

IMMUNITY OF GUINEA-PIGS TO DIPHTHERIA TOXIN
AND ITS EFFECT UPON THE OFFSPRING.

PART 2.

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C. *Immunity transmitted to young when both parents have been
injected with various mixtures of toxin and antitoxin.*

IN Part 1 results of the injection of the mothers only have been recorded: we shall now deal with the young of parents which have both received an injection of a toxin-antitoxin mixture. When this work was commenced a limited number of injected pigs were isolated for breeding and their number added to from time to time during the course of these investigations. A complete history was kept of each individual and full notes were taken of all events connected with them from the time of the injection of the parents until the testing of the young of several litters. The number of pigs that could be thus kept apart and individually watched was of necessity strictly limited. In order to obtain easily statistical data that would give indications for subsequent work we kept all survivals from routine toxin-antitoxin injections in runs for breeding without any attempt to isolate individual pigs. The only separation was into two groups—Cage 79 for all pigs that had received injections near the L+ doses, *i.e.* those that had given large local reactions, and Cage 80 for those near the L0 doses, giving little or no reactions. In these runs were put all pigs surviving, whether bucks or does. The general scale of immunity among the young of these pigs was no higher than that among the young from normal bucks and injected does.

This agrees with the generally recognised fact that there is no transmission of immunity through the male.

Figures from some 160 young were obtained from these two groups and results are given here as a confirmation of certain results in Part I.

Higher Immunity conferred by L+ than by L0 doses.

Higher immunity was obtained in the young from Cage 79 than in those from Cage 80, thus confirming results recorded in Tables I, II, III of Part I. The figures are given in Tables XVIII, XIX and XX.

TABLE XVIII.

Results of immunity of young divided according to the nature of the toxin-antitoxin mixture injected into the parents.

		Toxin-antitoxin mixture injected into parents	
		L0	L+
Deaths	Under 0.008 c.c.	11	2
	0.008 c.c. and under 0.010 c.c.	20	9
	0.010 " " 0.020 "	12	21
	0.020 " " 0.030 "	6	7
Survivals	0.008 c.c. and under 0.010 c.c.	4	13
	0.010 " " 0.020 "	21	22
	0.020 " " 0.030 "	8	8
	Over 0.030 c.c.	1	1

TABLE XIX.

Dose at or above which young survived and at or under which young died	Toxin-antitoxin mixture injected into parents			
	L0		L+	
	Survivals	Deaths	Survivals	Deaths
0.008 c.c.	34	11	44	2
0.010 "	30	31	31	11
0.020 "	9	43	9	32
0.030 "	1	49	1	39

TABLE XX.

Showing the percentage number of young surviving any particular dose.

Dose	Toxin-antitoxin mixture injected into parents	
	L0	L+
0.008 c.c.	75	95
0.010 "	49	73
0.020 "	17	21
0.030 "	2	2

As will be apparent, these tables differ in preparation from the corresponding ones in Part 1 since in the one case the mothers were all identified and it was possible to represent their immunity by the highest dose tolerated by, or the lowest dose fatal to, their young. In this way each mother was represented once in the tables. The tables now given are compiled from the degree of immunity shown by the individual young bred in one or other run containing L+ or L0 parents respectively. This difference in the tables is necessary because many of the young could not be identified with a particular mother and in no case was the father known as several bucks were present in each run.

From Table XX it will be seen that 73% of the young from L+ parents showed slight immunity (*i.e.* survived a dose of 0.010 c.c. toxin) while only 49% from L0 parents survived the same dose.

Rate of loss of immunity in the young.

Results of tests upon pigs of different ages (but of the same weight—250 grams) were tabulated and are given in Tables XXI, XXII and XXIII, corresponding to Tables IX, X and XI in Part 1.

TABLE XXI.

		Age of young in days					
		20 or less	21-30	31-40	41-50	51-60	Over 60
Deaths	Under 0.008 c.c.	1	1	2	3	4	2
	0.008 c.c. and under 0.010 c.c.	3	9	5	5	2	5
	0.010 " " 0.020 "	0	4	6	9	6	8
	0.020 " " 0.030 "	2	1	4	2	2	2
Survivals	0.008 c.c. and under 0.010 c.c.	4	9	3	1	0	0
	0.010 " " 0.020 "	5	13	10	10	3	2
	0.020 " " 0.030 "	1	7	5	1	0	2
	Over 0.030 c.c.	0	0	0	1	0	1

TABLE XXII.

Doses at or above which young survived and at or under which young died	Age of young in days											
	20 or less		21-30		31-40		41-50		51-60		Over 60	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0.008 c.c.	10	1	29	1	18	2	13	3	3	4	5	2
0.010 "	6	4	20	10	15	7	12	8	3	6	5	7
0.020 "	1	4	7	14	5	13	2	17	0	12	3	15
0.030 "	0	6	0	15	0	17	1	19	0	14	1	17

From Table XXIII it will be seen that the number of pigs surviving decreases with the increased age after 40 days from birth. The figures are not so regular as in Table XI because the numbers here are fewer. This is particularly the case with the pigs over 60 days old, three

TABLE XXIII.

Showing the percentage number of young of different ages that survived any particular dose.

Dose	Age of young in days					
	20 or less	21-30	31-40	41-50	51-60	Over 60
0.008 c.c.	90	96	90	81	42	71
0.010 „	60	66	68	60	33	41
0.020 „	20	33	27	10	0	16
0.030 „	0	0	0	5	0	5

exceptional pigs fully account for the irregularity of the figures. These pigs were recorded as three months old and yet showed considerable immunity. These cases are not specially emphasised as we cannot place absolute reliance upon individual results from these groups where the isolation and observation were not as rigid as in the pigs from which Part I results were obtained. If we assume that the rate of loss of immunity in the young is the same for the young of all injected does whether the bucks were normal or injected, then we can group the figures from both sets of pigs and obtain statistics from well over 500 pigs. These are given in Tables XXIV, XXV and XXVI. In all cases the pigs were tested directly they reached the weight of 250 grams.

From Table XXVI it will be seen that up to the age of 40 days at least 90% of young survived 0.008 c.c., from 41-50 days 88%, from 51-60 days 52%, and over 60 days 27%. The results in this table appear to

TABLE XXIV.

		Age of young in days					
		20 or less	21-30	31-40	41-50	51-60	Over 60
Deaths	Under 0.008 c.c.	3	5	4	4	11	16
	0.008 c.c. and under 0.010 c.c.	7	30	26	17	6	6
	0.010 „ „ 0.020 „	4	16	20	21	19	14
	0.020 „ „ 0.030 „	7	8	11	4	11	7
Survivals	0.008 c.c. and under 0.010 c.c.	13	19	7	2	1	0
	0.010 „ „ 0.020 „	13	30	28	19	6	3
	0.020 „ „ 0.030 „	2	14	15	5	5	2
	Over 0.030 c.c.	1	3	4	4	0	1

TABLE XXV.

Doses at or above which young survived and at or under which young died	Age of young in days											
	20 or less		21-30		31-40		41-50		51-60		Over 60	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0.008 c.c.	29	3	66	5	54	4	30	4	12	11	6	16
0.010 „	16	10	47	35	47	30	28	21	11	17	6	22
0.020 „	3	14	17	51	19	50	9	42	5	36	3	36
0.030 „	1	21	3	69	4	61	4	46	0	47	1	43

TABLE XXVI.

Showing the percentage number of young of different ages that survived any particular dose.

Dose	Age of young in days					
	20 or less	21-30	31-40	41-50	51-60	Over 60
0.008 c.c.	90	93	92	88	52	27
0.010 „	61	57	61	57	39	21
0.020 „	17	25	27	17	12	7
0.030 „	4	2	6	2	0	2

be almost uniform with any age up to 40 days with the tendency towards a maximum immunity among pigs between 30 and 40 days old rather than among younger pigs. All individual cases recorded indicate definitely that in pigs of the same litter (or in all pigs from the same mother if allowance is made for the progressive loss of immunity in the mother) the youngest pig gives the highest immunity without any such optimum age as would be indicated in Table XXVI. This apparent discrepancy would seem to show that the same causes that tend to yield high immunity in the mother also tend to delay the rate of growth in the subsequent offspring. If this were the case the majority of exceptionally young pigs tested would be the offspring of pigs of less immunity and the first two columns of Table XXVI would refer to pigs of a lower grade of immunity than those of subsequent columns.

D. Evidence of lowered resistance in young of injected parents.

While testing young for traces of immunity it was noticed that in some cases the young from injected parents died earlier than the controls. A few isolated instances of earlier deaths would be of no significance because marked differences in the lethal time are fairly frequent in normal pigs injected on the same day. It was noticed, however, that in

the case of certain does a large majority of their young injected with normal lethal doses died earlier than the controls. In such a case one is forced to conclude that either the immunity of the mother has passed off, that the immunity transmitted to the young by the mother has disappeared during the growth of the pig or that the mother failed to produce antitoxin in response to the injection, and that the absence of passive immunity then brings into evidence the damaging action of the toxin-antitoxin injection or of some disturbing factor other than the toxin in the toxin-antitoxin mixture. It is also possible that certain strains of guinea-pigs possess naturally either a greater or less resistance than the normal. This while possibly accounting for certain irregularities, cannot explain such results as are given in Tables XXIX to XXXII.

In Cage 79 where the young were bred from parents that had received L+ doses, only one case was noticed in which the young showed apparently a lowered resistance. This is given in Table XXVII.

TABLE XXVII.

Evidence of lowered resistance among young bred from L+ parents.

Cage	Litter	Age of young	Treatment of young		Control pig	
			Dose	Result	Dose	Result
79 F	1	20 days	0.0085 c.c.	Large reaction increasing 10 grms. in weight	—	—
	2	55 "	0.010 "	Died in 3 days	0.010 c.c.	Died in 5 days
		62 "	0.008 "	" 4 "	{ 0.008 "	" 7 "
		62 "	0.010 "	" 3 "		0.010 "

A 20-day old pig of the first litter easily survived a normal lethal dose. In the second litter where the pigs were 55 and 62 days old when tested two out of three died earlier than the controls. This result standing alone would be of very little significance but some marked instances occurred in Cage 80 recorded in Table XXVIII. The first litter of pig 80 CC was tested with too high a dose, but in the second litter one of the young died in five days after an injection of 0.005 c.c. when a control normal pig survived a higher dose (0.0055 c.c.). In the third litter all three of the offspring died earlier than the controls. Similar results were obtained with the 2nd, 3rd and 4th litters of 80 N. The greater susceptibility here shown cannot in every case be accounted for by the variation in resistance dependent on the age of the

pig; in 80 CC young of 28 days in one litter and 53 days in another showed lowered resistance while in Cage 80 N young of different litters 14 days and 53 days old died earlier than the controls.

A small summary of Table XXVIII will show that the lowering of resistance is quite definite. In Cage 80 CC four pigs are directly comparable with the controls, of these one died when control survived a higher dose and three died earlier than the normal pigs. In Cage 80 N of five pigs compared two died when controls survived the same dose and the other three died earlier.

It appears advisable to record here other evidence of lowered resistance in young bred from injected does (the case of the offspring of injected bucks and normal does will be dealt with later). Only a few instances were noted among the young of normal bucks and injected does and these are given in Table XXIX.

TABLE XXVIII.

Evidence of lowered resistance among young bred from L0 parents.

Cage	Litter	Age of young	Treatment of young		Control pig			
			Dose	Result	Dose	Result		
80 CC	1	49 days	0.016 c.c.	Died in 3 days	{	0.007 c.c.	Died in 4 days	
		49 "	0.012 "	" 2 "		0.007 "	" 4 "	
		49 "	0.008 "	" 3½ "		0.007 "	" 4 "	
	2	48 "	0.0055 "	" 3 "	{	0.006 "	" 3 "	
		53 "	0.0050 "	" 5 "		0.0055 "	Survived	
	3	28 "	0.007 "	" 6 "	{	0.007 "	Died in 7 days	
		28 "	0.008 "	" 4 "		0.008 "	" 6 "	
		35 "	0.008* "	" 4 "		0.008 "	" 7 "	
	80 N	1	21 "	0.016 "	" 7 "	{	0.007 "	Died in 5 days
			24 "	0.017 "	" 3 "		0.008 "	" 4 "
2		49 "	0.008 "	" 4 "	{	0.0065 "	Died in 4 days	
		49 "	0.012 "	" 3 "		0.007 "	Survived	
3		41 "	0.0055 "	" 6 "	{	0.0055 "	Died in 8 days	
		52 "	0.006 "	" 5 "		0.006 "	Survived	
4		14 "	0.007 "	" 4 "	{	0.007 "	Died in 5 days	
		14 "	0.008 "	" 4 "		0.008 "	" 14 "	

No instances were noticed among the young from normal bucks and does which had received L+ doses. The cases in Table XXIX were all from L0 does and it is decidedly significant that in each case the injected mixture contained at least $\frac{1}{2}$ c.c. of horse serum. It would

appear that in the usual routine toxin-antitoxin injections, where the quantity of horse serum would average 1/500 c.c., those doses that would affect the mother sufficiently to lower the resistance of the offspring must contain sufficient uncombined toxoid to produce considerable immunity. The degree of lowering of resistance is very small compared with the degree of immunity conferred and so it is rare that lowered resistance can be demonstrated. The effect is presumably non-specific and so could probably be shown if the young were tested

TABLE XXIX.

Evidence of lowered resistance among young bred from normal bucks, and does which had been injected with toxin-antitoxin mixtures.

Cage	Litter	Age of young	Treatment of young		Control pig	
			Dose	Result	Dose	Result
106 P	1	45 days	0·015 c.c.	Died in 2 days	0·0065 c.c.	Died in 5 days
	2	66 "	0·015 "	" 2 "	0·006 "	" 3 "
		80 "	0·006 "	" 2 "	0·006 "	" 5 "
		82 "	0·0055 "	" 3 "	0·0055 "	Survived
				0·006 "	Died in 5 days	
127 L	1	58 "	0·015 "	" 3 "	0·006 "	" 5 "
		66 "	0·006 "	" 5 "	0·006 "	Survived
	2	28 "	0·008 "	" 3 "	0·0065 "	Died in 8 days
		28 "	0·008 "	" 4 "	0·008 "	" 5 "
		31 "	0·008 "	" 5 "	0·008 "	" 5 "
					0·008 "	" 11 "
105 BB	1	25 "	0·012 "	" 3 "	0·006 "	Survived
	2	35 "	0·008 "	" 3 "	0·008 "	Died in 5 days
		35 "	0·008 "	" 4 "	0·008 "	" 5 "
		52 "	0·008 "	" 3 "	0·008 "	" 4 "

against a toxin other than that to which they are passively immune. A few pigs bred from mothers highly immune to diphtheria toxin were tested against living typhoid organisms but no reliable results were obtained owing to the great range of inaccuracy. It follows that, in dealing with young bred from normal bucks and injected does, lowered resistance can only be demonstrated in those cases where the effect on the general vitality of the mother is due to causes other than a simple toxin-antitoxin mixture, such as $\frac{1}{2}$ c.c. of horse serum as in the cases under consideration. It is possible that the cases in Tables XXVII and XXVIII were of does which had received injections containing large quantities of horse serum. It should again be stated that Tables XXVII

and XXVIII refer to pigs from cages 79 and 80 where the individual treatment was unknown.

In the course of experiments as to whether passive immunity in the mother could be transmitted to the young, various does were injected with 5 c.c. (5000 units) diphtheria antitoxic serum, before and during pregnancy, and again immediately after birth. The number of young surviving was not large and the results of all those tested for one lethal dose are recorded in Tables XXX, XXXI, XXXII.

TABLE XXX.

Evidence of lowered resistance among young bred from normal bucks, and does which had been injected with antitoxic serum alone.

Cage	Litter	Age of young	Treatment of young		Control pig	
			Dose	Result	Dose	Result
133	1	73 days	0.007 c.c.	Died in 7 days	0.007 c.c.	Survived
151	1	Died before reaching standard weight				
	2	69 days	0.006 c.c.	Survived, lost 65 grms.	0.006 "	Survived, lost 5 grms.
	3	43 "	0.008 "	Died in 4 days	0.008 "	Died in 4 days
	50 "	0.008 "	" 4 "	0.008 "	" 5 "	0.008 "

TABLE XXXI.

Evidence of lowered resistance among young from normal does injected with antitoxic serum during pregnancy.

Cage	Litter	Age of young	Treatment of young		Control pig	
			Dose	Result	Dose	Result
78	1	36 days	0.0085 c.c.	Died in 5 days
		59 "	0.013 "	" 3 "	0.013 c.c.	Died in 3 days
120	1	44 "	0.014 "	" 3 "	0.014 "	" 5 "
		44 "	0.014 "	" 5 "		
		44 "	0.015 "	" 4 "		
121	1	22 "	0.014 "	" 4 "
		22 "	0.016 "	" 4 "
		23 "	0.014 "	" 3 "	0.014 c.c.	Died in 8 days

In each table the young appear slightly less resistant than normal pigs. In all cases the conditions of breeding and growth of the young were the same as those for the normal control pigs except the injection of serum into the mother. If the resistance of these pigs was quite

TABLE XXXII.

Evidence of lowered resistance among young suckled by normal mothers injected with antitoxic serum immediately after birth of young.

Cage	Litter	Age of young	Treatment of young		Control pig	
			Dose	Result	Dose	Result
82	1	39 days	0.008 c.c.	Died in 2 days	0.008 c.c.	Died in 5 days
84	1	18 "	0.008 "	" 3 "	0.008 "	" 8 "
		20 "	0.008 "	" 3 "
122	1	22 "	0.014 "	" 3 "	0.013 c.c.	Died in 3 days
		25 "	0.013 "	" 7 "	0.015 "	" 3 "
123	1	24 "	0.014 "	" 3 "	0.013 "	Survived
		35 "	0.014 "	" 3 "	0.014 "	Died in 3 days
					0.016 "	" 3 "

normal we could expect that the majority would die in the same time as controls injected with the same dose, a few would die earlier, a few later. A summary of the results from the three tables shows that 14 young from injected mothers can be compared directly with normal control animals, that none died later than the controls, six died in the same time, five died earlier, two died when the control lived and one survived but lost 65 grams in weight where the control lost only 5 grams. Thus from 14 young from injected mothers, eight showed lowered resistance. It would appear that in each case where the vitality of the mother was slightly affected by the injection of a foreign protein the resistance of the young to diphtheria toxin was lowered to a slight extent.

E. Active immunity conferred by single injections of toxin in pigs that have been rendered passively immune by maternal transmission.

That the immunity conferred on the young of immune does is not transmitted to the next generation has been shown by all investigators who have taken up the subject of the transmission of immunity from parents to their offspring. This is in accord with the fact that the immunity so conferred is of a passive kind and is lost well before the age at which pigs commence to breed. Our own experiments were performed with five first-generation pigs from mothers whose other offspring had shown good immunity. These pigs were paired with normal bucks and the young tested as soon as they had attained the weight of 250

grams; neither immunity nor increased susceptibility was shown, all died within a short time of the controls, three slightly earlier, three at the same time and three slightly later.

We have already seen in Part I Section B that sublethal doses of toxin (without antitoxin) injected into normal pigs produce no appreciable immunity in their young. The following results show that a single injection of toxin into a doe rendered passively immune by maternal transmission may result in the young of such an animal exhibiting a considerable degree of immunity.

An example may be recorded of a doe in Cage 79S₂ bred from parents that had both received L+ doses. This pig was born 19. IV. '08 and on 1. VI. '08 when 250 grams in weight was injected with 0.0095 c.c. toxin, there was no local reaction and no change in weight. A control normal pig injected with the same dose on the same day died in seven days. The doe was paired with a normal buck on 29. VII. '08 and gave birth to two young on 4. XII. '08, to still-borns on 15. III. '09 and 21. V. '09, and to three young again on 6. VIII. '09. The results of the tests upon the young were as follows.

Born 4. XII. '08.	Tested when 33 days old.	0.0055 c.c.	Large reaction and increased 20 grms. in weight.
" "	" "	0.010 "	Large reaction and lost 10 grms. in weight.
	Control pig	0.0055 "	Died in 7 days.
Born 6. VIII. '09.	Tested when 25 days old.	0.008 "	Very large reaction, increased 25 grms. in weight.
" "	" "	0.015 "	Very large reaction, lost 25 grms. in weight.
" "	" "	0.030 "	Died in 4 days.
	Control pig	0.007 "	" 5 "

In this case an approximate normal lethal dose only was injected into the immune pig of the first generation, with the result that considerable immunity was conferred on the young of the second generation.

Contrasted with this case is that of a doe in Cage 79 P₁, also bred from parents that had both received L+ doses. This pig was born 1. V. '08 and on 4. VI. '08 when 250 grams in weight was injected with 0.012 c.c. toxin. There was a large local reaction and a loss of 40 grams in weight. A control normal pig was injected with 0.011 c.c. toxin and died in 9 days. The doe was paired with a normal buck on 8. I. '09 and young were born 25. V. '09 and 17. VIII. '09. The results of the tests upon the young were as follows.

Born 25. v. '09.	Tested when 14 days old.	0.008 c.c.	Very large reaction, lost 30 grms.
" "	" "	" "	" " " " 40 "
	2 Controls	" "	Both died in 5 days.
Born 17. viii. '09.	Tested when 45 days old.	0.010 c.c. 98 B ¹ .	Very large reaction, lost 65 grms.
" "	" "	0.016 "	" " " " Died in 3 days.
" "	" "	0.020 "	" " " " 3 "
	Control	0.016 "	" " " " 7 "
	"	0.020 "	" " " " 4 "

In this case second generation young only 14 days old showed very slight immunity, while older pigs of the next litter showed distinct evidence of lowered resistance.

The main difference between the two cases was the degree to which the mother was affected by the dose of toxin. Little or no immunity was transmitted when the mother was severely affected, while there was considerable immunity in the young whose mother easily tolerated the dose of toxin given. Similar results were noted in many instances and these are recorded in detail in Tables XXXIII, XXXIV and XXXV, divided according to the degree to which the mother was affected.

In Table XXXIII are recorded results of tests upon the young of seven immune does, all of which easily survived the dose given. In all cases the young showed decided immunity. As much as 10 fatal doses of toxin jar 967 A was tolerated by the young of 164 L₂.

Table XXXIV gives the results for eight does moderately affected by the toxin injected. Two cases, 164 S and 153 S, show little or no immunity.

The size of the local reaction and the change in weight are only general indications of the degree to which a pig is affected by an injection. The changes in weight recorded are those that take place in five days after the injection, a big decrease in weight (25 grams or more) or an increase are significant, but the

¹ The supply of test toxin 98 A, with which all the earlier tests were carried out, ran short in August 1909, and toxin 98 B, and in a few instances 967 A, was then used. In all results given so far no toxin number has been recorded because 98 A was used throughout, in all subsequent tables the toxin number is given. The relative values of the toxins used are as follows:

Original m.f.d. 98 A and B under 0.003 c.c.	} Average m.f.d. when used 98 A, 0.008 c.c.
Original m.f.d. 967 A 0.002 c.c.	
	" " " " 967 A, 0.004 "

98 A and B were two bottles filled at the same time with the same toxin prepared in 1900, 967 A was prepared in March 1909. The L₀ doses were similar, that of 98 A 0.27 c.c. and that of 967 A 0.32 c.c.

TABLE XXXIII.

Results of second generation tests when immune mothers easily survived the toxin injected.

Cage	Injection of mother		Time elapsing between injection of mother and birth of young	Age of young	Injection of young		Control pig	
	Dose (c.c.)	Reaction			Dose (c.c.)	Toxin	Dose (c.c.)	Result
100 J	0.010	Nil	7 months	30 days	0.012	98 A	0.0055	Died in 5 days
		+10 grms.		59 "	0.020	"	0.0060	" 6 "
		No change	13 "	10 "	0.016	"	0.0070	" 6 "
79 S ₂	0.0095	Nil	6 "	33 "	0.0055	"	0.0055	" 7 "
		-	14 "	25 "	0.010	"		
				25 "	0.0080	"		
				25 "	0.015	"		
				25 "	0.080	"		
127 BB	0.009	Trace	10 "	19 "	0.016	98 B	0.016	" 7 "
94 NN	0.016	Large + 20	3 "	31 "	0.020	"	0.016	" 7 "
		+25 grms.	6 "	21 "	0.080	"	0.016	" 7 "
94 BB	0.016	Very large	11 "	14 "	0.040	"
	0.020 ²	+45 "						
		+40 "						
164 L ₂	0.024	"	9 "	55 "	0.080	"
		+40 "	13 "	35 "	0.080	"		
				35 "	0.050	"		
				35 "	0.040	987 A		
94 C	0.020	Large	5 "	35 "	0.006	98 A	0.004	" 5 "
		+10 "		35 "	0.010	"	0.006	" 4½ "
			9 "	21 "	0.009	"	{ 0.009	" 5 "
			12 "	31 "	0.009	"	{ 0.009	" 6 "
				31 "	0.009	"	{ 0.009	" 6 "
				31 "	0.018	"	0.008	" 4 "

¹ In this and subsequent tables under column headed "Result" is given size of local reaction and number of grams gained or lost in weight during 5 days after the injection.

² A second injection given 10 days after the first.

TABLE XXXIV.

Results of second generation tests when immune mothers were moderately affected by toxin injected.

Cage	Injection of mother		Time elapsing between injection of mother and birth of young	Age of young	Injection of young		Control pig			
	Dose (c.c.)	Reaction			Dose (c.c.)	Toxin		Dose (c.c.)	Result	
164 L ₁	0.040	Very large	No change	6 months	34 days	0.020	98 B	0.016	Died in 7 days	
						0.030	"	"	"	
						0.030	"	"	"	
79 B ₁	0.010	Small	-25	11 "	25 "	0.008	98 A	0.007	" 6 "	
						0.020	98 B	0.014	" 7 "	
						0.020	"	Very large -15	0.016	" 19 "
						0.020	"	Died in 2 days	0.016	" 7 "
						0.008	967 A	" 2 "	0.0085	" 4 "
						0.016	98 B	Very large +10	0.016	Very large -50
19 F	0.010	Very large	-10	7 "	46 "	0.020	"	0.016	Died in 7 days	
						0.008	967 A	Died in 3 days	0.0085	" 4 "
79 AR	0.015	Large	-5	7 "	32 "	0.020	98 B	0.014	" 7 "	
						0.040	"	"	0.018	" 7 "
79 B ₂	0.016	Very large	-15	13 "	28 "	0.016	98 A	0.008	" 5 "	
						0.030	"	Died in 4 days	0.008	" 5 "
						0.020	98 B	Very large -25	0.016	" 7 "
						0.016	"	" +30	...	" ...
164 S	0.022	Large	-5	11 "	17 "	0.016	98 A	0.080	Died in 4 days	
						0.0080	"	Very large -15	0.0080	" 5 "
158 D	0.022	"	-5	8 "	21 "	0.0080	"	0.0080	" 3 "	
155 S	0.024	"	No change	11 "	38 "	0.015	"	0.0070	" 5 "	
						0.0080	"	" 5 "	0.0080	" 5 "
						0.0080	98 B	Very large -50	...	" ...

TABLE XXXV.

Results of second generation tests when immune mothers were markedly affected by toxin injected.

Cage	Injection of mother		Change of weight	Time elapsing between injection of mother and birth of young	Age of young	Injection of young		Control pig			
	Dose (c.c.)	Reaction				Dose (c.c.)	Toxin	Dose (c.c.)	Result		
79 P ₁	0.012	Large	-40	11 months	14 days	0.008	98 A	Very large - 30	0.008	Died in 5 days	
					14 "	0.008	"	"	"	0.008	" 5 "
					45 "	0.010	98 B	"	"	0.016	" 7 "
					45 "	0.016	"	Died in 3 days	0.016	" 4 "	
106 PP	0.018	Very large	-50	13 "	21 "	0.008	98 A	"	0.008	" 4 "	
					25 "	0.008	"	"	"	"	"
					23 "	0.009	"	"	"	"	"
					17 "	0.008	"	"	"	"	"
46 A	0.020	"	-20	9 "	28 "	0.008	"	"	0.008	Died in 5 days	
					38 "	0.008	"	"	"	0.008	" 7 "
					42 "	0.008	"	"	"	0.008	" 5 "
					52 "	0.020	"	"	"	0.070	" 11 "
127 LL	0.020	"	-45	11 "	56 "	0.008	"	"	0.008	Very large + 15	
					56 "	0.007	"	"	"	0.008	Died in 4 days
					56 "	0.007	"	"	"	0.007	" 5 1/2 "
					42 "	0.008	"	"	"	0.008	" 3 "
79 PP	0.020	"	-25	6 "	52 "	0.020	"	"	0.070	" 5 "	
					45 "	0.008	"	"	"	0.008	" 5 "
					18 "	0.007	"	"	"	0.007	" 6 "
					21 "	0.008	"	"	"	0.008	" 4 "
153 Z	0.026	"	-20	15 "	56 "	0.012	98 B	"	0.008	" 7 "	
					56 "	0.016	"	"	"	0.008	" 7 "
					21 "	0.009	"	"	"	0.016	" 5 "
					21 "	0.009	"	"	"	0.009	" 3 "
80 X	0.030	"	-55	21 "	11 "	0.018	98 A	Very large + 5	0.0090	"	
					11 "	0.018	98 B	"	"	0.018	Very large - 65

meaning of a small decrease is not clear unless the pig is kept under observation for a considerable time until it regains its normal rate of growth. A small decrease (5 or 10 grams) would indicate a severe reaction if the general conditions of time of year, feeding, etc. were conducive to rapid growth, and if the weight of the pig did not rise considerably for some time. On the other hand, if conditions are unfavourable for rapid growth, as in the winter time, a small decrease in weight in five days, followed immediately by a normal rise, indicates very little constitutional disturbance. Thus it follows that Table XXXIV may include pigs affected with markedly different degrees of severity and that if these pigs had been kept under close observation for a longer time after the injection, some would have been included in Table XXXIII and some in Table XXXV. The main comparison, therefore, must be made between Tables XXXIII and XXXV.

In Table XXXV results are given for the young of eight does, all of which were severely affected. Three only of these produced young which survived a normal lethal dose. These cases are as follows.

Cage 79 P₁, 14 days old young of first litter survived, older pigs of a subsequent litter showed no immunity.

Cage 153 Z. Only one test made on a 21 day old pig.

Cage 80 X. Only one test made on an 11 day old pig.

The young from the other five does showed no trace of immunity whatever, on the contrary there was evidence of an increased susceptibility as shown in Cage 79 PP, where five out of six young died earlier than the controls injected with the same dose.

From the three cases where very young offspring survived the normal lethal dose it would appear that pigs of this group may transmit a trace of immunity to their young. This trace is rapidly lost and any effect on the vitality of the offspring due to the severity of the reaction caused by the injection of the parent is then exhibited by a condition of greater susceptibility to the injection of toxin.

From these results it would appear that immunity in the second generation depends considerably, if not entirely, upon the ease with which the first generation doe tolerated the toxin injected. This accords with the experience gained in the course of general work on immunisation in which it is found that the most effective response to the production of antibodies is obtained when the dose administered produces a moderate disturbance only; if this is much exceeded so that the animal is severely affected its capacity for producing antibodies in response to the injection is greatly impaired.

F. Immunity in young of the third generation.

The results of the preceding section indicate a possibility of producing very high immunity in young whose female ancestors have received suitable stimuli in each generation. It would also seem possible to produce young of very low resistance in a similar way. Experiments have been commenced with these ends in view and the results thus far obtained are recorded in Tables XXXVI, XXXVII, XXXVIII and XXXIX, where the history of each generation is given. The two second generation pigs surviving in Cage 79 P₁ recorded in Table XXXV were each paired with a normal buck and their young showed indications of lowered resistance. These are detailed in Tables XXXVI and XXXVII. The two other cases of third generation pigs given in Tables XXXVIII and XXXIX were those whose parents and grandparents easily survived the doses given (see Table XXXIII, Cage 94 C and Table XXXIV, Cage 158 D). These pigs showed considerable immunity, particularly in the case of Table XXXIX, where a pig 47 days old gained in weight after an injection of 0.060 c.c. jar 98 B. This dose represents nearly a $\frac{1}{4}$ of the L0 dose, *i.e.* 50 binding units of toxin.

At the time of going to press another third generation result has been obtained confirming those of Tables XXXVIII and XXXIX. Two pigs in cage 223 LA survived and gained in weight after injections of 6 and 10 fatal doses of a fresh toxin. The male parents were normal in each generation and of the females the great-grandmother was injected with a toxin-antitoxin mixture near to the L+ dose causing a large local reaction and loss in weight. The grandmother survived 0.024 c.c. of toxin 98 A with a large increase in weight, the mother survived 0.030 c.c. 98 B with a slight loss in weight.

TABLE XXXVI.

Doe injected with an L+ mixture during 1907.
Paired with a similarly injected buck.



Doe born 1. v. '08, injected 4. vi. '08 with 0.012 c.c. 98 A. Large reaction, lost 40 grms. in weight. (Control injected with 0.011 c.c., died in 9 days.)
Paired with a normal buck.



Doe born 25. v. '09, injected 8. vi. '09 with 0.008 c.c. 98 A. Very large reaction, lost 30 grms. in weight. (Control injected with 0.008 c.c., died in 5 days.)
Paired with a normal buck.



Born 21. xii. '09, injected when 45 days old with 0.016 c.c. 98 B, died in 4 days.
(Control injected with 0.016 c.c., died in 5 days.)

TABLE XXXVII.

Doe injected with an L+ mixture during 1907.
Paired with a similarly injected buck.



Doe born 1. v. '08, injected with 0.012 c.c. 98 A. Large reaction, lost 40 grms. in weight. (Control injected with 0.011 c.c., died in 9 days.)
Paired with a normal buck.



Doe born 25. v. '09, injected with 0.008 c.c. 98 A. Very large reaction, lost 40 grms. in weight. (Control injected with 0.008 c.c., died in 5 days.)
Paired with a normal buck.



Born 18. i. '10. Injected when 31 days old with 0.012 c.c. 98 B. Very large reaction, lost 45 grms. in weight. (Control injected with 0.014 c.c., lost 45 grms.)

Born 3. v. '10. Injected when 21 days old with 0.016 c.c. 98 B. Very large reaction, lost 80 grms. in weight. (Control injected with 0.018 c.c., lost 75 grms.)

Born 12. viii. '10. Injected when 25 days old with 0.012 c.c. 98 B. Died in 8 days.

" " " " 25 " 0.018 " " " 4 "

(Control injected with 0.018 c.c., died in 7 days.)

TABLE XXXVIII.

Doe injected with L0 dose 22. i. '08.
Paired with a normal buck.

↓

Doe born 29. v. '08, injected 6. vii. '08 with 0.020 c.c. 98 A. Large reaction and increase of 10 grms. in weight. (Control injected with 0.016 c.c., died in 3 days.)
Paired with a normal buck.

↓

Doe born 20. vii. '09, injected 20. viii. '09 with 0.010 c.c. 98 A. Small reaction and increase of 10 grms. in weight.
Paired with a normal buck.

↓

Born 8. iii. '10, injected when 35 days old with 0.030 c.c. 98 B. Very large reaction. No change in weight. (Control injected with 0.016 c.c., died in 7 days.)
Born 1. viii. '10, injected when 32 days old with 0.050 c.c. 98 B. Very large reaction. Lost 10 grms. in weight. (Control injected with 0.016 c.c., died in 3 days.)
Born 1. viii. '10, injected when 32 days old with 0.030 c.c. 98 B. Large reaction. Gained 15 grms. in weight. (Control injected with 0.016 c.c., died in 3 days.)

TABLE XXXIX.

Doe injected with an L0 mixture 15. ii. '08.
Paired with a normal buck.

↓

Doe born 28. viii. '08. Injected 1. ix. '08 with 0.022 c.c. 98 A. Large reaction. Lost 5 grms. in weight. (Control injected with 0.012 c.c., died in 3 days.)
Paired with a normal buck.

↓

Doe born 21. v. '09. Injected 11. vi. '09 with 0.008 c.c. 98 A. No reaction. Gained 10 grms. in weight. (Controls injected with 0.008 c.c., died in 3 and 4 days.)
Paired with a normal buck.

↓

Born 12. v. '10. Injected when 43 days old with 0.040 c.c. 98 B. Medium reaction. Gained 10 grms. (Control injected with 0.018 c.c., died in 6 days.)
Born 12. v. '10. Injected when 47 days old with 0.060 c.c. 98 B. Very large reaction. Gained 5 grms. (Control injected with 0.018 c.c., died in 7 days.)
Born 6. ix. '10. Injected when 24 days old with 0.040 c.c. 98 B. No reaction. Gained 30 grms. (Control injected with 0.020 c.c., died in 4 days.)
Born 6. ix. '10. Injected when 24 days old with 0.060 c.c., no reaction. Gained 40 grms. (Control injected with 0.020 c.c., died in 4 days.)

SUMMARY.

(1) The young of parents both of which have been injected with an immunising mixture of diphtheria toxin and antitoxin, show immunity of the same order as that of young from similarly treated mothers and normal fathers.

(2) The injection of certain foreign substances into a female guinea-pig appears to have a direct effect on the offspring in diminishing their resistance to diphtheria toxin, shown equally well by the young of mothers injected, (a) before the attainment of sexual maturity, (b) during pregnancy, and (c) after birth during the period of lactation.

(3) A single injection of diphtheria toxin may give rise to a condition of active immunity (as tested by the resistance of the young) in guinea-pigs possessing hereditarily transmitted passive immunity. Should this injection of toxin give rise to great constitutional disturbance, the young may show lowered resistance, whereas, if it give rise to but slight constitutional disturbance, the young show a high degree of immunity. These effects appear to be accentuated if similar injections are repeated in the next generation.