DURATION OF PASSIVE IMMUNITY.

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PART III.

(With Charts XIII–XVI.)

Parts I and II of this paper have dealt with the three phases in the course of elimination of passive immunity in rabbits injected with diphtheria antitoxin obtained from a horse. The present section deals with other normal animals injected with horse serum, normal rabbits injected with serum from animals other than the horse, and with animals injected with homologous antitoxin.

A number of normal guinea-pigs were injected subcutaneously with 5 c.c. of horse serum containing 2500 units of diphtheria antitoxin, and blood was withdrawn from the heart on successive days after the injection. The results of antitoxin titration of the samples of serum so obtained are given in Table XXIII. Because each value recorded represents a different guinea-pig, there

TABLE XXIII.

Showing the antitoxic value of the serum of a number of guinea-pigs bled at different intervals of time after the subcutaneous injection of 5 *c.c. unconcentrated horse serum containing* 2500 *units of diphtheria antitoxin.*

Guinea-pig	Days after injection	Weight in grams	Units of antitoxin per c.c.
A	1	280	30
B	2	355	25
C	2	300	35
D	3	260	40
F	5	300	25
H	6	280	12
K	7	325	7
L	8	280	7
N	9	310	4
O	10	260	0·3
P	13	285	0·03
R	14	250	0·03
S	15	300	0·005
T	16	305	0·02
W	22	285	<0·001

is considerable variation among the values obtained, but the figures are sufficient to show that the course of elimination of antitoxic horse serum from the guinea-pig closely resembles that of similar serum from the rabbit. A guinea-pig tested 22 days after injection, contained in its circulation less than 1/100,000 of the total antitoxin injected. On comparing the general trend of

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the figures in Table XXIII with those in Table XVIII and Chart XI in Part II it will be seen that after subcutaneous injection of both rabbits and guinea-pigs with antitoxic horse serum the maximum concentration is obtained in the blood two or three days after the injection, absorption appears complete by the third or fourth day and the curve of loss then follows the normal course of Phase B. Precipitin formation represented by Phase C commences slightly later in guinea-pigs; guinea-pig *O* on the tenth day contained only one-tenth of the antitoxin seen in guinea-pig *N* on the ninth day, showing that in guinea-pig *O* Phase C had commenced by the tenth day. This result is in line with the generally accepted facts of anaphylaxis in guinea-pigs. One guinea-pig, *T*, showed an exceptionally high antitoxic value on the sixteenth day after injection.

TABLE XXIV.

Showing the antitoxic content of rabbits at different intervals of time after the intravenous injection of diphtheria antitoxin contained in goat serum.

Rabbit ...	88	118	45
Weight in grams	1700	1340	1760
Immunity ...	Normal	Normal	Horse sensitive
Volume injected	5·0 c.c.	5·0 c.c.	5·0 c.c.
Total units ...	125	125	50
Time interval	Antitoxic value in units per c.c.		
15 minutes	—	2·5	0·5
1 day	0·9	0·9	0·3
2 days	0·55	0·55	0·25
3 ,,	—	—	0·18
4 ,,	0·35	0·18	0·14
5 ,,	0·08	0·08	0·12
6 ,,	<0·0005	0·006	—
7 ,,	—	<0·001	0·015
8 ,,	—	—	0·001
9 ,,	—	—	<0·0005

TABLE XXV.

Showing the percentage daily loss in antitoxic value of the blood of rabbits at different intervals of time after the intravenous injection of diphtheria antitoxin contained in goat serum.

Time interval	Rabbit 88		Rabbit 118		Rabbit 45	
0–1 day	—	Phase A	64·0	Phase A	40·0	Phase A
1–2 days	38·9	Phase B	38·9	Phase B	16·7	Phase B
2–3 ,,	—	Average	—	Average	28·0	Average
3–4 ,,	20·2	29·5	42·8	40·8	22·2	20·3
4–5 ,,	77·1	Phase C	55·5	Phase C	14·3	
5–6 ,,	99·3	Average	92·5	Average	—	Phase C
6–7 ,,		88·2		74·0	64·6	Average
7–8 ,,	—		—		93·3	78·9

Three rabbits were injected intravenously with diphtheria antitoxin obtained from a goat. The results are recorded in Tables XXIV and XXV and Chart XIII. The outstanding features of each of the three curves 47, 48 and 49 is the early appearance of Phase C and the shortness of the period during which antitoxin was present. Rabbit 45, already sensitised to horse serum, did not

eliminate goat serum with the rapidity with which similarly sensitised rabbits eliminate horse serum, and showed less, rather than more, activity of response to goat serum than did the normal rabbits 88 and 118. The curve for rabbit 45 exhibits the typical three phases of elimination; the absence of a 15-minute reading for rabbit 88 and the early appearance of Phase C obscure the actual course of Phase B in Curve 47. Curve 48 is strongly suggestive of Curve 14 on Chart IV in Part II representing the course of elimination of horse serum from a rabbit injected only 11 days after a previous injection at a time when excess precipitin would still be present in the circulation. This suggestion of existence of circulating precipitin in one rabbit, and the early appearance and

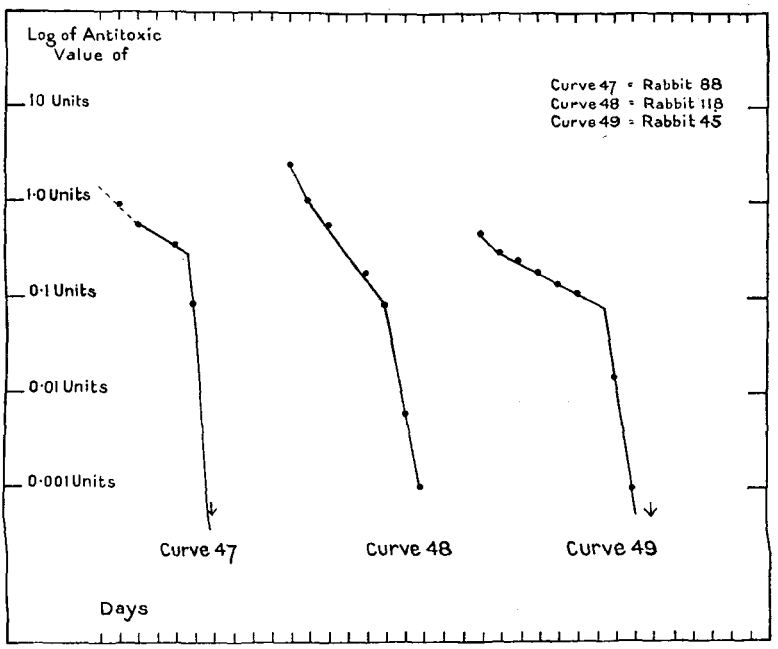

Chart XIII.

short duration of Phase C in all three rabbits shows that rabbits are more responsive to goat serum than to horse serum. Table XXVIII, showing the time taken for antitoxic values to fall below certain levels, also shows, when compared with Table XIX, that goat serum is eliminated more rapidly than horse serum. It must be pointed out that if immunity to animal protein is specifically acquired, the evidence suggests that the particular rabbits tested had more opportunity of acquiring immunity to goat serum than similar rabbits to horse serum. Unfortunately, no record was kept as to which of these rabbits had been acquired from outside sources; rabbits 45 and 88 were known to have been tended by assistants closely associated with the care of goats.

Tables XXVI and XXVII and Curves 50 to 55 on Chart XIV record the results of injecting rabbits intravenously with antitoxic serum obtained from men, guinea-pigs and cows. The rate of loss of antitoxin from these rabbits

and also from those recorded in Table XXIV are summarised in Table XXVIII. The antitoxin injected had been actively produced. Rabbit 91 is of special interest, the antitoxic content 15 minutes after injection was less than half the calculated value, and the loss during the next 24 hours was 80·6 per cent. These facts are strongly suggestive of the presence of circulating precipitin. The general results obtained indicate that the rabbits used in these experiments were particularly responsive to the serum for goat, guinea-pigs and men, less so to serum from horses and still less to serum from cows. These rabbits

TABLE XXVI.

Showing the antitoxic content of rabbits at different intervals of time after the intravenous injection of diphtheria antitoxin contained in cow, guinea-pig and human serum.

Rabbit	87	47	91	117	78	89
Weight	1560	1420	1110	1390	—	1190
Immunity	Normal	Horse sensitive	Normal	Normal	Normal	Normal
Serum injected	Cow	Cow	Human	Human	Guinea-pig	Guinea-pig
Volume in c.c.	5·0	5·0	5·0	5·0	10·0	5·0
Total units	750	750	22·5	10	200	100
Time interval	Antitoxic value in units per c.c.					
15 minutes	8·5	11·5	0·17	0·11	2·75	1·62
1 day	5·25	7·5	0·033	0·055	1·75	0·9
2 days	3·75	5·75	0·025	0·045	1·5	0·7
3 ,,	—	4·75	—	0·035	—	—
4 ,,	2·25	3·5	0·01 –0·04	0·03	0·9	0·55
5 ,,	0·9	2·75	0·01 –0·02	—	—	0·33
6 ,,	0·05	—	0·007	0·018	—	0·04
7 ,,	0·015	0·55	0·002–4	0·008	0·3	0·005
8 ,,	0·004	0·17	0·002	0·0045	—	<0·001
9 ,,	0·002	0·02	?0·0008	0·0015	—	—
10 ,,	—	0·01	—	0·0015	0·022	—
11 ,,	—	0·007	—	<0·001	Died	—
12 ,,	<0·0005	0·004	?0·0008	—	—	—
13 ,,	—	—	—	—	—	—
14 ,,	—	0·002	?<0·0005	—	—	—
15 ,,	—	0·002	—	—	—	—
16 ,,	—	0·0015	—	—	—	—
17 ,,	—	0·0015	—	—	—	—
18 ,,	—	0·0015	—	—	—	—
19 ,,	—	0·0015	—	—	—	—
20 ,,	—	—	—	—	—	—
21 ,,	—	0·0007	—	—	—	—
22 ,,	—	<0·0005	—	—	—	—

were in relatively close contact with goats, guinea-pigs and men, but not in direct contact with horses, while their connection with cows was even more remote. Considerably more work must be done before any positive proof can be obtained as to whether rabbits can acquire immunity to protein of other animals, but the facts at present known are sufficiently suggestive to warrant the mention of such a possibility.

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TABLE XXVII.

Showing the percentage daily loss in antitoxic value of the blood of rabbits at different intervals of time after the intravenous injection of diphtheria antitoxin contained in cow, human and guinea-pig serum.

Time interval in days	Rabbit 87		Rabbit 47		Rabbit 91		Rabbit 117		Rabbit 78		Rabbit 89	
0– 1	38·2	Phase A	34·8	Phase A	80·6	Phase A	50·0	Phase A	36·4	Phase A	44·4	Phase A
1– 2	28·5	Phase B	23·3		24·2		18·2		14·3		22·2	
2– 3	—	Average	17·4	Phase B	—	Phase B	22·2	Phase B	—	Phase B	—	Phase B
3– 4	22·5	25·5	26·3	Average	—	Average	14·3	Average	22·5	Average	11·4	Average
4– 5	60·0		21·4	22·1	—	25·7	—	19·3	—	22·5	40·0	24·5
5– 6	94·4	Phase C	—		27·2		22·5		—		87·8	Phase C
6– 7	70·0	Average	55·3		42·7	Phase C	55·5	Phase C	30·6		87·5	Average
7– 8	70·0	70·0	69·1	Phase C	—	Average	43·7	Average	—			87·6
8– 9	55·5		88·2	Average	51·5	c. 50	66·7	53·8	—	Phase C		—
9–10		—	50·0	55·9		—	(49·2)		58·2			—
10–11		—	30·0			—		—		—		—
11–12		—	42·8			—		—		—		—
12–13		—	—			—		—		—		—
13–14		—	29·3			—		—		—		—
14–15		—	(20·6)	Phase D		—		—		—		—
15–16		—	(25·0)	Average		—		—		—		—
16–17		—	(13·4)	17·4		—		—		—		—
17–18		—	(9·1)			—		—		—		—
18–19		—	(6·9)			—		—		—		—

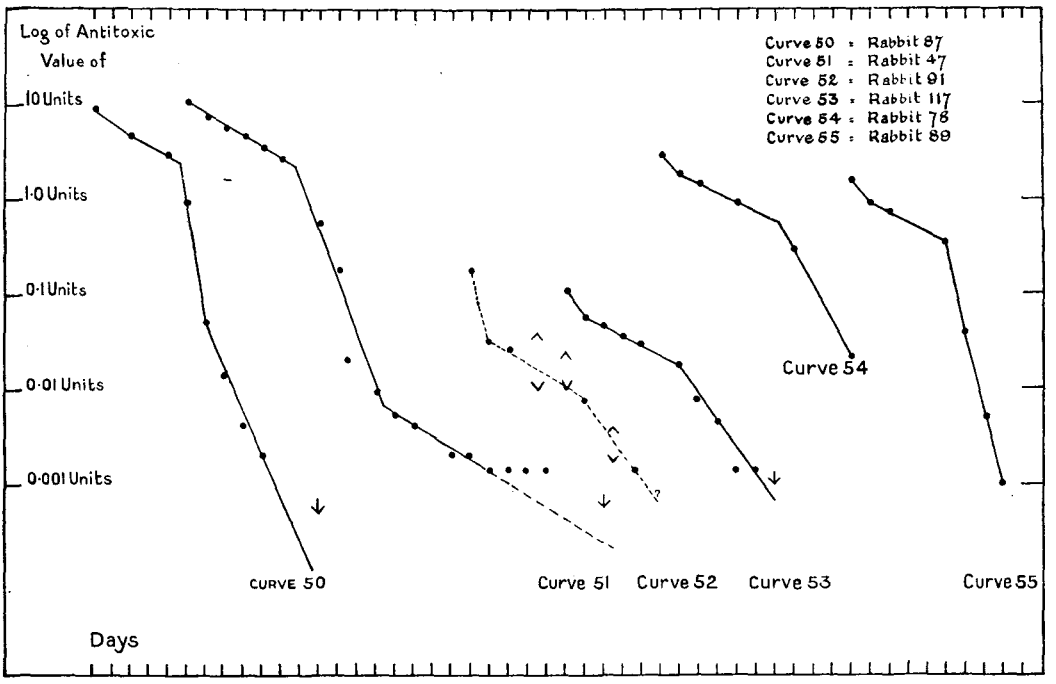

Chart XIV.

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TABLE XXVIII.

Showing the number of days taken for the antitoxin content of the rabbits recorded in Tables XXIV and XXVI to fall below various stated fractions of the total amount injected.

Rabbit	Serum injected	Weight	1/10	1/100	1/1000	1/10,000
88	Goat	1700	5	6	6	—
118	,,	1340	4	6	7	—
45	,,	1760	6–7	7	8	—
87	Cow	1560	6	6	8	10–12
47	,,	1420	6–7	9	10	20–21
91	Human	1110	6	9	—	—
117	,,	1390	7	12	—	—
78	Guinea-pig	?	8	9–10	—	—
89	,,	1190	6	7	8	—

The rate of loss of passive immunity produced by injection of homologous antitoxin may now be considered. A rabbit was injected intravenously with antitoxin actively produced in another rabbit and three horses were injected with antitoxin contained in horse serum freed from euglobulin and from albumen. Details relating to these animals are given in Tables XXIX and XXX and Curves 56 to 59 in Chart XV. Two curves are given for rabbit 77; in order to show in detail the course of antitoxic loss in such a form as to be comparable with preceding curves, Curve 56 A has been drawn on a larger scale (showing daily readings) than Curve 56 showing weekly readings to compare with 57, 58 and 59. The table of percentage loss and curve 56 A show that the rate of loss is not quite uniform. Throughout the whole period except for the first reading, involving, as it does, Phase A, the average weekly loss of rabbit serum injected into a rabbit is 57·2 per cent., equivalent to a daily loss of 11·1 per cent. of each day's content. For the first 17 days a greater loss takes place, 69·2 per cent. per week representing 15·5 per cent. per day, or taking the actual readings of 0·14 of a unit on the 17th day and 1·87 of a unit on the first day and abstracting the 16th root we get the daily loss as 15·0 per cent. The reason for this change in rate of loss after 17 days is not apparent. The loss during the first 24 hours representing Phase A was 42·4 per cent.; the loss during the next 24 hours was 26·7 per cent. compared with 9·7 per cent. per day for the next two days and 14·5 per cent. for the following three days. The high figure for the second day loss is suggestive of an extended Phase A due to the relatively large volumes (10 c.c.) of serum injected.

The horses were tested at weekly intervals only. Curve 57 for horse 980 shows a marked drop representing Phase A and then a steady loss until the animal is again injected at the sixth week. This injection was followed again by a Phase A loss, and then a steady fall averaging 17·5 per cent. per week. Individual readings for weekly differences vary considerably from this average, but, with so many points determined upon a single curve, it was unnecessary to titrate accurately each sample taken. From this weekly loss and the calculated loss during the first seven days it would appear that Phase A consisted of approximately a loss of 50 per cent. The curve of loss of antitoxin in horse 981

TABLE XXIX.

Showing the antitoxic content of one normal rabbit and three normal horses at different intervals of time after the intravenous injection of diphtheria antitoxin contained in homologous serum.

		Horse 980	Horse 981	Horse 982
Animal	Rabbit 77	Purified globulin from horse serum		
Injection volume	Rabbit serum 10·0 c.c.	100 c.c.		
Units	145	100,000		
Time interval	Antitoxic value in units per c.c.			
15 minutes	3·25	4·0	3·25	3·25
1 day	1·87	—	—	—
2 days	1·37	—	—	—
4 ,,	1·12	—	—	—
7 ,,	0·7	1·75	2·5	2·25
1½ weeks	0·5	—	—	—
2 ,,	0·2	1·25	1·5	1·5
2½ ,,	0·14	—	—	—
3 ,,	0·11	1·25	1–1·5	1·25
3½ ,,	0·07	—	—	—
4 ,,	0·04	1·0	1–1·25	1·0
4½ ,,	0·35	—	—	—
5 ,,	0·02	0·8	·75–1·0	·8–1·0
5½ ,,	0·017	—	—	—
6 ,,	0·009	4·5*	·6	·6
6½ ,,	0·007	—	—	—
7 ,,	0·0045	2·5	·6	·5
7½ ,,	0·003	—	—	—
8 ,,	?0·0035	2·0	·4	·5
8½ ,,	?0·001	—	—	—
9 ,,	0·0015	1·75	·25	·4
9½ ,,	0·0015	—	—	—
10 ,,	0·0007	1·25	·15	·4
10½ ,,	0·0005	—	—	—
11 ,,	<0·0005	1·25	·16	·25
12 ,,	—	1·0	·142	·2
13 ,,	—	·8	·11	·16
14 ,,	—	·8	·125	·14
15 ,,	—	·6	·09	·16
16 ,,	—	·5	·067	·14
17 ,,	—	·4	·05	·125
18 ,,	—	·33	·04	·125
19 ,,	—	·25	·03	·11
20 ,,	—	·25	·025	·1
21 ,,	—	·2	·022	·07
22 ,,	—	·17	·018	·07
23 ,,	—	·14	·014	·07
24 ,,	—	·12	·011	·05
25 ,,	—	·1	·008	·04
26 ,,	—	·08	·007	·04
27 ,,	—	·06	·0045	·044
28 ,,	—	·045	·004	·035
29 ,,	—	·045	—	·025
30 ,,	—	·035	·002–4	·025
31 ,,	—	·025	·002	·025
32 ,,	—	·025	·001–2	·025
33 ,,	—	·018	·001–2	·025
34 ,,	—	—	·001–2	·02
35 ,,	—	·016	·001–2	·02
36 ,,	—	—	·001	·012
37 ,,	—	·01	·001	·025
38 ,,	—	—	·001	·033
39 ,,	—	·006	—	·033
40 ,,	—	·006	—	·025
41 ,,	—	·006	—	·033
42 ,,	—	·004	—	·025
43 ,,	—	·004	—	·02
44 ,,	—	·003	—	·025
45 ,,	—	·003	—	·067
46 ,,	—	·002	—	·067
47 ,,	—	·0015	—	·25
48 ,,	—	—	—	·33
49 ,,	—	·0015	—	1·25
50 ,,	—	—	—	1·5
51 ,,	—	·0015	—	1·25
52 ,,	—	—	—	—

* Reinjected, value before injection 0·8 unit.

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TABLE XXX.

Showing the percentage weekly loss in antitoxic value of the blood of one normal rabbit and two normal horses at different intervals of time, after the intravenous injection of diphtheria antitoxin contained in homologous serum.

Time interval	Rabbit 77	Horse 980	Horse 981
0 –1 week	78·5	56·2	23·1
½–1½ weeks	61·0		
1 –2 ,,	71·4	28·5	40·0
1½–2½ ,,	72·0		
2 –3 ,,	45·0	0·0	(0–33·3)
2½–3½ ,,	50·0		
3 –4 ,,	63·6	20·0	(8·7–18·4)
3½–4½ ,,	50·0		
4 –5 ,,	50·0	20·0	(12·6–20·6)
4½–5½ ,,	51·4		
5 –6 ,,	55·0	0·0	(20·0–40·0)
5½–6½ ,,	58·8		
6 –7 ,,	50·0	44·4	0·0
6½–7½ ,,	57·1		
7 –8 ,,	(22·2)	20·0	33·3
7½–8½ ,,	(66·7)		
8 –9 ,,	(57·1)	12·5	37·5
8½–9½ ,,	(+50·0)		
9 –10 ,,	53·3	28·6	0·0
9½–10½ ,,	66·7		
10 –11 ,,	—	0·0	36·0
11 –12 ,,	—	20·0	11·3
12 –13 ,,	—	20·0	(22·5)
13 –14 ,,	—	0·0	(+12·7)
14 –15 ,,	—	25·0	25·5
15 –16 ,,	—	16·7	25·3
16 –17 ,,	—	20·0	20·0
17 –18 ,,	—	17·5	25·0
18 –19 ,,	—	24·2	16·7
19 –20 ,,	—	0·0	12·0
20 –21 ,,	—	20·0	18·2
21 –22 ,,	—	15·0	22·2
22 –23 ,,	—	17·6	21·4
23 –24 ,,	—	14·3	27·3
24 –25 ,,	—	16·7	12·5
25 –26 ,,	—	20·0	35·7
26 –27 ,,	—	25·0	11·1
27 –28 ,,	—	25·0	—
28 –29 ,,	—	0·0	—
29 –30 ,,	—	22·2	(20·5)
30 –31 ,,	—	28·5	—
31 –32 ,,	—	0·0	—
32 –33 ,,	—	28·0	—
33 –34 ,,	—	—	—
34 –35 ,,	—	(5·7)	—
35 –36 ,,	—	—	—
36 –37 ,,	—	(21·0)	—
37 –38 ,,	—	—	—
38 –39 ,,	—	(22·5)	—
39 –40 ,,	—	0·0	—
40 –41 ,,	—	0·0	—
41 –42 ,,	—	33·3	—
42 –43 ,,	—	0·0	—
43 –44 ,,	—	25·0	—
44 –45 ,,	—	0·0	—
45 –46 ,,	—	33·3	—
46 –47 ,,	—	25·0	—
	Average 56·5 %	Average 17·5 %	Average 21·5 %

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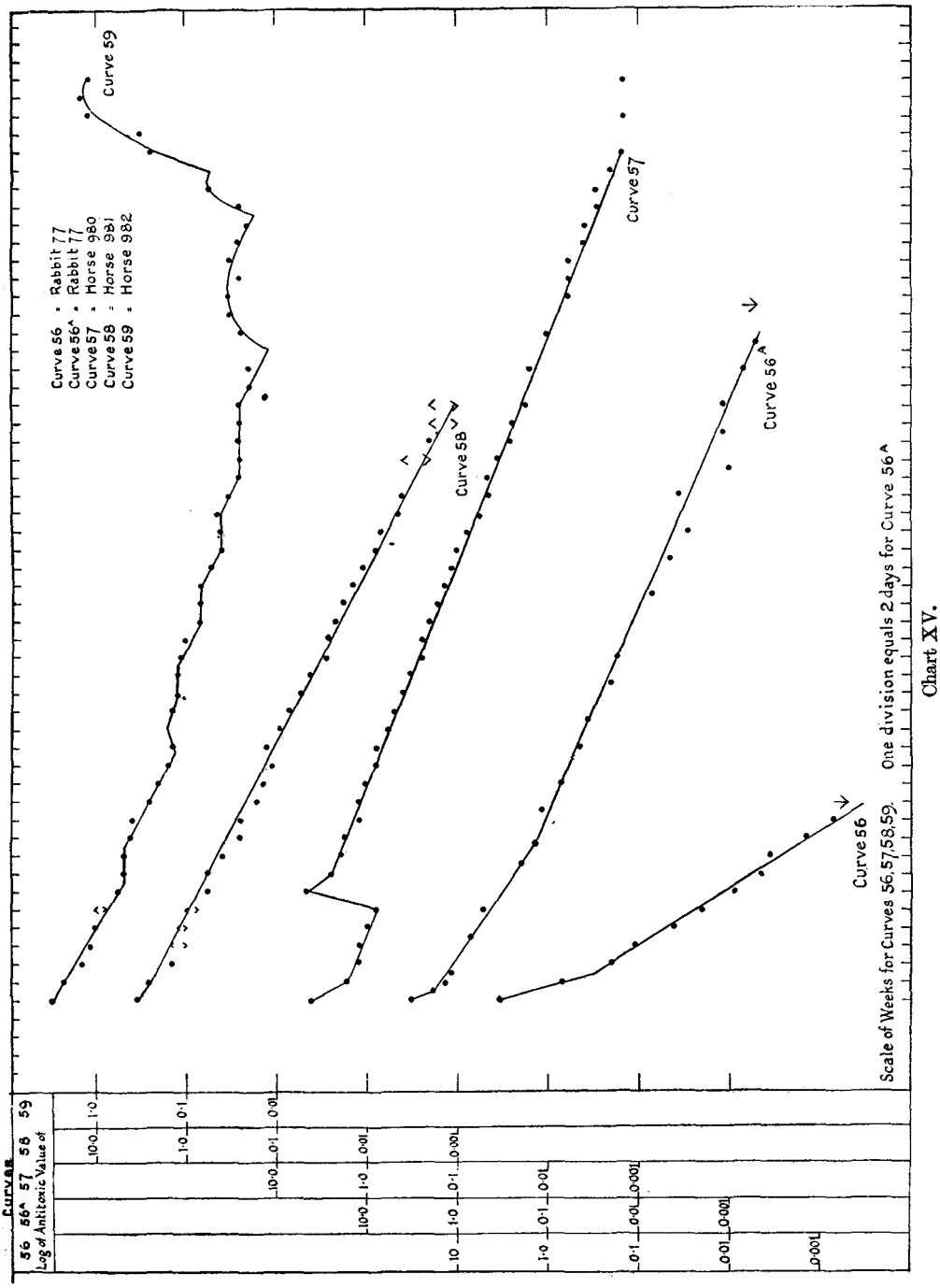

Chart XV.

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again shows a straight line when the log of antitoxic content is plotted against time; this curve of loss is slightly steeper than that for horse 980, and represents a weekly loss of 21·5 per cent. Phase A is not evident in horse 981, but the high percentage loss from the first to the second week would indicate that the reading for the antitoxic content of the sample of blood, taken seven days after the injection, was too high. All antitoxin injected had disappeared about 35 weeks after injection. Horse 982, however, still showed a high antitoxic content over 40 weeks after injection and little or no loss occurred from the 29th to the 40th week and then a marked increase in antitoxic values was seen. The various readings of the antitoxic content of the weekly samples are very irregular and do not at first sight fall upon a recognisable curve, for the logs of the antitoxic content plotted against time do not fall upon a straight line. It is, however, possible to draw, as we have done in Curve 59, a series of short sections parallel to Curve 58; these sections are linked with lines roughly parallel to the base line or showing a tendency to rise. Each such interrupted section represents an active immunity response. At the time of the injection of diphtheria antitoxin this horse already possessed a normal antitoxic value of 0·008 of a unit per c.c.; the other two horses, 980 and 981, both contained less than 0·0005 of a unit. Horse 982, already actively immune, could respond to any accidental stimulus by a rapid production of antitoxin, thus showing an irregular curve made up of constantly diminishing passive immunity and frequently varying active immunity. This horse affords a good illustration of the course of production of active immunity naturally acquired by an animal. At first the responses are small, and the rate of loss of apparent immunity is delayed only two weeks, but later the responses increase until eventually an increase in antitoxic content of one unit may occur within a week.

An examination of Curve 58, representing horse 981, also shows a marked tendency for a series of observed values to lie on one side of the straight line representing the average rate of loss of antitoxin. It is possible that these variations are of significance; it would be possible to draw a series of interrupted sections, *e.g.* 0–6, 7–13, 14–19 weeks, with small increases of active immunity between the sections.

The apparent delay between the 32nd and the 38th week before the antitoxic content fell below one-thousandth of a unit per c.c. may have been due to some small residual actively produced antitoxin; on the other hand, it must be stated that at the time these tests were made we were encountering some little difficulty in detecting small amounts of antitoxin. This detection of very small amounts of antitoxin depends upon the potency of the test toxin used and upon the relatively high content of toxin compared with toxoid. The minimal reacting dose (M.R.D.) of our test toxin at the commencement of our experiments was sufficiently small to enable one two-thousandth of antitoxin per c.c. to be detected with ease. Gradual deterioration of toxin into toxoid increased the M.R.D. in relation to L_R so that a reaction no longer appeared when tests were made at the L_R/20,000 level (see Glenny

and Allen, 1921, and Hartley and Hartley, 1922). In later experiments no attempt was made to titrate for less than one-thousandth unit per c.c. with this toxin, and for a short time, until another toxin was available, it was found difficult to ascertain definitely whether one-thousandth unit was present or not.

The majority of apparently normal horses possess some diphtheria antitoxin, in their blood, probably actively produced as a result of a succession of naturally acquired stimuli. The immunity responses to these stimuli gradually increase as may well be seen in the case of horse 982. It is possible, therefore, to regard horse 981 (and possibly horse 980) as in an earlier stage of actively acquired immunity to diphtheria, showing only very small responses to minute accidental stimuli. If this is so then the rate of loss of homologous serum in a horse must be represented by a line slightly steeper than Curve 58. If, however, the slight variations in the titration of values for horses 980 and 981 are of no significance and the apparent delay in reaching our zero line is due to difficulty in testing for small amounts of antitoxin, then Curves 57 and 58 must be taken as representing the true rate of loss of homologous serum in the horse. The slope of Curve 56 shows how much faster homologous serum may be eliminated from a rabbit than from horses.

TABLE XXXI.

Showing antitoxic value at different ages of three guinea-pigs of the same litter passively immune by maternal transmission.

Guinea-pig	Age	Antitoxic value in units per c.c.	Weight in grams	Weight × unit value
C	At birth	2·6	100	260
F	,,	2·6	100	260
C	21 days old	0·46	270	124
C	24 ,,	0·44	300	132
D	24 ,,	0·54	255	138
C	43 ,,	0·11	440	48
D	43 ,,	0·12	360	43
F	43 ,,	0·12	375	45
C	61 ,,	0·04	530	21
F	68 ,,	<0·04	475	< 18

The remaining experiments recorded in this part were performed by one of us (A. T. G.) in conjunction with our colleague Dr H. J. Sudmersen in 1912 and 1913 and hitherto unpublished. Table XXXI shows the rate of loss of homologous serum in guinea-pigs. A litter of three guinea-pigs borne by an actively immune mother were bled within a few hours of birth, and their blood was found to be of the same antitoxic strength as that of their mother. One or other of the young was bled at different ages, until, when ten weeks old, no antitoxin could be detected. Antitoxic values were not titrated by the intradermic method and the least detectable strength depended upon the amount of blood available; with small quantities of blood obtainable from guinea-pigs it was not possible to detect less than one twenty-fifth unit per c.c.; with larger quantities obtainable from larger animals one-hundredth unit could be titrated. With growing guinea-pigs the antitoxic content is continually

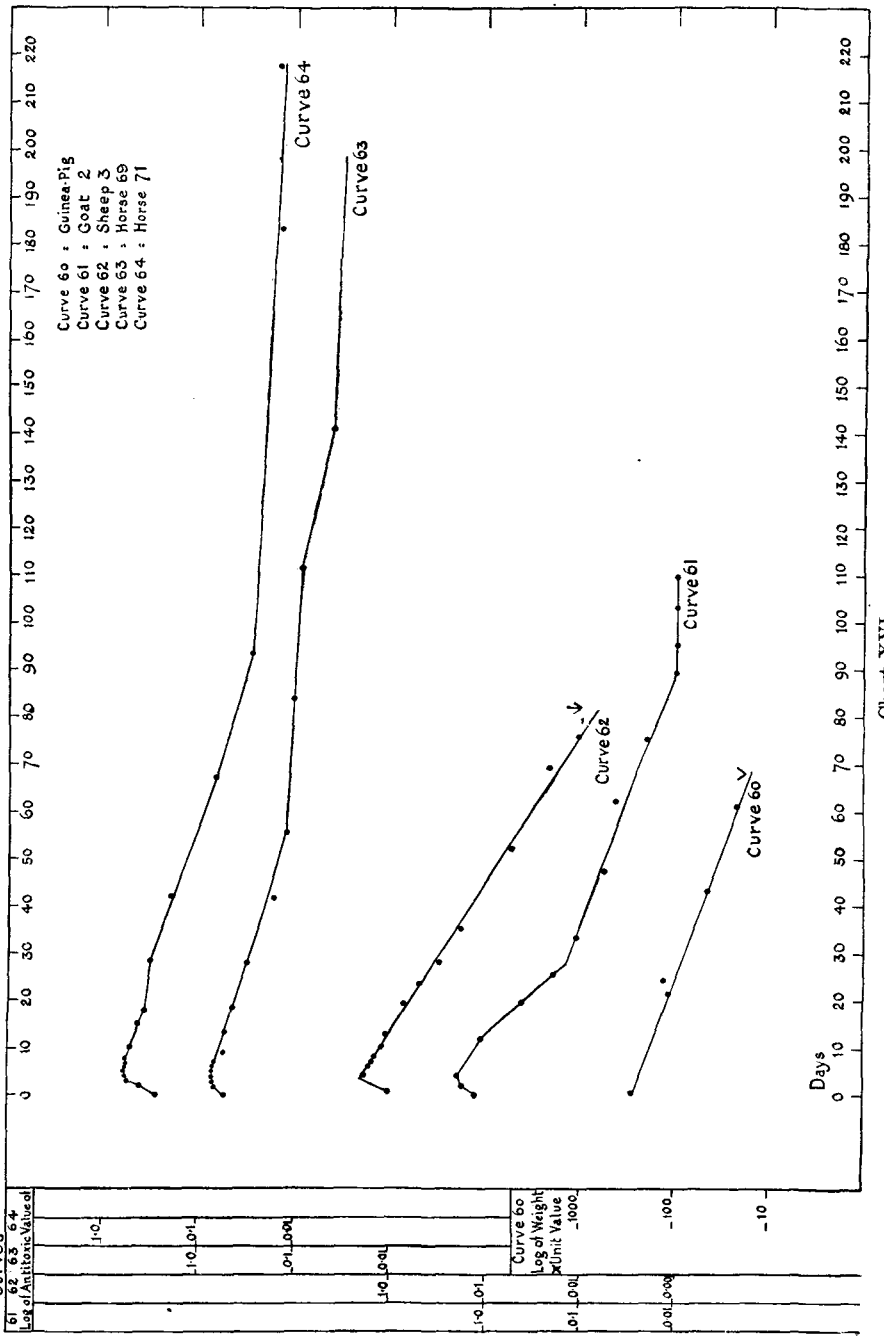

Chart XVI.

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diluted because of the increasing blood volumes, Curve 60 on Chart XVI has therefore been constructed by plotting the log of "Weight × unit value" (*i.e.* some fixed multiple of the total antitoxic content) against time. From this curve the rate of weekly loss of homologous antitoxin in guinea-pigs has been calculated as 25 per cent., *i.e.* of the same order as the rate of loss of homologous antitoxin in horses.

TABLE XXXII.

Showing the antitoxic value of a goat, a sheep and two horses at different intervals of time after the subcutaneous injection of unconcentrated horse serum containing diphtheria antitoxin.

Animal	Goat 2	Sheep 3	Horse 69	Horse 71
Injection volume	10 c.c.	10 c.c.	50 c.c.	50 c.c.
Total units	5500	7000	26,500	26,000

Antitoxic value in units per c.c. at different intervals

Interval	Unit value	Interval	Unit value	Interval	Unit value	Interval	Unit value
Before injection	0·00	Before injection	0	Before injection	0·03	Before injection	0·015
4½ hrs.	0·20	2 hrs.	0·07	12 hrs.	0·45	3 hrs.	0·04
6½ ,,	0·33	4 ,,	0·15	24 ,,	0·55	6 ,,	0·06
24 ,,	1·3	6 ,,	0·22	36 ,,	0·62	9 ,,	0·09
2 days	1·8	1 day	1·0	2 days	0·67	12 ,,	0·14
4 ,,	1·95	2 days	1·6	3 ,,	0·70	24 ,,	0·28
6 ,,	1·75	3 ,,	2·0	4 ,,	0·70	36 ,,	0·35
12 ,,	1·05	4 ,,	1·8	5 ,,	0·70	2 days	0·42
19 ,,	0·40	6 ,,	1·6	6 ,,	0·70	3 ,,	0·55
26 ,,	0·18	7 ,,	1·5	7 ,,	0·62	4 ,,	0·57
33 ,,	0·12	8 ,,	1·45	9 ,,	0·57	5 ,,	0·60
47 ,,	0·07	10 ,,	1·25	13 ,,	0·52	6 ,,	0·57
62 ,,	0·04	13 ,,	1·05	18 ,,	0·45	7 ,,	0·55
75 ,,	0·02	19 ,,	0·70	20 ,,	0·42	8 ,,	0·55
89 ,,	0·01	23 ,,	0·50	28 ,,	0·32	10 ,,	0·52
103 ,,	0·01	28 ,,	0·30	42 ,,	0·18	15 ,,	0·42
110 ,,	0·01	35 ,,	0·17	56 ,,	0·13	18 ,,	0·37
117 ,,	0·00	52 ,,	0·05	84 ,,	0·10	28 ,,	0·32
—	—	69 ,,	0·02	112 ,,	0·09	42 ,,	0·20
—	—	75 ,,	0·01	141 ,,	0·04	67 ,,	0·07
—	—	82 ,,	<0·01	201 ,,	0·033	94 ,,	0·03
—	—	—	—	278 ,,	0·030	183 ,,	0·015
—	—	—	—	—	—	217 ,,	0·015

Table XXXII records the antitoxic values of a goat, a sheep and two horses after the subcutaneous injection of horse serum containing diphtheria antitoxin. Samples of blood were withdrawn at somewhat irregular intervals. Table XXXIII has been compiled therefore to show the weekly rate of loss during successive intervals between the withdrawal of samples of blood. Curves for the antitoxic content of these animals and of the guinea-pigs recorded in Table XXXI are shown on Chart XVI (Curves 60 to 64). The two horses recorded both possessed a small amount of normal antitoxin and the curves recorded are therefore complicated by actively acquired immunity. Curve 63 consists of a continuous straight line from the first to the eighth week, with an average rate of loss of 20·2 per cent. per week; some slight natural stimulus intervened and for the next eight weeks the indicated loss, due to the differences between passive immunity lost, and active immunity gained, is extremely

small. From the 16th to the 20th weeks, the rate of loss of passive immunity is only slightly reduced and shows a weekly rate of 18·2 per cent. Curve 64 shows a fairly uniform line from the 8th to the 94th day with an average rate of loss of 20·7 per cent. per week; the list of weekly percentage loss recorded in Table XXXIII shows one discrepant figure, that of 9·7 per cent. for the period between the 18th and 28th days. If this is regarded as due to active immunity the observed points lie on two parallel lines showing a weekly loss of 22·5 per cent.

TABLE XXXIII.

Showing the percentage weekly loss in antitoxic value of the blood of a goat, a sheep and two horses at different intervals of time after the subcutaneous injection of unconcentrated horse serum containing diphtheria antitoxin.

Goat			Sheep			Horse 69			Horse 71		
Interval in days	Percentage weekly loss		Interval in days	Percentage weekly loss		Interval in days	Percentage weekly loss		Interval in days	Percentage weekly loss	
6– 12	44·8	Average 59·3	6– 7	36·3	Average 33·8	7– 9	25·5	Average 20·2	8–10	17·8	Average 23·0
12– 19	61·9		7– 8	21·1		9– 13	14·8		10–15	25·8	
19– 26	55·0		8–10	40·5		13– 18	18·3		15–18	25·6	
26– 33	33·3	Average 28·0	10–13	33·4		18– 20	21·5		18–28	9·7	
33– 47	23·6		13–19	37·7		20– 28	21·2		28–42	20·9	Average 22·0
47– 62	23·0		19–23	44·5	Average 46·3	28– 42	25·0		42–67	25·5	
62– 75	31·1		23–28	51·1		42– 56	15·0		67–94	19·7	
75– 89	29·1		28–35	43·3		56– 84	6·4	Average 9·1	—		—
89–103	—	—	35–52	39·6	Average 36·8	84–112	2·6		—		—
		—	52–69	31·4		112–140	18·2		—		—
		—	52–75	38·7		—	—	—	—		—

Antitoxic horse serum injected into a goat was detected with certainty 75 days after subcutaneous injection. Readings were not taken with sufficient frequency to determine the exact duration of phases, but Curve 61 on Chart XVI and the rates of loss given in Table XXXIII show that it is probable that Phase C started between the 6th and 12th day and ended between the 26th and 33rd day. The average weekly rate of loss between the 6th and 26th day was 53·9 per cent.; from the 26th to the 89th day the loss was fairly uniform and averaged 28·0 per cent. Antitoxic horse serum was also detected for 75 days after subcutaneous injection into a sheep, but Phase C was not very evident. The rate of loss of antitoxin appeared slightly greater between the 19th and 35th days, but the difference was not very marked. The long duration of horse serum in goats and sheep is of interest when compared with the shorter duration of rabbit serum in the one rabbit tested.

Table XXXIV has been compiled to show the time taken for the antitoxic values of the animals recorded in Tables XXIX, XXXI and XXXII to fall below one-tenth, one-hundredth and one-thousandth of the amount injected in the animals injected intravenously and of the highest recorded content in the animals injected subcutaneously. The figures show that 10 per cent. of homologous antitoxin remained in all four horses and three guinea-pigs until from 8 to 12 weeks after injection. Homologous serum injected into a

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rabbit had fallen below 10 per cent. in two weeks, but 10 per cent. of horse serum remained in both a goat and a sheep for three weeks.

TABLE XXXIV.

Showing the number of weeks taken for the antitoxic content of the animals recorded in Tables XXIX, XXXI and XXXII to fall below various stated fractions of the total amount injected.

Animal	Serum injected	1/10	1/100	1/1000
Rabbit 77	Rabbit	1½–2	5	8
Horse 980	Horse	11	24	38
,, 981	,,	9	20	30
,, 69	,,	8 –12	—	—
,, 71	,,	9	—	—
Guinea-pig *C, D, F*	Guinea-pig	8	—	—
Goat 2	Horse	3	10	—
Sheep 3	Horse	3	9	—

SUMMARY.

1. The course of disappearance of passive immunity in rabbits injected with diphtheria antitoxin obtained from goats, men, guinea-pigs and cows, consists of the same three phases that follow the injection of horse serum.

2. The rabbits examined were more responsive to goat, human and guinea-pig serum than to horse and cow serum.

3. The course of disappearance of passive immunity in rabbits, horses and guinea-pigs injected with homologous antitoxin, consists of Phases A and B only, and Phase B is far slower than when heterologous serum is injected into rabbits.

4. Sheep and goats eliminate antitoxin obtained from a horse at a very slow rate, and Phase C is hardly detectable.

5. Natural immunity of horses to diphtheria toxin is gradually acquired by a number of increasing responses to external stimuli.

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