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

Part 1.

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Since the time Ehrlich(1) established on a scientific basis many of the main facts regarding the transmission of immunity as exhibited in mice to the vegetable poisons, abrin, ricin and robin, many investigators have concerned themselves with the subject of transmission of immunity to various poisons and bacterial infections. Ehrlich first showed conclusively that the immunity transmitted from the mother was of a passive nature, due to the transference of the antibodies from the body fluids of the mother to the foetus through the placenta, and also in the case of the mouse by means of the milk during lactation. He found that the immunity thus transmitted disappeared some three months after birth; no trace of immunity was detected in the next generation; neither was an immunised father capable of transmitting immunity to his offspring. Ehrlich concluded from these results that there exists no true hereditary transmission of immunity in mice, a conclusion further supported in both mice and guinea-pigs immunised against tetanus(2).

These results have in the main been confirmed by all later observers. The fact that the young of mice become immunised through the milk is exceptional and not generally shown by other animals experimented upon. Carrière(3) obtained results in guinea-pigs inoculated with products of the tubercle bacillus which show that not only is immunity in the strict sense of the term not transmitted to the offspring but on the contrary a condition of increased susceptibility and a general lowered condition of vitality of the offspring resulted.

Lustig(4) similarly demonstrated that fowls inoculated with abrin produce offspring of less than normal resistance to this poison.

Anderson(5) appears to be the first to have shown that the young of guinea-pigs treated with a mixture of diphtheria toxin and antitoxin exhibit immunity to diphtheria toxin, no such immunity being shown in the young of those treated with toxin only. The immunity was not transmitted by all mothers treated with the toxin-antitoxin mixture, and this he compared with the fact that horses respond very differently to diphtheria toxin in their capacity to produce antitoxin. Grandchildren of immune mothers were not more resistant than normal. Theobald Smith(6) arrived independently at results confirming those of Anderson.

In a second paper Theobald Smith(7) arrived at the following conclusions:

Active immunity to a relatively high degree can be produced in guinea-pigs by the injection of diphtheria toxin-antitoxin.

Neutral mixtures are less effective in producing immunity than mixtures which contain excess of toxin capable of giving rise to local lesions.

We have been engaged on observations on somewhat similar lines for a number of years. We have worked on a larger aggregate of material, and by methods differing in some important particulars from those adopted by the above observers. Although, therefore, our results to a large extent correspond with those which have been published meanwhile by others independently engaged with the question, we think it desirable to place them on record. We have, however, delayed publication until various other questions which had arisen in connection with certain phenomena occurring in the second and third generation could be followed out.

Our method of determining the degree of immunity conferred on the offspring differed from that of Theobald Smith who ascertained the L+ dose on animals of a definite age, while our results were based upon the effect of the injection of toxin only as soon as the young had reached the weight of 250 grams. The degree of immunity depending presumably upon the quantity of circulating antitoxin is more accurately expressed by the neutralising value in terms of the volume of toxin (indicating the number of binding units) rather than the number of fatal doses tolerated. In all cases tabulated the same toxin (98 A) was use throughout.

The seasonal variation(8) of susceptibility of guinea-pigs which affects so markedly the apparent minimal fatal dose of a toxin is barely notice-

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able in testing neutralising values. In the same way the volume of toxin tolerated by immune young (*i.e.* neutralised by their antitoxin) shows no great variation at different times of the year except in those cases where the volume of toxin tested was very close to the normal fatal dose. In testing the immunity of a pig only one test is permissible, so that it is seldom possible to determine accurately the immunity value, beyond saying that it is above or below a certain value. When there are three or four pigs in one litter, the first pigs reaching the standard weight can be used as an index for the value to test for in the other pigs of the same litter, and so a more accurate immunity value can be obtained.

In tabulating the results of immunity conferred upon their young by immunised does the degree of immunity is measured by the largest dose of toxin tolerated by any of the offspring, or if the doses given were in all cases too high and none of the young survived, the immunity is taken to be less than the lowest dose that killed. In practice it was found generally unworkable to investigate a particular point by performing definite experiments avoiding all other variables. The possibilities of the injected pigs dying before any young were born, or of the young dying before they reached standard weight, or of there being an insufficient number of young to fix the degree of immunity with any accuracy, were so great that it was found best to obtain our main results by keeping isolated and breeding from a large number of guinea-pigs that survived injections of toxin or toxin and antitoxin mixture given in the course of the regular laboratory routine rather than by specially injecting a limited number of pigs and endeavouring to breed from them. The results obtained in testing the immunity of the offspring from these pigs could then be tabulated. In each set of tables where the results are divided according to a particular variable the other variables are ignored. When it would appear that the results of one variable would overwhelm those of another, individual cases are dealt with.

The first part of our results deals only with the first generation of the young from injected does and normal bucks.

A. *Immunity transmitted to young by mother injected with various mixtures of toxin and antitoxin.*

The variables upon which the immunity of the young may depend are:

1. Nature of the injection received by the mother.
2. Individual differences of different mothers.

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3. Time elapsing between injection of mother and birth of young.
4. Age of young when tested for immunity.
5. Time of year and climatic conditions when test was made.
6. Individual differences in young.

These variables are dealt with individually below:

1. *Nature of injection received by mother.*

1 *a*. The constitution of the mixture and the degree to which the mother was affected may be indicated by the local reaction produced by the injection. In Table I the results are divided according to whether the local reaction produced was nil, small or large. The results of immunity conferred by the mother are indicated by the largest dose of toxin tolerated by any of their young, or if none of the young survived the dose given, then the lowest dose that killed is recorded, provided that death occurred within 10 days of the test and that the test was made upon young born within 12 months of the injection of the mother. It should be remembered that in these tables each mother is represented only once although tests may have been made upon many of its offspring.

TABLE I.

Results of immunity of young divided according to the local reaction produced by the immunising mixture when injected into the mother.

		Size of local reaction in mother		
		Nil	Small	Large
Lowest dose killing young	Under 0·008 c.c. ...	4	2	0
	0·008 and under 0·010 c.c.	9	2	0
	0·010 ,, 0·020 c.c.	8	1	1
	0·020 ,, 0·030 c.c.	2	0	0
Highest dose tolerated by young	0·008 and under 0·010 c.c.	3	0	0
	0·010 ,, 0·020 c.c.	6	3	6
	0·020 ,, 0·030 c.c.	4	1	3
	Over 0·030 c.c. ...	3	2	2

To summarise these results it is necessary to consider the proportion of does whose young tolerated a particular dose or more of toxin to those whose young were killed by that or a lower dose of toxin. Thus in Table I of the mothers showing nil swelling, eight had young killed by a dose of toxin of 0·010 to 0·020, nine had young killed by a dose

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between 0·008 and 0·010 and four below 0·008, therefore the young of these 21 would all have died if injected with 0·020 and in the same way seven had young capable of surviving an injection of 0·020 c.c.

TABLE II.

Dose at or above which young survived and at or under which young died	Size of local reaction in mother: Nil		Small		Large	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0·008 c.c.	16	4	6	2	11	0
0·010 c.c.	13	13	6	4	11	0
0·020 c.c.	7	21	3	5	5	1
0·030 c.c.	3	23	2	5	2	1

TABLE III.

Showing the percentage numbers of mothers whose young survived any particular doses.

Dose	Size of local reaction in mother: Nil	Small	Large
0·008 c.c.	80	75	100
0·010 c.c.	50	60	100
0·020 c.c.	25	37	83
0·030 c.c.	11	28	66

From Table III it is seen that mixtures nearer L+ than L0 confer higher immunity—the young of over 80 °/₀ of the does injected with toxin-antitoxin mixtures producing large local reactions tolerated 0·020 c.c. of toxin, while the young of only 25 °/₀ of the does injected with mixtures producing no local reaction tolerated the same dose. It should be pointed out that these tables represent the results from 62 mothers but at least 400 young from these mothers were tested. A somewhat noticeable exception to the figures occurs in the case of two mothers with small local reactions whose young died with doses less than 0·008 c.c. In both cases the pigs were used for testing very weak antitoxic serum and were injected with a test dose of toxin together with ½ c.c. of serum. This quantity of serum in itself is enough to account for a small local reaction on the pig. In both cases the rapid growth of the pigs after the injection indicates that so far as the toxin reaction is concerned the swelling should be considered as nil. Table IV gives the results as they would read if this alteration were made.

1 *b*. Probably the most essential feature of the toxin-antitoxin mixture injected into the mother is the amount of toxin (or toxoid)

dissociated, and this is best indicated by the excess of antitoxin present in the mixture above that necessary to protect life until the 5th day when injected with one test dose of toxin. In many cases the mothers had been used for giving only a rough approximation of the antitoxic value of a serum and so the exact value of the serum used was not known in all cases. The figures in Tables V, VI and VII give the results for all mothers where it was known that the excess of antitoxin in the mixture was less than 20 %, less than 50 %, or over 50 %.

TABLE IV.

Dose	Size of local reaction in mother: Nil	Small	Large
0·008 c.c.	72	100	100
0·010 c.c.	46	75	100
0·020 c.c.	23	50	83
0·030 c.c.	10	40	60

TABLE V.

Results of immunity of young divided according to the excess of antitoxin present in the toxin-antitoxin mixture injected into the mother.

		Excess of antitoxin: Over 50 %	Under 50 %	Under 20 %
Lowest dose killing young	Under 0·008 c.c. ...	2	1*	0
	0·008 and under 0·010 c.c.	3	1	0
	0·010 ,, 0·020 c.c.	3	2	0
	0·020 ,, 0·030 c.c.	1	0	0
Highest dose tolerated by young	0·008 and under 0·010 c.c.	0	0	0
	0·010 ,, 0·020 c.c.	2†	7	3
	0·020 ,, 0·030 c.c.	0	2	2
	Over 0·030 c.c.	0	3	0

* This pig was paralysed and consequently its responsive power was impaired.

† One of these figures is the result of only a single test upon one young and may be a case of accidental survival through leakage of injection. The other refers to a pig that gave a most unusual result of no local reaction but a marked drop in weight, indicating that the test was "out" in some way.

TABLE VI.

Dose at or above which young survived and at or under which young died	Excess of antitoxin: Over 50 %		Under 50 %		Under 20 %	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0·008 c.c.	2	2	12	1	5	0
0·010 c.c.	2	5	12	2	5	0
0·020 c.c.	0	8	5	4	2	0
0·030 c.c.	0	9	3	4	0	0

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TABLE VII.

Showing the percentage numbers of mothers whose young survived any particular dose.

Dose	Excess of antitoxin		
	Over 50 %	Under 50 %	Under 20 %
0·008 c.c.	50	92	100
0·010 c.c.	28	85	100
0·020 c.c.	0	55	100
0·030 c.c.	0	42	—

Table VII shows very definitely that the immunity conferred by a doe upon the young depends upon the amount in the toxin-antitoxin mixture of toxin (or toxoid) dissociated and so available for immunisation. In the case of eight does where the excess of antitoxin was greater than 50 % none of their young could tolerate 0·020 c.c. of toxin, while the young from five out of nine mothers receiving under 50 % excess of antitoxin tolerated this dose.

1*c*. Another variable in the constitution of the mixture injected into the mother is the constitution of the toxin used. The majority of the results recorded were from mothers injected with the same test toxin but in the few cases where other toxins were used there was a strong indication that a fresh toxin does not give such high immunity as an older toxin where there is more toxoid dissociated in a neutral mixture. The results obtained are too few to form any definite conclusion unless all other variables can be eliminated.

1*d*. Another variable in the constitution of the mixture is the amount of toxin injected. The results recorded so far are from cases where the mother received one test dose of toxin together with antitoxin. In testing the antitoxic value of the serum obtained from apparently normal horses it is often necessary to supply 1/5 or 1/10 of the test dose of toxin. The young bred from mothers used for these tests did not as a general rule give very high immunity. In one case a moderately high degree of immunity was shown, this however was not to be compared with the highest degrees of immunity reached by the young of mothers treated with a full test dose of toxin.

2. *Individual differences in parents.*

In most animals individuals of the same species appear to vary in their responsiveness to immunisation. Various slight irregularities in the foregoing tables are sufficient to show that such variability does exist in guinea-pigs. A definite example is given below.

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Two pigs 100 Z and 105 AA were injected with a test dose of the same toxin and 1·3 unit antitoxin. Nine months after the injection the young were tested with 0·015 c.c. toxin, that of 100 Z gave no local reaction, while that of 105 AA gave a very large local reaction and a drop of 35 grams in weight on the fifth day after injection. Subsequent tests of later litters showed that the young of 100 Z would tolerate 0·050 c.c. of toxin better than those of 105 AA would tolerate 0·015 c.c.

3. *Time elapsing between injection of mother and birth of young.*

In the majority of cases the young of the first and possibly the second litter were tested with doses well above or well below the dose they would just tolerate, consequently very few examples illustrate the degree of immunity at all stages in the life of the mother. That a gradual diminution in immunity or at least in the power of transmitting immunity occurs in the mother is shown in the individual cases given in Table VIII.

TABLE VIII.

Cage	Litter	Time elapsing between injection of mother and birth of young	Age of young	Dose injected	Date of death	Local reaction	Change in weight
47 A	1st	13 months	32 days	0·016 c.c.	...	Very large	−35 grm.
			37 ,,	0·018 ,,	...	,,	−50 ,,
	2nd	20 ,,	35 ,,	0·006 ,,	...	,,	−15 ,,
			35 ,,	0·010 ,,	6th day	...	...
105 B	1st	5 ,,	40 ,,	0·022 ,,	...	Very large	−30 grm.
			44 ,,	0·024 ,,	...	,,	−55 ,,
			44 ,,	0·026 ,,	...	,,	−45 ,,
	2nd & 3rd	...	Still borns	...	...	...	...
	4th	16 months	56 days	0·018 c.c.	3rd day	...	...
			56 ,,	0·015 ,,	4th ,,	...	...
153 F	1st	6 ,,	42 ,,	0·012 ,,	...	Very large	+10 grm.
			42 ,,	0·024 ,,	...	,,	−10 ,,
	2nd	...	Still borns	...	...	...	...
	3rd	14 months	35 days	0·028 c.c.	4th day	...	...
			35 ,,	0·025 ,,	5th ,,	...	...
153 AA	1st	9 ,,	18 ,,	0·015 ,,	...	Very large	−55 grm.
	2nd	12 ,,	32 ,,	0·008 ,,	6th day	...	...
94 B	1st	11 ,,	38 ,,	0·020 ,,	...	Very large	−5 grm.
	2nd	14 ,,	35 ,,	0·016 ,,	...	,,	−15 ,,
			42 ,,	0·018 ,,	6th day	...	...
	3rd	20 ,,	50 ,,	0·015 ,,	4th ,,	...	...
			61 ,,	0·012 ,,	5th ,,	...	...
	4th	25 ,,	21 ,,	0·008 ,,	...	Very large	−20 grm.
			28 ,,	0·008 ,,	...	,,	−10 ,,

The cases recorded in Table VIII show more or less rapid loss of immunity in the mother, but they cannot be closely analysed in consequence of the variation of immunity according to the age of the young which is to be dealt with next.

We have demonstrated immunity to the extent of tolerating 10 fatal doses of toxin in young born 25 months after the injection of the mother.

4. *The age of the young when tested for immunity.*

In all cases the young are tested when they have reached the weight of 250 grams, the time taken for pigs to reach this weight varies from 10 days to 120 days. Tables IX, X, XI, give the results of over 300 young whose exact ages were known. It should be noted that these tables differ from I, II, III and V, VI, VII as all the young are given irrespective of the treatment of the parents while the earlier tables recorded the mothers and not the young.

TABLE IX.

		Age of young in days					
	Dose tested	20 or less	21—30	31—40	41—50	51—60	Over 60
Deaths	Under 0·008 c.c. ...	2	4	2	1	7	14
	0·008 and under 0·010 c.c.	4	21	21	12	4	1
	0·010 ,, 0·020 c.c.	4	12	14	12	13	6
	0·020 ,, 0·030 c.c.	5	7	7	2	9	5
Survivals	0·008 and under 0·010 c.c.	9	10	4	1	1	0
	0·010 ,, 0·020 c.c.	8	17	18	9	3	1
	0·020 ,, 0·030 c.c.	1	7	9	4	5	0
	Over 0·030 c.c. ...	1	3	4	3	0	0

TABLE X.

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.	19	2	37	4	35	2	17	1	9	7	1	14
0·010 c.c.	10	6	27	25	31	23	16	13	8	11	1	15
0·020 c.c.	2	10	10	37	13	37	7	25	5	24	0	21
0·030 c.c.	1	15	3	44	4	44	3	27	0	33	0	26

Table XI shows that immunity has almost disappeared at the end of two months. But that it may occasionally be present, however, to a considerable extent after this period will be shown later when we record cases in connection with another section of the subject.

TABLE XI.

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

Dose	Age of young in days: 20 or less	21—30	31—40	41—50	51—60	Over 60
0·008 c.c.	90	90	94	94	56	6
0·010 c.c.	62	51	57	55	47	6
0·020 c.c.	16	21	26	21	17	0
0·030 c.c.	6	6	8	10	0	0

Table XII gives individual cases of the effect of the age of the young.

TABLE XII.

Cage	Time elapsing between injection of mother and birth of young	Age of young	Dose injected	Date of death	Local reaction	Change in weight
47 B	14 months	51 days	0·009 c.c.	3rd day	...	...
		58 ,,	0·007 ,,	5th ,,	...	...
	17 ,,	52 ,,	0·006 ,,	3rd ,,	...	...
		73 ,,	0·0055,,	...	Very large	– 45 grm.
	24 ,,	28 ,,	0·008 ,,	...	,,	– 45 ,,
		32 ,,	0·008 ,,	7th day	...	...
		35 ,,	0·009 ,,	14th ,,	...	...
100 N	9 ,,	50 ,,	0·015 ,,	15th ,,	...	...
	14 ,,	31 ,,	0·020 ,,	...	Very large	– 15 grm.
105	6 ,,	56 ,,	0·030 ,,	7th day	...	...
		70 ,,	0·030 ,,	3rd ,,	...	...
106 D	6 ,,	29 ,,	0·026 ,,	...	Large	+ 25 grm.
	9 ,,	60 ,,	0·020 ,,	...	Very large	– 25 ,,
	14 ,,	36 ,,	0·020 ,,	...	,,	+ 25 ,,
106 L	6 ,,	60 ,,	0·025 ,,	...	Large	– 10 ,,
	11 ,,	29 ,,	0·025 ,,	...	Very small	No change
153 A	5 ,,	50 ,,	0·018 ,,	...	Very large	– 10 grm.
		87 ,,	0·018 ,,	3rd day	...	...

It will be seen from Table XII that in some cases, notably in cage 100 N, young pigs of subsequent litters will tolerate a larger dose than older pigs of earlier litters owing to the more rapid loss of immunity in the young compared to that in the mother. This result is what would have been expected from the fact that passive immunity is more transient than active. The smaller dose tolerated by older pigs is due to loss of antitoxin and not to any weakness of the pig indicated by the longer time taken to reach the standard weight, because we have shown(8) that for normal guinea-pigs of the same weight the minimal fatal dose increases with age. This is also seen in Table XIII giving the time taken by 0·008 c.c. toxin to kill normal guinea-pigs of different ages during the months of May, June and July, 1909.

TABLE XIII.

	Age of guinea-pigs		
	20—30 days	31—40 days	41—50 days
Time taken to kill	3 days	3 days	4 days
	3 „	3 „	7 „
	3 „	4 „	11 „
	4 „	6 „	
	4 „		

5. *Time of year and climatic conditions.*

This has been shown to have a marked effect on normal guinea-pigs, and a similar effect was noticeable on young showing little or no immunity. The effect has not been demonstrated clearly in the case of young showing considerable immunity. The effect would be quite masked by other variables in any attempt to show the effect by statistical methods.

6. *Individual differences in young.*

Throughout our work there has been evidence that slight individual differences do occur, but great variations in immunity have not been observed. The differences noted were small and mostly to be accounted for by the age of the pigs. It seldom happened that a direct comparison could be made on different individuals of the same litter reaching the standard weight on the same day. In one case however (cage 105 TT) three pigs of the same litter were tested when 35 days old and 250 grams in weight. The dose used was 0·015 c.c. toxin and the results were

Doe ... ... died in three days.
Buck ... ... „ „ seven „
Buck ... ... Survived with very large local reaction and a loss of 50 grams in weight on the fifth day.

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General notes on results obtained with young from mothers injected with toxin-antitoxin mixtures.

Results obtained with cage 19 afford a very good series showing the combined influence of the age of young when tested and the time elapsing between injection of mother and birth of young. The figures are given in Table XIV.

TABLE XIV.

Litter	Time elapsing between injection of mother and birth of young	Age of young	Dose injected	Date of death	Local reaction	Change in weight
1 2 }	All still born	...	...	...	...	...
3	11 months	49 days	0·018 c.c.	...	Large	- 5 grm.
		56 ,,	0·020 ,,	4th day	...	...
4	14 ,,	57 ,,	0·014 ,,	...	Very large	- 25 grm.
		62 ,,	0·016 ,,	4th day	...	...
5	19 ,,	70 ,,	0·010 ,,	...	Very large	- 10 grm.
		84 ,,	0·012 ,,	5th day	...	...
6	22 ,,	34 ,,	0·012 ,,	...	Large	+ 15 grm.
		45 ,,	0·015 ,,	...	,,	- 15 ,,

In each litter the results are sufficiently clear to enable us to fix a fatal dose as an index of the immunity possessed by the young of that litter.

TABLE XV.

Litter	Time elapsing between injection of mother and birth of young	Average age of young	Minimum fatal dose for litter
3	11	52½ days	0·019 c.c.
4	14	59½ ,,	0·015 ,, -
5	19	77 ,,	0·011 ,, +
6	22	39½ ,,	0·016 ,,

From Table XV it will be seen that the degree of immunity of each litter is in order of the average age of the young of that litter if allowance is made for a slight decrease owing to loss of immunity in mother.

As would be expected the amount of different toxins tolerated by immune young depends not upon the number of fatal doses but upon the binding units. Immune young will tolerate many more fatal doses of a fresh toxin containing little toxoid than of an old toxin rich in toxoids.

The results recorded so far were made with toxin (98 A) of which the average fatal dose for the year could be taken as 0·008 c.c. Another bottle (98 B) of the same toxin was found to have diminished more rapidly in toxicity so that at the time of testing (eight years after the preparation of the toxin) the average fatal dose was 0·014 c.c. It was found that the volumes of 98 A and 98 B tolerated by highly immune young were almost identical. The highest number of fatal doses that immune young survived when tested with 98 A was 7, with another toxin (967 A) the highest number was 14. In volume the highest amounts tolerated were similar and the binding unit content of the two toxins were similar although the fatal dose of 98 A (eight years old) was 0·008 c.c. and that of 967 A (two years old) was 0·004 c.c.

B. *Immunity transmitted to young by mother injected with toxin only.*

It has already been demonstrated clearly by Anderson and Theobald Smith that single injections of sub-lethal doses of toxin in does will not produce immunity in the young. A set of six does was put aside for breeding to confirm this and in no case could we demonstrate immunity in the young. A further set of three does was treated with a series of gradually increasing doses of toxin and again no immunity could be detected in the young. Two of the does treated in this way received similar injections (1/5, 2/5, 2/3 and 1 M.F.D. at weekly intervals). At the end of the treatment one (100 A) had decidedly decreased in weight and the other (100 B) increased. In the case of 100 A only one out of seven young from the first two litters survived while in 100 B four out of six lived. The young of 100 B all showed normal susceptibility to toxin but those of 100 A showed a lowering of resistance as seen in Table XVI. The comparison between the two pigs is shown in Table XVII.

Lustig has shown that immunity to abrin is not transmitted by fowls to their offspring. We shall similarly show in a subsequent section of this investigation that there is evidence against transmission of immunity to diphtheria toxin by the effect on the germ cell before fecundation. Immunity in the young must depend therefore upon the transference of passive immunity from the mother either during pregnancy or by means of the milk. Several experiments were made but only one case was recorded in which a normal pig suckled by a highly immune doe survived the normal lethal dose. In two cases out of three there

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appeared to be very slight lowering of the immunity when immune young were suckled by normal does. Thus it appears that immunity is mainly transferred through the placenta during pregnancy. A few experiments were performed to see whether immunity passively acquired by the mother could be so transferred. In eight cases where does were injected at different times during the course of pregnancy with 2000 units of diphtheria antitoxin; no immunity could be detected in the young. This failure may be due to the more rapid loss of passive immunity conferred by the inoculation with antitoxin obtained from another species of animal.

TABLE XVI.

Young from 100 A		Normal young	
Dose	Date of death	Dose	Date of death
0·008 c.c.	5th day	0·009 c.c.	4th day
0·009 ,,	3rd ,,	0·009 ,,	5th ,,
0·009 ,,	3rd ,,	0·009 ,,	5th ,,
		0·009 ,,	6th ,,

TABLE XVII.

Pig	Change in weight during treatment	Resistance of young to Diphtheria toxin	Percentage survivals in first two litters	Percentage survival during total time of breeding
100 A	– 20 grm.	Lowered	14	46
100 B	+ 40 ,,	Normal	66	75

SUMMARY.

1. Diphtheria toxin-antitoxin mixtures induce a higher immunity in guinea-pigs than sub-lethal doses of toxin; one injection of the mixture being sufficient to produce an immunity lasting in some cases for a period of over two years, as shown by the passive immunity conferred on the offspring.

2. The highest immunity is produced by toxin-antitoxin mixtures containing the most uncombined toxoid.

3. The active immunity of the mother is transferred passively to the offspring.

4. The passive immunity thus transferred usually disappears at the end of two months after birth, and only in rare instances has been recognised after three months.

5. Immunity is mainly transmitted in utero, and only to a slight extent during lactation.

6. Young bred from does that have been used for a single routine antitoxin test may be able to tolerate 14 times the dose of diphtheria toxin fatal for a normal guinea-pig.

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