for any rabbit after the antitoxic content had fallen below this level is impossible to determine. With the majority of rabbits this level bore roughly the same relationship to the total amount of antitoxin present as in the case of rabbit 73 in the table under review, *i.e.*, about one fifteen-thousandth of the total antitoxin injected. Rabbits 103, 104, 105 injected with 7–10 times the usual amount of antitoxin could be followed out over a longer range than other rabbits, the least amount detectable being approximately

Table XV.

Showing the percentage daily loss in antitoxic value of the blood of normal rabbits at different intervals of time after the intravenous injection of diphtheria antitoxin contained in purified horse pseudo-globulin.

Time interval	Rabbit 73		Rabbit 103		Rabbit 104		Rabbit 105	
0–1 day	58·3	Phase A						
1–2 days	12·0							
2–3 ,,	13·6	Phase B						
3–4 ,,	26·3	Average						
4–5 ,,	—	21·0						
5–6 ,,	32·0							
6–7 ,,	—		23·0	Phase B	45·8		50·0	
7–8 ,,	73·8	Phase C	42·5		50·0		40·0	
8–9 ,,	96·8	Average	47·8		50·0		44·4	
9–10 ,,	71·4	80·7	81·7	Phase C	61·7		56·0	
10–11 ,,	—		90·0	Average	51·6	Phase C	69·1	Phase C
11–12 ,,	—		—	64·8	—	Average	—	Average
12–13 ,,	—		76·7		50·0	51·0	31·4	49·3
13–14 ,,	—		50·0		33·3		37·5	
14–15 ,,	—		(29·3)	Phase D	70·0		70·0	
15–16 ,,	—		(20·6)	Average	46·7		(45·2)	
16–17 ,,	—		33·3	27·7	12·5		33·3	
17–18 ,,	—		—		14·3		20·0	
18–19 ,,	—		—		—		—	
19–20 ,,	—		—		29·3		25·0	
20–21 ,,	—		—		—		—	Phase D
21–22 ,,	—		—		18·3	Phase D	5·7	Average
22–23 ,,	—		—		—	Average	—	19·6
23–24 ,,	—		—		13·4	15·9	13·4	
24–25 ,,	—		—		—		—	
25–26 ,,	—		—		—		—	
26–27 ,,	—		—		(5·5)		20·5	
27–28 ,,	—		—		—			
28–29 ,,	—		—		18·3			
29–30 ,,								
30–31 ,,								

one hundred-thousandth of the total antitoxin injected. The comparable level of least detectable amounts, one fifteen-thousandth, was passed for these three rabbits between the twelfth and the seventeenth day before the existence of Phase D could be determined with certainty. This suggests the possibility of Phase D being of frequent occurrence although infrequently detected. On the other hand it must be pointed out that in the next tables another rabbit is recorded that was injected with 7500 units of antitoxin without exhibiting signs of Phase D while rabbit 74 in Table XVI received 15,000 units and Phase D was not detected. The most probable explanation is that when relatively large amounts of serum are injected the amount of precipitin produced is insufficient to eliminate all the precipitinogen.

Tables XVI and XVII and Curves 39, 40, 41 on Chart X record the results of injecting three rabbits from the same litter at the same time with different amounts of diphtheria antitoxin contained in purified horse globulin. The exceptionally high figures for percentage loss during Phase A are probably due to loss by withdrawal of blood; these three rabbits were all bled seven times

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in the first 24 hours after injection in an endeavour—to be reported later—to explore more fully Phase A. The total amount of blood withdrawn during this period was from 20 to 30 per cent. of the total blood content of the rabbits. The extent of loss during Phase A is again in the same order, *i.e.*, the greatest loss occurs in the rabbit receiving the smallest injection. The short duration of the presence of any detectable antitoxin in rabbit 106 and the long duration in the rabbit 108 shows an artificial difference because the least detectable amount of antitoxin is relatively smaller in proportion to the amount injected in the rabbit receiving the large injection. These tables and the two preceding

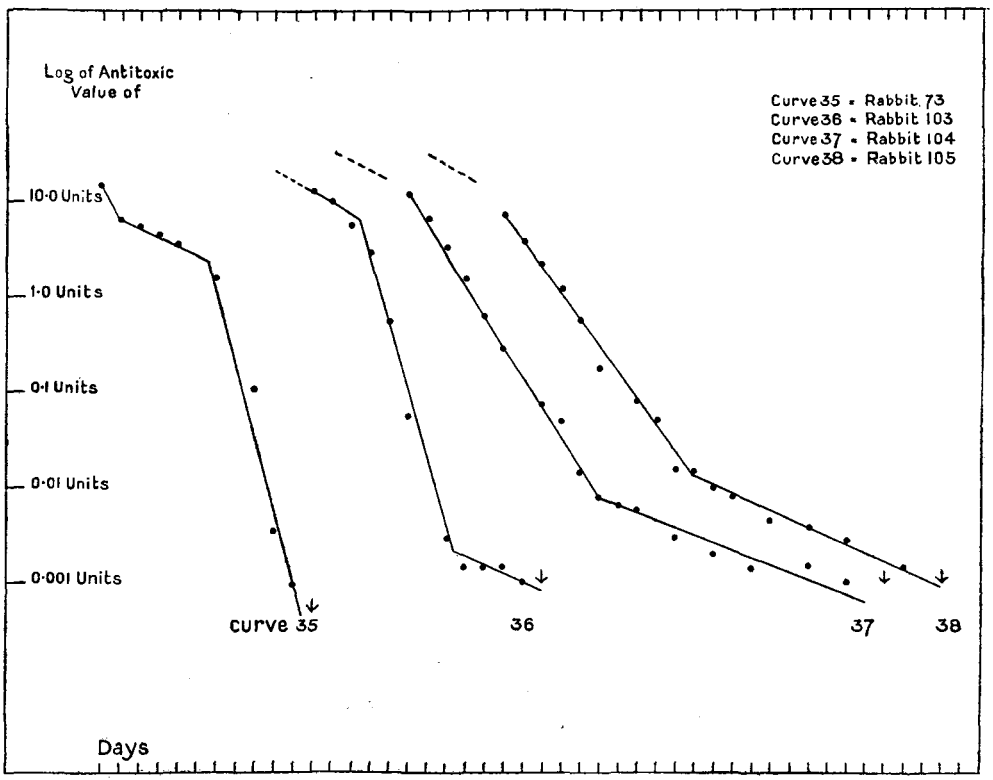

Chart IX.

tables show that the immunity reaction of rabbits to horse serum, in so far as elimination of the antitoxin carrying pseudo-globulin is concerned, is the same whether the rabbit is injected with whole serum or with purified pseudo-globulin.

All results so far recorded have dealt with rabbits injected intravenously. Two normal rabbits 55 and 63 were injected subcutaneously, one with the usual amount of horse serum (0·5 c.c. containing 750 units of antitoxin) and the other with ten times that amount. The results are recorded in Table XVIII and Chart XI. Owing to the slow rate of absorption after subcutaneous

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Table XVI.

Showing the antitoxic content of three normal rabbits from the same litter at different intervals of time after the intravenous injection of different quantities of diphtheria antitoxin contained in purified horse pseudo-globulin.

Rabbit	106	107	108
Weight	990	880	920
Age	11 wks.	11 wks.	11 wks.
Units of antitoxin injected	75	750	7500
Volume	0·05 c.c.	0·5 c.c.	5·0 c.c.
Time interval			
15 minutes	1·67	16·0	130·0
1 day	0·58	6·5	55·0
2 days	0·45	4·5	37·0
3 ,,	0·3	3·5	30·0
4 ,,	0·18	2·75	22·0
5 ,,	0·14	2·25	17·5
6 ,,	0·11	0·58	12·0
7 ,,	0·045	0·1	9·0
8 ,,	0·02	0·02	4·7
9 ,,	—	—	—
10 ,,	<0·001	0·004	0·8
11 ,,	—	—	0·18
12 ,,	—	0·0015	0·05
13 ,,	—	<0·001	0·015
14 ,,	—	—	0·006
15 ,,	—	—	0·003
16 ,,	—	—	—
17 ,,	—	—	0·001
18 ,,	—	—	—
19 ,,	—	—	<0·001

Table XVII.

Showing the percentage daily loss in antitoxic value of the blood of three normal rabbits from the same litter at different intervals of time after the intravenous injection of different quantities of diphtheria antitoxin contained in purified horse pseudo-globulin.

Time interval	Rabbit 106		Rabbit 107		Rabbit 108	
0–1 day	65·2	Phase A	59·4	Phase A	57·7	Phase A
1–2 days	22·4		30·7	Phase B	32·7	
2–3 ,,	33·3	Phase B	22·2	Average	18·9	Phase B
3–4 ,,	40·0	Average	21·4	23·1	26·7	Average
4–5 ,,	22·2	27·8	18·2		20·4	25·8
5–6 ,,	21·4		74·2		31·4	
6–7 ,,	59·1	Phase C	82·7		25·0	
7–8 ,,	55·5	Av. 57·3	80·0	Phase C	47·7	
8–9 ,,		—	—	Average	—	
9–10 ,,		—	55·3	66·2	58·7	
10–11 ,,		—	—		77·5	
11–12 ,,		—	38·8		72·2	Phase C
12–13 ,,		—		—	70·0	Average
13–14 ,,		—		—	60·0	59·8
14–15 ,,		—		—	50·0	
15–16 ,,		—		—	—	
16–17 ,,		—		—	42·3	
17–18 ,,		—		—	—	
18–19 ,,						

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injection, all appearance of Phase A is obliterated and a considerable portion of Phase B is also masked. The chief interest of the experiment lies in the comparison between the curves of antitoxic content of rabbits injected subcutaneously, and those of rabbits injected intravenously. On Chart XI the standard curve of total antitoxic content has been reproduced from Chart V. Curve 43, representing rabbit 63, has been scaled down to one-tenth for comparison. It will be seen that during the first two days the antitoxic content of the rabbit injected subcutaneously is markedly below the average content of rabbits injected intravenously. After that time there is no great difference. For a period of three days the Phase B section of the curve follows closely

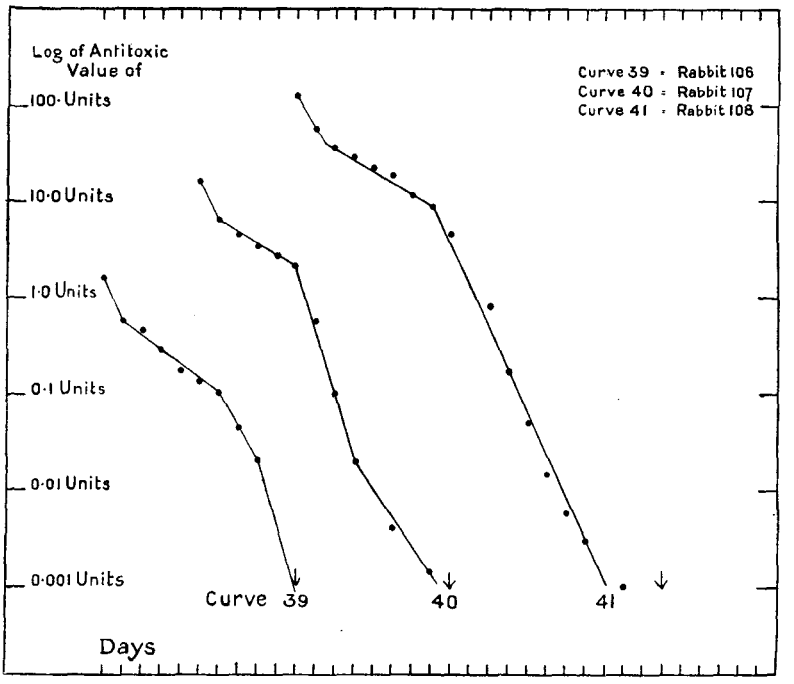

Chart X.

that of the standard curve for intravenous injection; the Phase C section follows closely that of curve 19 representing rabbit 41. The time of appearance and steepness of slope of Phase C show that the production of precipitin follows equally well the intravenous or the subcutaneous injection of horse serum. The relative antitoxic content after intravenous or subcutaneous injection is also seen in Table XVIII by comparing the actual values of rabbit 63 injected subcutaneously with those given for rabbit 72. As already recorded in Table XII, rabbit 72 was injected with 5 c.c. of horse serum; because only one-tenth of the serum was antitoxic horse serum of the same unit value as that injected into rabbit 63, the observed values of rabbit 72 have been multiplied by ten. The differences of weight of the two rabbits is

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Table XVIII.

Showing the antitoxic content of the blood of two rabbits injected subcutaneously with antitoxic horse serum.

Rabbit	55	63	72
Weight	1930	1790	1590
Volume of serum injected	0·5 c.c.	5·0 c.c.	5·0 c.c.
Number of units injected	750	7500	750
Method of injection	Subcutaneously		Intravenously
Time interval	Antitoxic value in units per c.c.		10 times antitoxic value in units per c.c.
1 hour	—	0·25	—
2 ,,	—	0·35	75·0
3 ,,	—	1·3	—
4 ,,	—	2·25	70·0
5 ,,	—	3·25	—
6 ,,	—	4·25	65·0]
7 ,,	—	—	—
8 ,,	—	7·0	—
1 day	2·75	19·0	45·0
2 ,,	3·5	28·0	32·5
3 ,,	3·5	28·0	27·5
4 ,,	2·75	19·0	22·5
5 ,,	2·75	—	13·2
6 ,,	—	11·0	7·0
7 ,,	0·70	6·0	2·5
8 ,,	0·0008	2·75	1·4
9 ,,	0·00015	1·3	0·8
10 ,,	<0·0005	0·45	0·3
11 ,,	—	0·022	0·1
12 ,,	—	—	0·06
13 ,,	—	<0·0005	—

not sufficient to affect the following comparison. Two hours after injection, the antitoxic value per c.c. of blood of a rabbit injected intravenously was 200 times that of a rabbit injected subcutaneously with the same dose; four hours after injection the value was 30 times, six hours after, 15 times, 24 hours after injection twice, two days after injection 15 per cent. above, and not until the third day did the values coincide. Although many workers have called attention to the advantage of intravenous injection of antitoxin over subcutaneous injection, the importance of this point does not yet seem to be fully realised. Curve 43A on Chart XI shows the rate of absorption of antitoxin during the first eight hours after injection. Very little antitoxin is absorbed during the first two hours and then about 1 per cent. of the total injected is absorbed per hour. The highest concentration in the blood, two and three days after injection, represents only one-third of the total antitoxin injected.

It is convenient at this stage to consider the variations in response of the normal rabbits injected with horse serum.

Table XIX has been prepared in order to summarise one aspect of the varied responses of different rabbits to horse serum. The rabbits have been arranged in order of weight which, to a certain extent, indicates age, and to

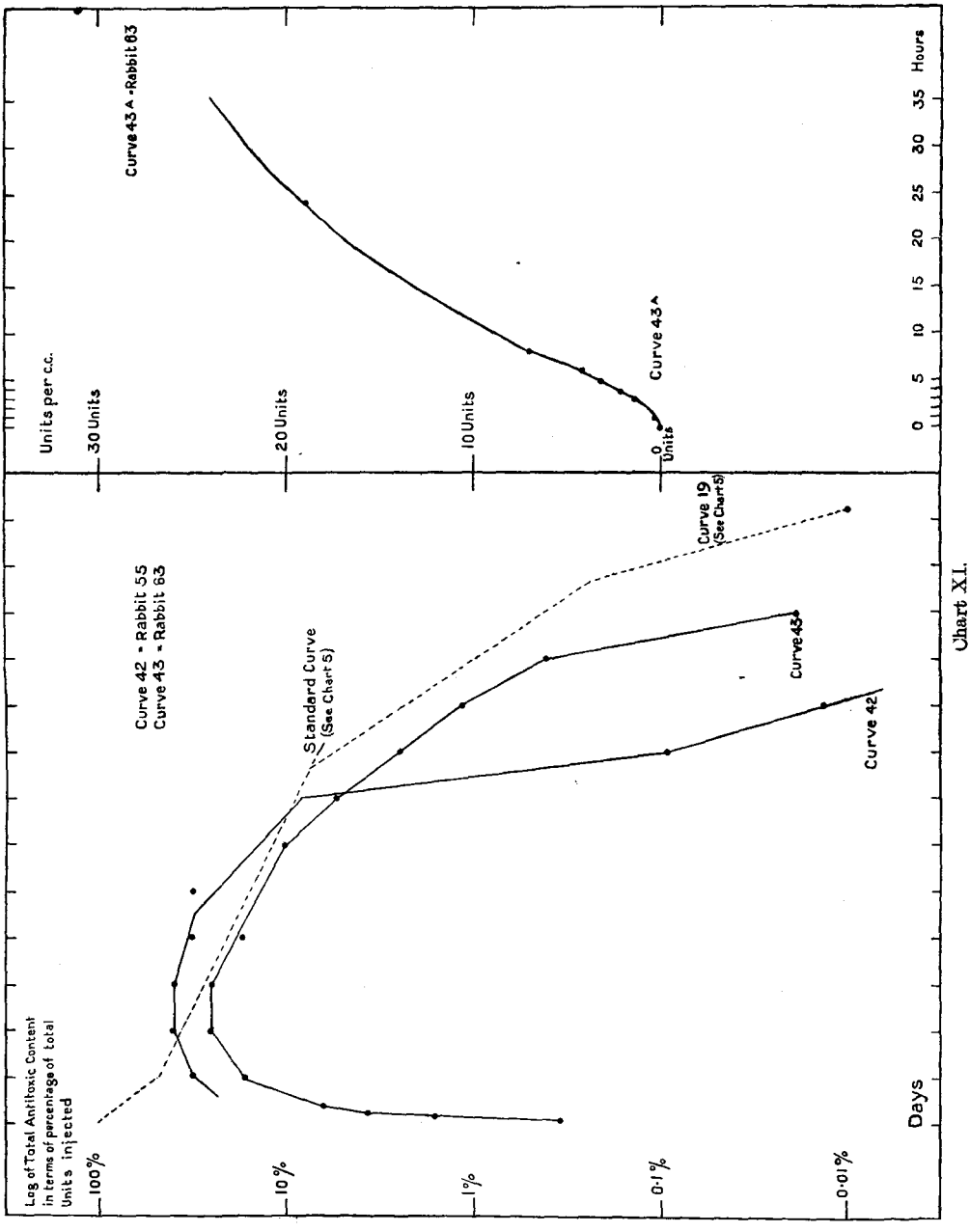

Chart XI.

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Table XIX.

Showing the number of days taken for the antitoxic content of all normal rabbits recorded in preceding tables to fall below various stated fractions of the total amounts injected.

Rabbit	Table	Weight	1/10	1/100	1/1000	1/10,000	1/100,000
84	10	400	7	11	12	13–14	—
76	8	510	7	13–15	19	26	—
86	8	680	6–7	12	19	27–30	—
79	8	740	6–7	9	10	12	—
107	16	880	6	7	9–10	12	—
94	10	910	6	6	7	9	—
108	16	920	6	9–10	12	14	17
106	16	990	5	9-10	9–10	—	—
93	8	1020	6	8–9	12	16	—
75	12	1190	6–7	9	10	11–12	—
73	14	1390	5	7–8	9	10	—
71	12	1470	6	10	12	—	—
72	12	1590	6	9	12	*c.* 14	—
105	14	1620	6	10	12–13	17	28–29
103	14	1640	7	10	11	12–13	17
41	1	1810	7	11	12–13	14–16	—
50	1	1870	8	10	—	—	—
49	1	1930	7	10	12	12	—
104	14	1930	7	10	12–13	16	29
42	5	2040	8–9	11	13	21–23	—
61	5	2040	5	7	8	8	—
74	12	2380	6–7	8–9	9	10	12
110	10	2810	5	6	6	7	—
109	10	2840	6	6	7	8	—
83	10	3240	6–7	6–7	7	8	—

each rabbit is marked the number of days taken for the antitoxic content to fall below one-tenth, one-hundredth, one-thousandth, etc. of the original content. It will be seen that 25 normal rabbits injected with antitoxic horse serum intravenously lost 90 per cent. of the total antitoxin in from 5 to 9 days, while 19 of the 25 rabbits lost this amount by the sixth or seventh day; no marked connection can be seen between variations from the average and the weight of the animal. The next column of the table shows that various rabbits have taken from 6 to 13 or 15 days to lose 99 per cent. of the antitoxin injected. The majority of the rabbits have taken from 10 to 11 days to lose this quantity. The only rabbits to take more than 11 days are quite small, although four out of eight of those weighing less than one kilo. took less than ten days. The times taken to lose 99·9 per cent. of the antitoxin injected, *i.e.* for the antitoxic content to fall below one-thousandth of the original value give more consistent results in accordance with weight or age. The average time taken is from 10 to 13 days; of the rabbits weighing less than one kilo. four took 10 days or less, two took 12 days and two 19 days; of the rabbits weighing between one and two kilos. one took under 10 days, nine took from 10 to 13 days, and none longer; five out of six of those weighing over two kilos. took less than 10 days. The time taken to fall to one ten-thousandth of the original value was more varied; the average time would appear to be about 12 to 16 days but this time varied from seven days in a heavy rabbit to between 27 to 30 days in a light rabbit. The rabbit giving the most abnormal results was rabbit 94;

although under one kilo. at the time of injection and only seven weeks old it responded so markedly to horse serum that all detectable antitoxin was lost in nine days.

There is already sufficient evidence to show that older rabbits tend to exhibit an increased capacity for response to an injection of horse serum, which may be regarded as an acquired active immunity. It would be of interest to determine how this active immunity can be acquired.

It is possible that active immunity may have been acquired by feeding, but if immunity may be acquired with such ease then it would appear possible for rabbits to acquire an active immunity before birth or to have absorbed the antigen. Tables XX and XXI and curves 44 to 46 on Chart XII record the results obtained from three rabbits that may have some bearing on this point. Rabbits 80 and 92 were both born of the same litter from a mother highly immune to horse serum and injected several times during pregnancy. Rabbit 80 injected with horse serum when only five weeks old eliminated serum more rapidly than was usual for young rabbits, but rabbit 92 injected when ten weeks old eliminated even more rapidly. If the rapid elimination of antitoxic horse serum was due to passive immunity inherited from the mother, the loss due to Phase A would have been greatly increased by elimination due to circulating precipitin and the older rabbit should have appeared less immune.

It is possible that rabbit 80 possessed circulating precipitin at the time of injection; a first day loss of 55·5 per cent. recorded in Table XXI was succeeded by a second day loss of 54·2 per cent., but as reference to Table XX shows

Table XX.

Showing the antitoxic content of three rabbits at different intervals of time after the intravenous injection of 750 *units of diphtheria antitoxin contained in* 0·5 *c.c. of horse serum.*

Rabbit	80	92	85
Weight	510	1110	2380
Age	5 wks.	10 wks.	—
Previous history	Rabbits of the same litter born from a mother highly immune to horse serum		Fed with horse serum 3 weeks previously
Time interval			
15 minutes	27·0	15·0	8·0
1 day	12·0	7·5	4·0
2 days	5·5	5·25	—
3 ,,	—	—	2·5
4 ,,	5·5	3·25	2·25
5 ,,	3·0	1·25	0·7
6 ,,	—	0·11	0·02
7 ,,	0·22	0·015	<0·001
8 ,,	0·14	0·002	—
9 ,,	0·08	0·001	—
10 ,,	0·018	—	—
11 ,,	0·008	—	—
12 ,,	<0·0025	<0·0005	—
13 ,,	—	—	—
14 ,,	0·0014	—	—
15 ,,	<0·0005	—	—

Table XXI.

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

Time interval	Rabbit 80		Rabbit 92		Rabbit 85	
0–1 day	55·5	Phase A	50·0	Phase A	50·0	Phase A
1–2 days	(54·2)?		30·0	Phase B	—	Phase B
2–3 ,,	—	Phase B	—	Average	21·0	Average
3–4 ,,	(22·9)	Av. 22·9	21·3	25·6	10·0	15·5
4–5 ,,	45·4		61·5		68·9	Phase C
5–6 ,,	—		91·2	Phase C	97·1	Av. 83·0
6–7 ,,	72·9		86·4	Average		
7–8 ,,	36·4		86·7	75·1		
8–9 ,,	42·9	Phase C	50·0			
9–10 ,,	77·5	Average				
10–11 ,,	55·5	53·0				
11–12 ,,	68·7					
12–13 ,,	—					
13–14 ,,	25·2					

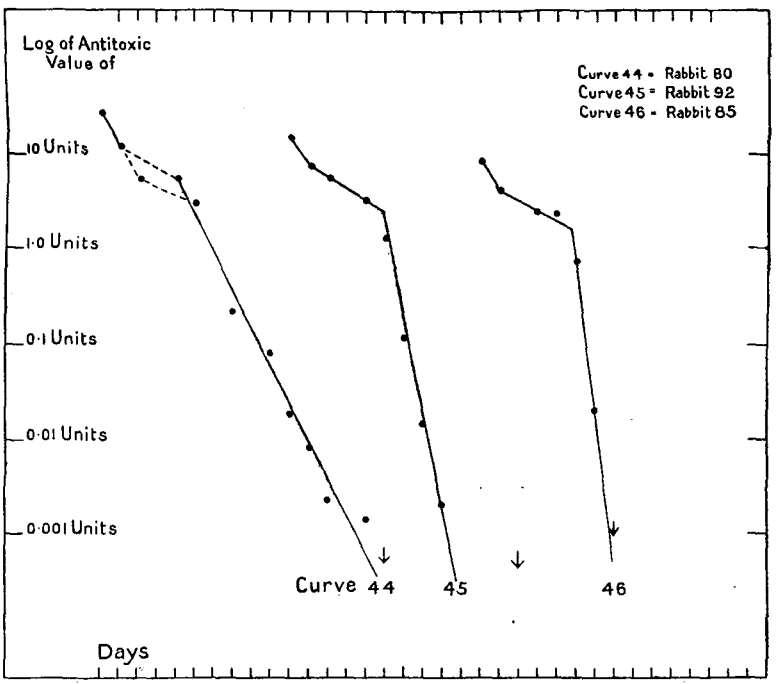

Chart XII.

that the antitoxic values recorded both for the second and for the fourth day was 5·5 units per c.c., it is obvious that one value recorded was an incorrect reading. If the second day reading is correct, then Phase A augmented by elimination due to circulating precipitin extended over two days, Phase B ends on the fifth day and the average rate of loss calculated from the readings of 5·5 units on the second day and 3·0 on the fifth day was 26·2 per cent. On the other hand if the fourth day reading is correct then passive immunity

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inherited from the mother was already lost when the rabbit was injected at the age of five weeks, and Phase B ended on the fourth day and showed an average loss of 22·9 per cent. calculated from the first day reading of 12·0 unit and the fourth day reading of 5·5 unit.

Table XXII shows the number of days taken for the total antitoxin content of these rabbits to fall below one-tenth, one-hundredth, one-thousandth, and one-ten-thousandth of the total amount injected. This table read in conjunction with Table XIX compiled in a similar manner for normal rabbits, emphasises the fact that rabbit 80 eliminated antitoxin more quickly than the majority of rabbits weighing less than 1 kilo., while rabbit 92 (weighing only 1100 grams) exhibited a greater rate of loss than any of the eleven normal rabbits weighing between one and two kilos.

Table XXII.

Showing the number of days taken for the antitoxic content of the rabbits recorded in Table XX to fall below various stated fractions of the total amount injected.

Rabbit	Weight	1/10	1/100	1/1000	1/10,000
80	510	6–7	6–7	10	12
92	1110	5	6	7	9
85	2380	5	6	7	7

Rabbit 85 had been fed with horse serum three weeks before the injection and showed a high degree of immunity. This may indicate the possibility that active immunisation to horse serum readily arises from feeding or simply that older rabbits may, for some reason not understood, be highly immune.

That some degree of sensitiveness (*i.e.* immunity) to horse serum may be acquired by rabbits in some accidental way may not appear an unreasonable hypothesis when we consider the remarkable instances of sensitiveness of different human beings to different proteins.

Summary of Part II.

1. Normal rabbits injected intravenously with diphtheria antitoxin obtained from a horse vary considerably both in regard to the duration of Phase B and the duration and the intensity of Phase C.

2. Phase C, the phase of accelerated loss due to formation of precipitin appears sooner and is more pronounced in older rabbits.

3. The early appearance and more rapid formation of antibody (precipitin) in certain rabbits is indicative of naturally acquired immunity.

4. The highest concentration in the blood of rabbits injected subcutaneously with antitoxic horse serum is seen after 2 to 3 days, when it reaches approximately the same value as that of rabbits injected intravenously, and represents only one-third of the total antitoxin injected. Two hours after subcutaneous injection the antitoxic content is only one two-hundredth of that of a rabbit injected intravenously.