NOTES ON THE PRODUCTION OF IMMUNITY TO DIPHTHERIA TOXIN.

By A. T. GLENNY, B.Sc. and H. J. SÜDMERSEN, Ph.D.

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

(With 15 Charts.)

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INTRODUCTION.

THE present paper deals with some of the results of our work on immunity, extending over a period of many years.

During the period occupied by this research, publications have appeared at various times dealing with similar lines of work or reaching similar conclusions. We, however, do not consider that any part in which conclusions agree with those already published should be omitted, since each section of the paper has a bearing on some other section.

Though we do not bring forward anything that is fundamentally new, we believe that, on many points and particularly in connection with what we have called the "Secondary Stimulus Phenomenon," our observations have attached a clear and quantitative value to an idea that is foreshadowed in several places in the literature of immunity, but of which we cannot find a clear description in any of the modern text-books.

A review of the field of work covered will be of more general interest, for a full knowledge of this field has a very definite practical bearing on such problems as those involved in the active immunisation by toxin-antitoxin mixtures of people susceptible to diphtheria, in the protection of horses exposed to the risk of tetanus by mixtures of tetanus toxin and antitoxin, the long protection afforded by vaccination with vaccine lymph and by typhoid vaccine, etc.

Review.

The chief point of interest in this paper is the marked contrast between the immunity response to the primary stimulus and the response to the secondary stimulus.

Primary Stimulus is the term that we apply to the initial injection of an antigen into a non-immune animal. The primary stimulus causes a very slow production of antibody; so delayed is the response that many weeks may elapse before the presence of antitoxin can be detected in the circulating blood.

In no instance have we detected an immunity response in less than two weeks after the primary stimulus nor has the highest immunity been attained in less than five weeks. The average length of latent period following a primary stimulus is three weeks and the maximum immunity is usually reached in about eight weeks (see statement 21 and Table XXIV).

Secondary Stimulus is the term that we apply to the injection of toxin into an actively immunised animal. In contrast to the primary stimulus this causes a rapid response, the latent period is about four days and the maximum immunity is reached in about ten days (see statement 32 and Table XXXV). The secondary stimulus phenomenon has been demonstrated in guinea-pigs, rabbits, sheep, goats and horses. The injection of toxin into naturally immune horses and into one naturally immune human was followed by a similar response to that caused by a secondary stimulus in an animal artificially immunised by a primary stimulus (see statement 37 and Table XLI).

That certain animals in domestication or in intimate association with man, such as the horse, frequently possess diphtheria antitoxin in their blood, while other animals, such as the guinea-pig and the rabbit, do not possess antitoxin, are now well-known facts.

It has also been established that a large proportion of human beings who have never had clinical diphtheria, possess normal antitoxin in their blood. The question of the origin of diphtheria antitoxin in the normal individual is obscure and therefore the subject of theoretical speculation. Some would hold that its possession is an innate quality and hereditarily transmitted, while others, basing their opinion on the finding of the diphtheria bacillus in healthy people, and at the seat of superficial ulceration in horses, believe that diphtheria antitoxin in the healthy normal individual has arisen in all cases in direct response to the presence of the diphtheria bacillus at some time or other during life, although its presence may have given rise to little or no appreciable alteration in the normal condition of health.

By comparison of the tables and curves, it will be seen that the behaviour of animals possessing normal antitoxin, to an injection is the same as that of animals previously immunised by the injection of the specific toxin. This gives strong support to the theory that the origin of normal antitoxin is by active response at some time or other to an infection by the diphtheria bacillus.

That natural immunity is an active immunity produced by a natural infection has long been accepted although it has been difficult to account for the natural immunity present in the majority of horses. The only recorded case of *B. diphtheriae* found in a horse was that by Cobbet (*Lancet*, 25 Aug.

1900) until very recently when Minett found the organism in limb lesions of a number of horses (*Journal of Comparative Pathology*, Dec. 1920).

Intermediate Stimulus is the term that we apply to the injection of toxin into an animal that is only partially immune. It is obvious that there cannot be a sharp division between normal animals capable only of a primary response and actively immune animals fully equipped for a secondary response. A second injection of toxin given when the primary response is commencing to develop is followed by an immunity response intermediate in character between primary and secondary responses (see statement 38 and Table XLII).

The quantity of toxin that can be injected without injury to a non-immune animal is very small; in order to increase the primary stimulus it is necessary therefore to use toxin the lethal power of which has been weakened by age, by chemical agents such as iodine or formalin, or almost completely neutralised by antitoxin. The effect of certain chemical agents is to destroy the lethal power without altering appreciably the antigenic value of a toxin. Under statement 3 in Part I A there are recorded the only instances in this paper in which such a toxin has been used. In the majority of cases the primary stimulus has consisted of a toxin-antitoxin mixture and the secondary stimulus of a greater amount of toxin without antitoxin. The difference in response to the two stimuli is not due to the difference in intensity of stimulus. Theobald Smith (*Journal of Experimental Medicine*, 1909) has shown that greater immunity is produced by a single injection of multiple doses of a toxin-antitoxin mixture than by a single dose; that this does not account for the difference between the primary and secondary phenomena is shown in Table XXXIII where both primary and secondary stimuli consisted of toxin-antitoxin mixtures of identical composition, and in Table XXVII where the secondary stimulus consisted of a less toxic mixture than the primary stimulus. It is at present a matter of theoretical speculation as to the amount of toxin in a toxin-antitoxin mixture that is available as an antigen. Mixtures lying within the "Differential Region" (Ehrlich) or within the "visible spectrum of the toxin-antitoxin effects" (Theobald Smith) contain uncombined toxoid and some antigenic power is thus explicable; why completely neutralised and over neutralised mixtures also act as antigens is less clear. There appear to be three possible explanations, that the combined toxin-antitoxin complex acts in itself as an antigen, that dissociation occurs between the toxin and antitoxin, or that there is present a variety of toxoid uncombined that lies outside the visible spectrum.

Instances of the negative phase are seen in Tables XXII, XXXI, XXXVI, XXXVIII. The negative phase is distinct from the latent period and consists of a fall in antitoxic value after a secondary stimulus greater than can be accounted for by combination between the circulating antitoxin and the injected toxin.

The primary and secondary stimulus phenomena have been demonstrated in this paper for guinea-pigs, rabbits, goats, horses and humans injected with

diphtheria toxin. Similar phenomena occur in animals injected with the toxins of the pathogenic anaerobes (work yet to be published by one of us). The response of many different animals to five different toxins follows the same definite course; the primary and secondary stimulus phenomena may yet be found of universal application to immunity and not limited only to antitoxin production.

Methods.

Except where otherwise stated, Toxin 1915 was used throughout. The m.l.d. of this toxin was 0·005 c.c. and its L0 and L + doses were 0·32 c.c. and 0·38 c.c. respectively.

Toxin J 176 is occasionally referred to; this toxin is of considerable interest as the L + dose has remained unchanged for 13 years. The toxin has been kept in the liquid form in an ice chest and has been in constant use. Under certain conditions, therefore, diphtheria toxin may remain stable.

The results of any injections into an animal are recorded by noting the day of death, or, if the animal survives for five days, by noting the size of the local reaction and the loss or gain in weight during the five days following the injection. The size of the local reaction is classified as follows:

No reaction	nil	Medium swelling	MS
Very small swelling	trace	Large swelling	LS
Small swelling	SS	Very large swelling	VLS

The numbers following the size of the swelling indicate in grammes the rise or fall in weight during the five days following the injection.

When only small quantities of blood were available for examination as from the ear of a guinea-pig, we invariably collected the blood in a Thoma Zeiss red corpuscle counting pipette which afforded a convenient apparatus for the accurate measurement of small volumes. A 4 per cent. solution of sodium citrate was drawn up into the pipette directly after the required volume of blood. The contents were then blown into a small glass containing the dose of toxin previously made up to 4 c.c. by the addition of normal saline and about 0·15 c.c. of sodium citrate solution. The pipette was washed out by drawing the fluid up and blowing out a few times. The blood and toxin were mixed by gentle shaking. We found that a much larger quantity of sodium citrate than that used in testing by this method had no influence on either toxin or antitoxin. A series of control experiments was made by injecting a number of guinea-pigs with varying amounts of antitoxin mixed with different fractions of a test dose of toxin. From the table of results obtained it was possible to interpolate intermediate results and to interpret the results obtained with test animals in terms of fractions of a unit of antitoxin in the blood tested. When only small quantities of blood were available the smallest amount of antitoxin that could be detected was 0·04 unit per c.c., only values greater than this can be accepted with any confidence. When testing larger animals such as the horse not only was more serum available

for each test but a larger number of tests could be made upon the serum obtained from each test bleeding; it follows that such results are more accurate and a smaller amount of antitoxin could be detected. Values of 0·01 unit per c.c. are however of doubtful significance. It might be pointed out here that since this work was done it has been found possible to test for as little as 0·0005 unit per c.c. by means of the intracutaneous method.

Arrangement of results.

The results are divided into three parts:

Part I. Injection of toxin into animals with no normal antitoxin.

A. Injection of toxin alone.

B. Injection of toxin under cover of antitoxin.

Part II. Injection of toxin into actively immune animals.

Part III. Injection of toxin into animals possessing normal antitoxin.

In each part the results with different species of animal are given separately. For the sake of ease of reference each result is prefaced by a statement of the fact subsequently demonstrated by the results recorded. An index of parts, subdivisions and statements is given at the beginning of the paper.

PART I.

INJECTION OF TOXIN INTO ANIMALS WITH NO NORMAL ANTITOXIN.

A. INJECTION OF TOXIN ALONE.

Guinea-pigs.

(1) *Sublethal doses of toxin increase the susceptibility of guinea-pigs to subsequent intoxication.*

A number of guinea-pigs that had survived sublethal doses of toxin were injected with 1 m.l.d. of toxin from 3–8 weeks later when they had regained approximately their original weight. It was found that the majority of those that had been seriously affected by the original injection died within three days. Normal guinea-pigs injected with the same dose of toxin usually died upon the fifth day; as will be seen from Table I, only 16 per cent. died on the

Table I.

The effect of a single fatal dose of toxin upon guinea-pigs previously injected with a sublethal dose of toxin.

	Total tested	Number dying within three days	Percentage number of early deaths
Normal guinea-pigs during corresponding period ...	19	3	16
Guinea-pigs previously injected with toxin causing loss in weight during the first five days of less than 30 grms.	10	2	20
30–40 grms.	16	4	25
40–50 grms.	7	2	28
Over 50 grms.	9	8	89

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third day or earlier, as compared with 89 per cent. among those that had been severely affected by the first injection.

Among other guinea-pigs which had increased in weight during the first five days after the first injection, a subsequent injection of 1 m.l.d. of toxin, 3–8 weeks later, gave the same results as in normal animals.

An increase in susceptibility after an injection of toxin altered to toxoid or in combination with antitoxin is also seen in Tables III and VI.

It is open to question whether this increased susceptibility is really specific. It is possible that, had the first injection been any other poison, *e.g.* tetanus toxin or a poisonous drug producing the same general lowering of vitality, similar increased susceptibility to a subsequent injection of diphtheria toxin would have been met with.

(2) *Guinea-pigs may be killed by less than* 1 *m.l.d. if small doses are given at frequent intervals. The effect produced by dividing the injection depends upon the size and the time interval of the dose.*

The results of a number of experiments are given in Table II.

Table II.

The effect of injecting fractions of a lethal dose of toxin at different intervals.

Dose given at each injection	Results obtained from: Daily injection	Results obtained from: Injection every two days	Fraction of normal m.l.d. required to kill the animal when injected: Daily	Fraction of normal m.l.d. required to kill the animal when injected: Every two days
2/5 m.l.d.	—	Died after 2 inj.	—	4/5
1/5 „	Died after 4 inj.	1 died after 5 inj. 3 „ 7 „	4/5	7/5
1/10 „	4 died after 6 inj. 1 „ 7 „ 2 „ 10 „ 1 survived 10 „	1 „ 12 „ 3 survived 12 „	about 8/10	greater than 12/10
1/20 „	1 died after 16 inj. 2 „ 24 „ 1 survived 24 „		about 24/20	

The number of experiments is very small but there are indications to show that, in order to kill a guinea-pig with a total quantity of less than the minimal lethal dose, an interval of two days may elapse between the injections when 2/5 of an m.l.d. is given at each injection; when 1/5 or 1/10 m.l.d. is given, the result is only brought about at a shorter interval of time and one day may elapse between the injections; again, when 1/20 m.l.d. is given at daily intervals, three out of four animals survive a total above the m.l.d. It probably follows that if an injection of 1/20 m.l.d. or less were given more frequently than at daily intervals, death would follow before an amount equal to 1 m.l.d. had been given. The intervals of time between the injections must be spaced according to the dose; they must be of longer duration when the dose is large but must not exceed a limit which becomes smaller as the dose is lessened.

The fact that very few experiments were performed under this section must again be stressed, but the indications are of sufficient interest to justify their inclusion in the paper.

From results given later, under statement 4, of attempts to immunise guinea-pigs by a series of injections of toxin alone, it will be seen that sub-lethal doses at weekly intervals are frequently fatal.

(3) *Toxin rich in "toxoid" may produce high immunity.*

The toxin used in this experiment had originally an m.l.d. of 0·005 c.c.; the toxin was changed into "toxoid" by treatment with formalin rendering the product atoxic, so that 5 c.c. would not kill.

Three weeks after a single dose of from 0·5 c.c. to 2·0 c.c. of an old formalinised toxin (L0 dose about 1·0 c.c.—m.l.d. over 5·0 c.c.) a number of guinea-pigs survived 2 m.l.d. of toxin without any local reaction.

Table III gives the results obtained by injecting four other guinea-pigs with increasing doses of the same formalinised toxin.

Table III.

The course of injection of four guinea-pigs with formalinised toxin.

Interval	Dose	Guinea-pig *V*	Guinea-pig *X*	Dose	Guinea-pig *W*	Guinea-pig *Z*
—	0·2 c.c.	SS + 20	trace + 15	0·5 c.c.	LS + 15	LS + 10
5 weeks	0·5 c.c.	SS – 10	LS – 5	1·0 c.c.	VLS – 25	LS – 20
2 „	1·0 c.c.	nil – 45	trace + 25	2·0 c.c.	nil + 40	nil + 50
2 „	2·0 c.c.	nil + 50	nil + 40	5·0 c.c.	nil + 40	trace + 65

N.B. For interpretation of symbols in this and all succeeding tables see explanation given under heading "Methods."

In all cases, two weeks after the last injection, antitoxin was demonstrated in the blood. *W* was the only animal whose antitoxic value was worked out and that was found to be 8·5 units per c.c. It will be noticed that in all cases the second injection was not tolerated so well as the first or subsequent ones. (See statement 1.)

(4) *Guinea-pigs have been immunised successfully on rare occasions by a series of injections of toxin alone.*

Attempts to immunise guinea-pigs against unmodified toxin gave variable results. In the majority of cases the animals failed to survive a long series of injections. Death may have been due to the poor condition of the guinea-pigs, the injections having rendered them more susceptible to adverse conditions. In a few cases, post-mortem examination showed that death was due to other causes than diphtheria intoxication.

Table IV records the results obtained by giving a number of guinea-pigs weekly injections of toxin (m.l.d. 0·005 c.c.).

In the case of guinea-pig *R* in which antitoxin was produced, the animal was immunised very slowly, no increase to 0·002 c.c. being made until after

ten injections at 0·001 c.c. (1/5 of m.l.d.). In another series, the animals were allowed to rest for nine weeks after the first injection and were then injected

Table IV.

The course of immunisation of six guinea-pigs receiving weekly injections of toxin, showing the number of weekly injections at each dose.

Dose	Guinea-pig *A*	Guinea-pig *C*	Guinea-pig *F*	Guinea-pig *P*	Guinea-pig *R*	Guinea-pig *S*
0·0005 c.c.	2	2	3	—	—	—
0·0006 c.c.	—	—	—	1	1	1
0·001 c.c.	8	4	1	9	10	9
0·002 c.c.	—	3	—	2	4	3
0·003 c.c.	—	—	—	—	2	—
0·004 c.c.	—	—	—	—	3	—
Result ...	Death	Death	Death	Death	Over 1 unit of antitoxin produced	Death

weekly. A number of deaths occurred, but in two cases the immunisation was successful. These two cases are recorded in Table V.

Table V.

The course of immunisation of two guinea-pigs receiving weekly injections of toxin at a long interval after an initial injection.

S 2. iv.

0·0005 c.c. (1/10 m.l.d.). Reaction SS + 10 grms.
Interval nine weeks.
Three weekly injections of 0·001 c.c.
Nine weekly injections of 0·002 c.c.
Antitoxic value of blood tested nine days after the last injection = 1·4 units per c.c.

W 2. iv.

0·0005 c.c. (1/10 m.l.d.). Reaction—trace—no change in weight.
Interval nine weeks.
Three weekly injections of 0·001 c.c.
Nine weekly injections of 0·002 c.c.
Three weeks later 0·02 c.c. (4 m.l.d.).
Five days later 0·05 c.c. (10 m.l.d.). Reaction—nil + 50 grms.
Antitoxic value of blood not tested.

In a further series larger doses and longer intervals were employed. Two guinea-pigs out of a number survived the course of injections and showed marked immunity. From Table VI, where these cases are recorded, it will be seen that guinea-pig *P* 8. xii. produced 70 units of antitoxin per c.c. of blood and was able easily to tolerate 2000 times the m.l.d. of toxin.

The successful immunisation of the animals recorded in Tables V and VI was probably due to the fact that the main course of injection was started about two months after the first injection, at a time when the animals were actively immune (see statement 21).

Table VI.

The course of immunisation of two guinea-pigs receiving injections of toxin at long intervals.

Dose	Result		Interval	Result
P 8. xii.				
0·005 (toxin J 2172)	MS	+25 grms.	7 weeks	—
0·003 (toxin J 1915)	LS	−30 „	3 „	No antitoxic value 10 days later
0·004 „	LS	−80 „	2 „	—
0·004 „	nil	+10 „	3 „	—
0·004 „	nil	+35 „	3 „	Antitoxic value detectable 4 days later
0·02 „	nil	−10 „	2 „	Over 8 units per c.c. 4 days later
0·1 „	nil	−15 „	2 „	—
10·0 (2000 m.l.d.)	trace	−20 „	—	About 70 units per c.c. 18 days later
S 15. iv.				
0·001	trace	+30 grms.	9 weeks	—
0·002	VLS	+25 „	3 „	—
0·004	VLS	+55 „	5 „	—
0·004		−65 „	2 „	—
0·004		− 5 „	5 „	—
0·02		+ 5 „	5 days	—
0·05 (10 m.l.d.)		nil	—	Antitoxic value not tested

Horses.

(5) *Horses possessing no normal antitoxin resemble guinea-pigs in their reaction to toxin and in the difficulty of immunisation.*

In our experience in the routine immunisation of horses for the production of diphtheria antitoxic sera, horses (over seven years old) normally possessing no detectable antitoxin (less than 1/500 units per c.c.) in their serum are rare. Such horses are extremely difficult to immunise with toxin alone.

B. INJECTION OF TOXIN UNDER COVER OF ANTITOXIN.

(*a*) ANTITOXIN INJECTED AT THE SAME TIME AS TOXIN.

Guinea-pigs.

(6) *A single injection of a toxin-antitoxin mixture may confer a high degree of immunity.*

About 350 guinea-pigs that had been used for routine testing of antitoxin against toxin were subsequently tested for immunity by the injection of toxin. It was found that a number were able to tolerate as much as 400 times the m.l.d. for a normal guinea-pig. They had all received mixtures containing one test dose of toxin together with varying amounts over one unit of antitoxin. The animals were divided into two groups—those that had received mixtures causing local reactions, and those that had received mixtures in which the excess of antitoxin was sufficient to prevent local reaction. In the former group, the constitution of the test mixture only varied between the

narrow limits of the "differential region" L0 to L +; while in the latter group, there was an excess of antitoxin over that contained in the L0 mixture. The results of injecting toxin into guinea-pigs of the two groups are recorded in Tables VII and VIII. Owing to individual variability it is impossible to predetermine for any particular guinea-pig an exact dose at which a reaction will occur. Since only one test of tolerance can be made on one guinea-pig, it will be seen that the large number of guinea-pigs mentioned above was required in order to arrive at any conclusion by this method. The results are divided according to the number of weeks elapsing between the injection of the toxin-antitoxin mixture and the injection of toxin. The tables give the number of guinea-pigs surviving on the fifth day after the injection of toxin out of the total number injected with any particular dose. The figures printed in heavy type indicate the nearest dose, for the interval of time, at which approximately equal proportion of deaths and survivals occur and which can be taken as an index of tolerance.

Table VII represents the first group, *i.e.* those that had received mixtures causing local reaction.

Table VII.

Showing ratio of survivors to the total number of guinea-pigs injected with any particular dose of toxin, divided according to the interval of time elapsing after the injection of a toxin-antitoxin mixture causing local reaction.

Dose of toxin	Weeks elapsing between injection of toxin-antitoxin mixture and injection of toxin only																			
c.c.	2		3		4		6		8				12				16			20
5·0	.		.		.		.		0/5				.				.			.
2·0	.		.		.		.		**7/14**				**7/14**				**0/2**			**1/1**
1·0	.		.		.		**1/6**		5/5				4/5				**1/4**			.
0·5	.		.		0/6		**5/10**		6/8				4/5				.			.
0·2	.		.		1/3		5/8		2/2				.				.			.
0·1	.		.		2/14		.		2/2				2/2				.			.
0·064	.		.		**11/18**		.		4/4				.				.			.
0·032	0/1		.		7/11		.		.				.				.			.
0·016	.		**6/16**		10/13		**1/1**		.				.				.			.
0·008	.		9/13		4/5		4/4		2/2				.				.			.
0·005	**15/18**		.		.		.		.				.				.			.

Table VIII represents the second group, *i.e.* those that had received a mixture in which the excess of antitoxin was sufficient to prevent local reaction. The results of the injection of toxin into animals of this group are of less importance than those of the first group as in many instances a very large excess of antitoxin may have been present in the mixture, while in other cases an exact L0 mixture may have been injected. It is shown later (in statement 9) that a large excess of antitoxin in the mixture injected reduces the extent of immunity produced.

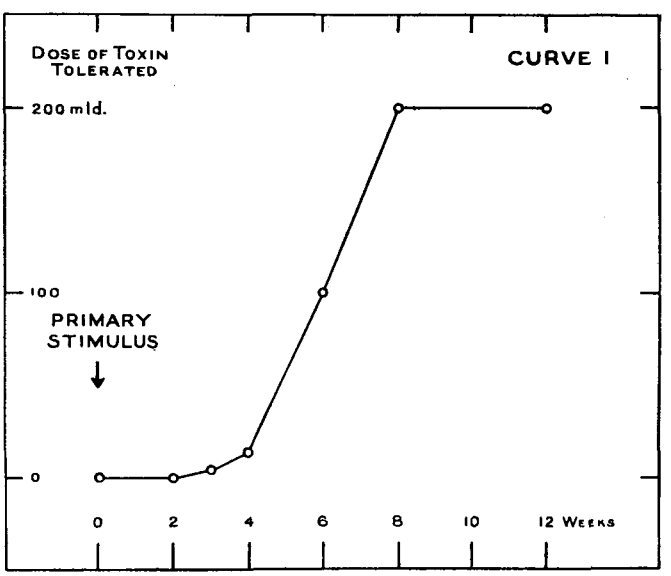
Table VIII.

Showing ratio of survivors to the total number of guinea-pigs injected with any particular dose of toxin, divided according to the interval of time elapsing after the injection of a toxin-antitoxin mixture causing no local reaction.

Dose of toxin	Weeks elapsing between injection of toxin-antitoxin mixture and injection of toxin only																			
c.c.	2		3		4		6		8				12				16			
2·0	.	.	.	.	.	.	.	.	1/4	.	.	.	1/5	.	.	.	0/1	.	.	
1·0	.	.	.	.	0/5	.	4/11	.	.	.	.	.	.	.	.	.	3/6	.	.	
0·5	.	.	.	.	.	.	4/12	.	.	.	0/1	.	.	.	.	.	.	.	.	
0·2	.	.	.	.	5/12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
0·1	.	.	1/7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
0·064	.	.	3/6	.	.	.	3/3	.	.	.	.	.	.	.	.	.	.	.	.	
0·032	.	.	3/4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
0·016	.	3/11	4/5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
0·008	.	5/10	2/5	.	.	.	1/2	.	.	.	.	.	.	.	.	.	.	.	.	
0·005	5/8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

(7) *Comparatively little immunity is produced for at least four weeks after the injection of a toxin-antitoxin mixture and the maximum is reached at eight weeks or later.*

The results in Tables VII and VIII show that a high degree of immunity, as shown by resistance to toxin, generally results from a single injection of a toxin-antitoxin mixture but the immunity is slow in making its appearance. It is of interest to note that two weeks after the injection of a mixture



CURVE 1. From results given in Table VII showing average resistance of guinea-pigs to diphtheria toxin after an injection of a toxin-antitoxin mixture causing local reaction.

Latent period: 3 weeks. Maximum height: 8–12 weeks.

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causing local reaction, some of the guinea-pigs injected will not tolerate 0·005 c.c. that is, the average m.l.d. for a normal 250 grm. guinea-pig, although these animals have increased in weight. The first two or three weeks may be considered as a latent period of no immunity. At the end of the third week, slight but definite immunity is shown, and after this it rises quickly to the sixth week, reaching its maximum at about the eighth week.

The average dose of toxin tolerated by guinea-pigs at different intervals of time after an injection of toxin-antitoxin mixture causing local reaction is shown in Curve 1.

A few guinea-pigs that had been used for routine testing of antitoxin and had shown local reaction as the result of the toxin-antitoxin mixture injected were tested for antitoxic value at various intervals of time after the injection. The results are recorded in Table IX.

Table IX.

The antitoxic value of the blood of guinea-pigs at different intervals after an injection of a toxin-antitoxin mixture.

Interval of time elapsing between toxin-antitoxin injection and testing of blood for antitoxin	Number of guinea-pigs tested	Antitoxic value of blood of individual guinea-pigs
4 weeks	2	0·0; 0·0 unit per c.c.
8 „	2	0·14; 0·14 unit per c.c.
12 „	5	0·07; 0·07; 0·14; over 0·15; 0·21 unit per c.c.

(8) *Individual guinea-pigs vary considerably in their immunity response to an injection of a toxin-antitoxin mixture.*

From Table VII, it will be noted that eight weeks after an injection of a toxin-antitoxin mixture, some animals (7 out of 14 tested) were found to tolerate 2·0 c.c. of toxin, while others (2 out of 8) were killed by one-quarter of the dose. Similar differences are shown at 12 weeks. At an earlier period, the differences are more marked, *e.g.* four weeks after the original injection, one guinea-pig out of three tested survived an injection of 2·0 c.c., while another guinea-pig (1 out of 5 tested) was killed with as small a dose as 0·008 c.c. (probably about the m.l.d. for a normal guinea-pig of the same weight). We are inclined to doubt whether such differences are to be ascribed to differences in the constitution of the toxin-antitoxin mixture which lay within the narrow range covered by the "differential region." Other individual differences are shown later in Table XI.

(9) *It would appear that a moderate excess of antitoxin in the immunising mixture does not affect materially the degree of immunity produced. A considerable excess of antitoxin is required to render a mixture incapable of producing immunity.*

A number of animals were injected with different mixtures containing one test dose of toxin and varying amounts of antitoxin from 1·2 units to

5·0 units. The mixture containing 1·2 units antitoxin and one test dose of toxin occasionally produced slight local reaction. This can be taken as being very close to the border of the L0 dose. At an interval of from 13 to 15 weeks, some of the guinea-pigs were injected with 2·0 c.c. (400 m.l.d.) of toxin. The results are given in Table X.

Table X.

Recording the number of guinea-pigs surviving 400 m.l.d. of toxin three months after the injection of a toxin-antitoxin mixture containing varying amounts of antitoxin.

Constitution of mixture		Number of guinea-pigs surviving 2·0 c.c. toxin	Number of guinea-pigs killed by 2·0 c.c. toxin
Toxin	Antitoxin		
1 test dose	1·2 units	3	1
1 „	1·3 „	1	2
1 „	1·5 „	4	1
1 „	2·0 „	0	4

From the small number of animals, it appears therefore that within the limits represented by 1·2 to 1·5 units of antitoxin in the mixture no marked differences are shown. A noticeable falling off is in evidence when the mixture contains 2·0 units of antitoxin, since all four guinea-pigs tested were killed by 2·0 c.c. of toxin. Such a mixture, however, produced quite definite immunity, for another guinea-pig tested survived an injection of 1·0 c.c. (100 m.l.d.) of toxin. A few guinea-pigs that had received mixtures over-neutralised to the extent of five units of antitoxin to one test dose of toxin, were injected with a single fatal dose of toxin and showed very little, if any, increased resistance.

The immunity conferred by toxin-antitoxin mixtures in guinea-pigs was also tested by ascertaining the antitoxic value of the blood of guinea-pigs after such an injection. The amount of antitoxin present was found to be extremely small and the results are somewhat inconsistent owing to the difficulty of testing small amounts of blood for traces of antitoxin.

The groups of guinea-pigs recorded above in Table X were tested for antitoxin at various times before the injection of toxin; it was found that little or no antitoxin was detectable four weeks after injection, and that for 2–3 months the highest values were obtained by the group injected with the mixture containing 1·2 units of antitoxin. The results are recorded in Table XI.

Table XI.

The antitoxic value of the blood of guinea-pigs 2–3 months after the injection of a toxin-antitoxin mixture containing varying amounts of antitoxin.

Constitution of mixture		Number of guinea-pigs showing			
		0·00	0·05	0·10	0·20
Toxin	Antitoxin	unit of antitoxin per 1 c.c. of blood			
1 test dose	1·2 units	0	2	1	2
1 „	1·3 „	1	1	1	0
1 „	1·5 „	1	3	1	1
1 „	2·0 „	3	1	2	0

(10) *The immunity conferred by a single injection of a toxin-antitoxin mixture is of long duration.*

Table XII.

Recording the antitoxic value, two years after injection, of guinea-pig *H* injected 3 Nov. 1911, with a mixture containing one test dose of toxin together with between 1·0 and 1·2 units of antitoxin. Large local reaction.

Date	Weight	Weeks after injection	A.T. value per c.c.
22 Jan. 1912	—	11	0·14 unit
1 Feb. 1912	570 grms.	13	0·15 „
3 Feb. 1912	—	13	0·17 „
18 Mar. 1912	565 „	19	0·17 „
21 Mar. 1912	545 „	20	0·15 „
22 May 1912	695 „	29	0·15 „
18 Nov. 1912	935 „	54	0·15 „
20 Aug. 1913	1075 „	94	over 0·09 „

It is important to note from this table that for one year if not for two years, the value maintained a constant level. The variations recorded from the thirteenth to the fifty-fourth week are all within the errors of experiment. This guinea-pig survived an injection of 2·0 c.c. toxin on the occasion of its last test, one year and nine months after its original injection. That the level of concentration of antitoxin in the blood is maintained during such a long period while growth is taking place and in the absence of any further stimulation is remarkable. During the period, the guinea-pig gave birth to eight young and thereby a large amount of the mother's antitoxin was lost. (In several experiments, we found that at birth the antitoxic value per c.c. of the blood of the offspring is the same as that of the mother.) It is extremely probable also the elimination of autogenous antitoxin is continually taking place since we know that antitoxin so closely related as that of the mother is soon got rid of in the offspring. From the increase in weight and consequently of blood content of the animal, it would appear therefore that the total antitoxin present steadily increased for at least a year after the injection.

Table XIII.

Recording the antitoxic value of the blood of guinea-pig *C* injected 6 Sept. 1911 with a mixture containing one test dose of toxin together with 1·2 units of antitoxin. No local reaction.

Date	Weight	Weeks after injection	A.T. value per c.c.
29 Dec. 1911	—	16	0·50 unit
2 Jan. 1912	—	17	0·60 „
5 Feb. 1912	510 grms.	22	0·33 „
18 Mar. 1912	740 „	28	0·21 „
1 April 1912	635 „	30	0·26 „
2 Aug. 1912	650 „	47	0·21 „
18 Nov. 1912	750 „	62	0·11 „
6 Sept. 1913	—	104	0·05 „

Another guinea-pig, *S* 10. x. 11, injected with a toxin-antitoxin mixture causing local reaction was found to contain 0·15 unit of antitoxin four months after the injection. Tested a year later, the level was found to be the same, and 22 months later the animal tolerated an injection of 2·0 c.c. toxin.

The guinea-pig recorded in Table XIII is given as an exceptional case, in that the immunity produced is high and the subsequent fall rapid.

Rabbits.

(11) *Rabbits injected with a toxin-antitoxin mixture give results similar to those produced in guinea-pigs.*

Rabbits are similar to guinea-pigs in that they possess no normal antitoxin and the m.l.d. is, weight for weight, the same as that for guinea-pigs. Owing to their larger weight, it follows that rabbits can tolerate toxin-antitoxin mixtures fatal to guinea-pigs. Five rabbits were injected with 0·36 c.c. toxin (J 176) and one unit of antitoxin, a mixture just fatal to guinea-pigs. The results are recorded in Table XIV.

Table XIV.

Antitoxic value of serum of rabbits after an injection of a toxin-antitoxin mixture containing one unit of antitoxin and 0·36 c.c. toxin, J 176.

Rabbit	Weeks after injection	A.T. value per c.c.
1	9	·07 unit
2	9	·12 „
.	12	·12 „
3	9	·10 „
11	2	·02 „
.	4	·04 „
.	6	·05 „
.	8	·04 „
12	2	·04 „
.	4	·10 „
.	6	·14 „
.	8	·14 „

All the rabbits recorded in Table XIV were eventually injected with 2·0 c.c. toxin (50–100 times the m.l.d. for a normal rabbit) and in all cases they survived at least five days. Nos. 1 and 11, with the lowest antitoxic value, were severely affected.

Several tests were made upon rabbits 11 and 12 at different intervals, and it will be seen that as in the case of the guinea-pigs (see statement 7) the highest value is not obtained until six or eight weeks after the injection.

(12) *More toxic mixtures produce higher immunity.*

Table XV gives the results obtained in two rabbits injected with a more toxic mixture than that used in the preceding table, *i.e.* 0·38 c.c. toxin + one unit of antitoxin. The degree of immunity produced in both rabbits was higher than that in any rabbit recorded in Table XIV.

Table XV.

Antitoxic value of serum of rabbits after an injection of a toxin-antitoxin mixture containing one unit of antitoxin and 0·38 c.c. toxin, J 176.

Rabbit	Weeks after injection	A.T. value per c.c.
5	8	0·21 unit
"	16	0·21 "
9	12	0·26 "

(13) *Splenectomised rabbits respond to injections of toxin-antitoxin mixtures.*

Two rabbits that had had their spleens removed a few months previously were injected with an L + mixture containing 0·36 c.c. toxin, J 176, and one unit of antitoxin. The results are given in Table XVI.

Table XVI.

Antitoxic value of serum of splenectomised rabbits after an injection of a toxin-antitoxin mixture.

	Antitoxic value of	
Weeks after injection	Rabbit 15	Rabbit 16
0	0·00 unit per c.c.	0·00 unit per c.c.
2	0·01 "	0·01 "
3	0·03 "	0·08 "
4	0·04 "	0·15 "
5	0·04 "	0·16 "
6	0·04 "	0·14 "
7	0·04 "	0·16 "
8	0·03 "	0·12 "
9	0·025 "	0·11 "
10	—	—
11	0·025 "	—
12	—	0·10 "
13	—	0·10 "

Both rabbits were subsequently injected with 2·0 c.c. (50–100 m.l.d.) toxin; rabbit 16 survived and rabbit 15 died three days after the injection. The immunity produced in rabbit 15 was lower than that of any rabbits recorded in Table XIV, and the animal failed to survive a dose of toxin tolerated by all the other rabbits. On the other hand, the immunity produced in rabbit 16 was higher than that of any of the non-splenectomised rabbits. The highest antitoxic value was reached at the fourth or fifth week, which appears to be earlier than is the case with normal rabbits.

Other animals.

(14) *Goats injected with toxin-antitoxin mixtures give results similar to those produced in guinea-pigs.*

Goat No. 12 which was found to contain no normal antitoxin was injected with ten times the L + mixture for a guinea-pig. The dose consisted of 3·8 c.c. of toxin, J 176, together with 10 units of antitoxin. One-tenth of

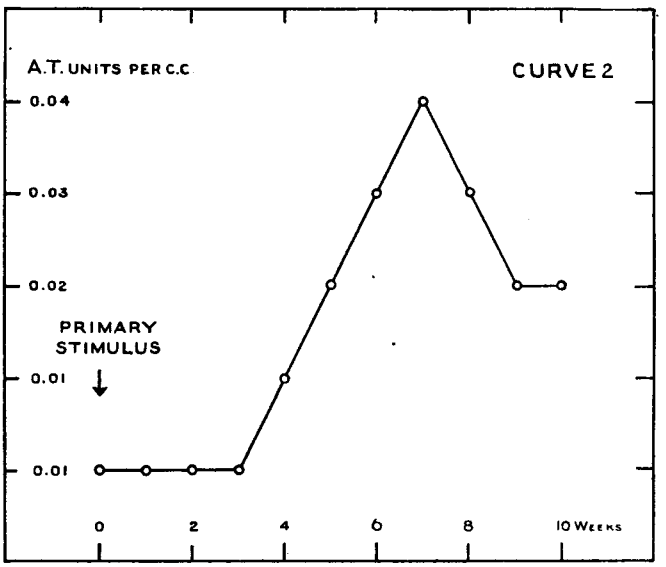
this mixture kills a guinea-pig in 60 hours. The results are recorded in Table XVII.

Table XVII.

Antitoxic value of goat No. 12 after an injection of a toxin-antitoxin mixture.

Weeks after injection	A.T. value per c.c.	Weeks after injection	A.T. value per c.c.
0	0·00 unit	6	0·03 unit
1	0·00 „	7	0·04 „
2	0·00 „	8	0·03 „
3	0·00 „	9	0·02 „
4	0·01 „	10	0·02 „
5	0·02 „		

No antitoxin was detected until the fourth week after the injection, and the highest antitoxic value was recorded at the seventh week. These results are shown graphically in Curve 2.



CURVE 2. From results given in Table XVII, showing the antitoxic value of the serum of a goat possessing no normal antitoxin after an injection of a toxin-antitoxin mixture.

Latent period: 3 weeks. Maximum height: 7 weeks.

(15) *Horses, possessing no normal antitoxin, injected with toxin-antitoxin mixtures give results similar to those produced in guinea-pigs.*

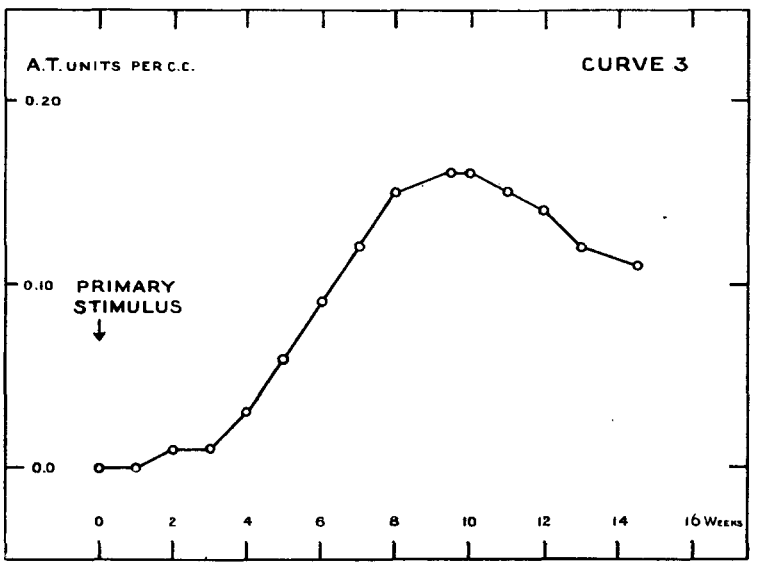
Horse 27 *Y*, in whose blood no normal antitoxin could be detected, since 1 m.l.d. of toxin mixed with 5 c.c. of its serum killed a guinea-pig, was injected with ten times a toxin-antitoxin mixture that was fatal to guinea-pigs. The dose consisted of 3·6 c.c. of toxin, J 176, together with 10 units of antitoxin. The results are recorded in Table XVIII.

Table XVIII.

Antitoxic value of horse 27 *Y* after an injection of a toxin-antitoxin mixture.

Weeks after injection	Antitoxic value of serum	Weeks after injection	Antitoxic value of serum
0	0·00 unit per c.c.	8	0·15 unit per c.c.
1	0·00 ,,	9½	0·16 ,,
2	0·01 ,,	10	0·16 ,,
3	0·01 ,,	11	0·15 ,,
4	0·03 ,,	12	0·14 ,,
5	0·06 ,,	13	0·12 ,,
6	0·09 ,,	14½	0·11 ,,
7	0·12 ,,		

These results are shown graphically in Curve 3.



CURVE 3. From results given in Table XVIII, showing the antitoxic value of the serum of a horse (possessing no normal antitoxin) after an injection of a toxin-antitoxin mixture. Latent period: 3 weeks. Maximum height: 10 weeks.

(16) *The amount of toxin injected, in relation to the size of the animal, is of importance in the production of immunity.*

A number of other animals that had been found to possess no normal antitoxin were injected with toxin-antitoxin mixtures as follows:

Table XIX.

Animal	Injection	Result
Goat No. 1	1 L + mixture (for a guinea-pig)	No antitoxin detected 9 weeks later
Sheep No. 4	1 ,, ,,	,, ,, 9 ,,
2 cows	1 ,, ,,	,, ,, 4 ,,
Horse No. 45	1 ,, ,,	,, ,, 14 ,,
,, No. 46	5 times ,, ,,	,, ,, 14 ,,
,, No. 47	1 ,, ,,	,, ,, 3 ,,

Horses Nos. 45, 46 and 47 were tested weekly.

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In all cases the dose employed was relatively small compared with the size of the animal; it is probable that some small amount of antitoxin was produced too small to be detected by the usual tests. That some degree of immunity was produced by these injections is evident from the results of a second injection into the goat, sheep and horse No. 47, recorded later under statements 29 and 31.

That the amount of toxin injected is of importance can be shown by the fact that among guinea-pigs tested for tolerance after an injection of a toxin-antitoxin mixture causing local reaction, we found in the course of our work that those that had received only a fraction of a test dose of toxin showed far less immunity than those receiving a full dose. This point was also brought out in connection with the immunity transmitted to the offspring ("Immunity of the Guinea-pig to Diphtheria Toxin and its Effects on the Offspring," *Journal of Hygiene*, vol. XI, No. 2, p. 226).

The importance of the amount of toxin injected may account for the difficulty of immunising with sublethal doses of toxin, and the comparative ease of immunising with toxin rich in toxoid where a number of binding units can be given at a single injection.

(*b*) Antitoxin injected before toxin.

Guinea-pigs.

(17) *Immunity may be produced by an injection of toxin into guinea-pigs that have previously received heterologous antitoxin; the results are not uniform.*

Five guinea-pigs that had been injected with antitoxic horse serum and subsequently injected with toxin were tested for immunity. Immunity was produced in one case only, recorded in Table XX and Curve 4.

Table XX.

Antitoxic value of guinea-pig *Z* 22. vii. injected with 1000 units diphtheria antitoxic horse serum, and ten days later (when very little antitoxin remained) injected with 0·1 c.c. toxin. Large local reaction, no change in weight.

Weeks after injection of toxin	Weight of guinea-pig		Antitoxic value of blood
2	340 grms.		0·0 unit per c.c.
3	365 „		0·0 „
4	395 „		0·0 „
5	430 „		0·05 „
6½	445 „		0·14 „
7	470 „		0·17 „
8	485 „		0·25 „
12	545 „	greater than	0·35 „
12½	575 „	„	0·84 „
13	—	less than	0·70 „
14	615 „		0·65 „
31	—		0·21 „

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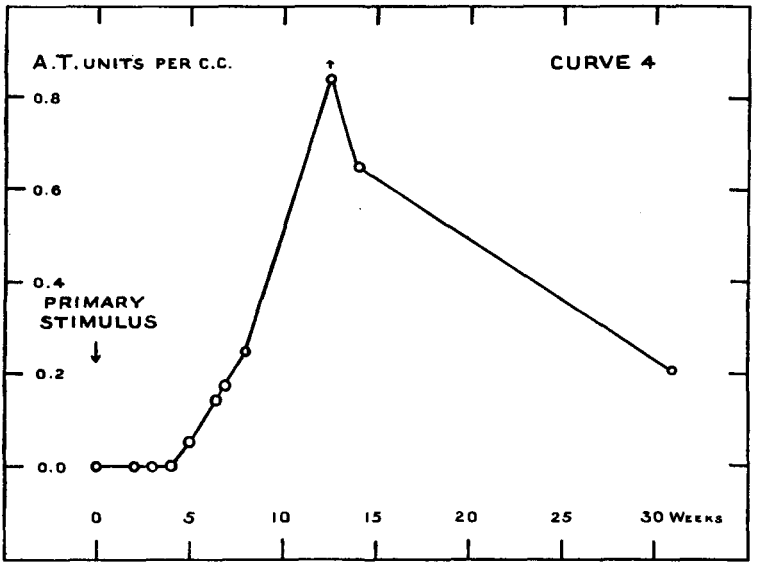
As will be seen from Table XX and from Curve 4, no immunity was evident until more than four weeks after the toxin injection, and the antitoxic value continued to rise until the maximum was reached sometime between the eighth and the thirteenth week.



CURVE 4. From results given in Table XX, showing the antitoxic value of the blood of a guinea-pig injected with toxin, ten days after it had received an injection of antitoxin.

Latent period: 4 weeks. Maximum height: 8th–12th week.

In four other cases, no immunity could be detected, due possibly to over-neutralisation of the toxin by the circulating antitoxin, or in some cases to too small a dose of toxin. The various guinea-pigs are compared below.

Table XXI.

Guinea-pig	A.T. value at time of injection	Volume of toxin	Result	Immunity
TT 14. iii. 12	Faint trace	0·01 c.c.	LS + 10	nil
P 22. vii. 12	Under 0·3 unit	0·1 c.c.	LS + 15	nil
Z 22. vii. 12	,, 0·5 ,,	0·1 c.c.	LS 0	Over 0·84 unit per c.c.
Z 30. ix. 12	,, 0·7 ,,	2·0 c.c.	LS – 5	nil
N 14. iii. 12	8·0 ,,	5·0 c.c.	trace 0	nil

In this method, toxin has been introduced under cover of circulating heterologous antitoxin which is eliminated at a rapid rate; the results are very uncertain. In the next section, the circulating antitoxin is homologous and is not eliminated so rapidly; in this case the results are fairly uniform.

Table XXII. Results of injection of toxin into ten guinea-pigs passively immune by maternal transmission. Arranged in order of the highest antitoxic value produced.

Guinea-pig	*A* 18.7 D	*OO* 12.7 C	*C* 6.9 B	*OO* 12.7 D	*A* 18.7 C	*OO* 12.7 A	*C* 6.9 A	*N* 21.7 C	*A* 18.7 B	*K* 11.7 A
Antitoxic value at time of injection units per c.c. of blood	0·04	0·26	0·04	0·26	0·04	1·5	0·04	0·07	0·04	0·92
Volume of toxin injected	0·1 c.c.	0·1 c.c.	0·05 c.c.	1·0 c.c.	0·5 c.c.	2·0 c.c.	0·1 c.c.	0·5 c.c.	0·1 c.c.	8·0 c.c.
Resulting local reaction	*trace*	*nil*	VLS	VLS	VLS	LS	SS	VLS	VLS	VLS
Change in weight in grms.	+20	+15	0	−20	+15	+5	+15	+25	−25	0

Weeks after toxin injection	Antitoxic value in units per c.c. of blood									
1	.	.	.	.	0·0	.	.	.	.	.
1½	.	.	.	.	.	0·0	0·0	.	.	0·0
2	.	0·04	0·0	0·04	0·0	0·0	.	.	.	0·0
2½	0·0	.	.	.	.	0·0	.	0·04	0·04	.
3	.	0·04	.	0·04	.	.	.	.	.	.
3½	0·0	.	.	.	0·04	0·0	.	0·09	.	.
4	.	.	.	.	.	.	.	0·17	.	.
4½	0·0	0·04	.	0·04	.	.	.	0·21	.	.
5	.	.	.	.	0·05	.	.	.	.	.
5½	.	0·04	0·05	0·05	0·11	.	.	0·32	0·35	.
6	.	0·055	.	0·055	.	0·0	.	0·70	0·70	.
7	0·0	0·11	.	0·14	0·17	.	0·07	.	.	.
8	.	.	0·11	.	.	.	0·10	0·75	0·70	0·35
9	.	.	0·21	.	.	0·0	0·21	.	.	0·86
10	.	0·06	0·21	0·09	.	0·05	0·36	.	.	1·4
12	0·0	.	0·20	.	.	.	0·46	.	0·81	1·5
14	.	.	.	.	0·35	0·11	.	.	0·87	.
16	.	0·09	.	0·25	.	0·17	.	.	0·97	.
18	.	.	.	.	.	.	0·44	.	.	.
20	.	.	.	.	.	0·21	.	0·48	0·94	.
25	.	.	.	.	.	0·30	0·44	.	.	.
30	.	.	.	.	.	0·38	.	.	.	1·3
40	.	.	.	.	.	0·38	0·15	.	.	.
90	.	.	.	.	.	0·20	.	.	.	.

Note. A recorded antitoxic value of 0·04 unit may be of no significance owing to the limits of accuracy in testing very small quantities of antitoxin with little blood available for each test.

(*c*) ANTITOXIN PRESENT IN THE FORM OF PASSIVE IMMUNITY TRANSMITTED FROM MOTHER.

Guinea-pigs.

(18) *A high degree of immunity sometimes results from a single injection of toxin in a guinea-pig passively immune by maternal transmission.*

Ten guinea-pigs that had been bred from actively immune mothers were injected with toxin as soon as they reached a weight of 250 grms., and the antitoxic value of their blood subsequently tested. The results are given in Table XXII. It will be seen that in several instances the antitoxic value of the blood was over $\frac{1}{2}$ unit per c.c., and in one case $1\frac{1}{2}$ units.

It should be noted that the guinea-pigs recorded above were passively immune by maternal transmission and had been slowly eliminating antitoxin since birth. Upon injection of toxin, further loss occurs owing to neutralisation; in no case was any appreciable antitoxin detected two weeks after the injection. If no toxin had been injected, antitoxin would certainly have been present in *OO* 12.7 A and *K* 11.7 A for a few weeks later.

The above results are summarised in Table XXIII.

Table XXIII.

Summary of results recorded in Table XXII, of injection of toxin into ten guinea-pigs passively immune by maternal transmission.

Guinea-pig	Vol. of toxin c.c.	Result	A.T. value at time of injection units per c.c. of blood	Highest value reached units per c.c. of blood	Latent period weeks	Interval before highest value reached weeks
A 18.7 D	0·1	trace + 20 grms.	0·04	nil	—	—
OO 12.7 C	0·1	nil + 15 ,,	0·26	0·11	$5\frac{1}{2}$	7
C 6.9 B	0·05	VLS 0 ,,	0·04	0·21	about $5\frac{1}{2}$	9
OO 12.7 D	1·0	VLS − 20 ,,	0·26	0·25	$4\frac{1}{2}$	about 16
A 18.7 C	0·5	VLS + 15 ,,	0·04	0·35	$3\frac{1}{2}$–5	about 14
OO 12.7 A	2·0	LS + 5 ,,	1·5	0·38	9	30
C 6.9 A	0·1	SS + 15 ,,	0·04	0·46	less than 7	12
N 21.7 C	0·5	VLS + 25 ,,	0·07	0·75	$2\frac{1}{2}$–$3\frac{1}{2}$	8
A 18.7 B	0·1	VLS − 25 ,,	0·04	0·97	$2\frac{1}{2}$–$5\frac{1}{2}$	16
K 11.7 A	8·0	VLS 0 ,,	0·92	1·5	2–8	12

The figures given in this table cannot be taken as exact because in several cases the curve was not fully worked out, and some figures depend upon one result only.

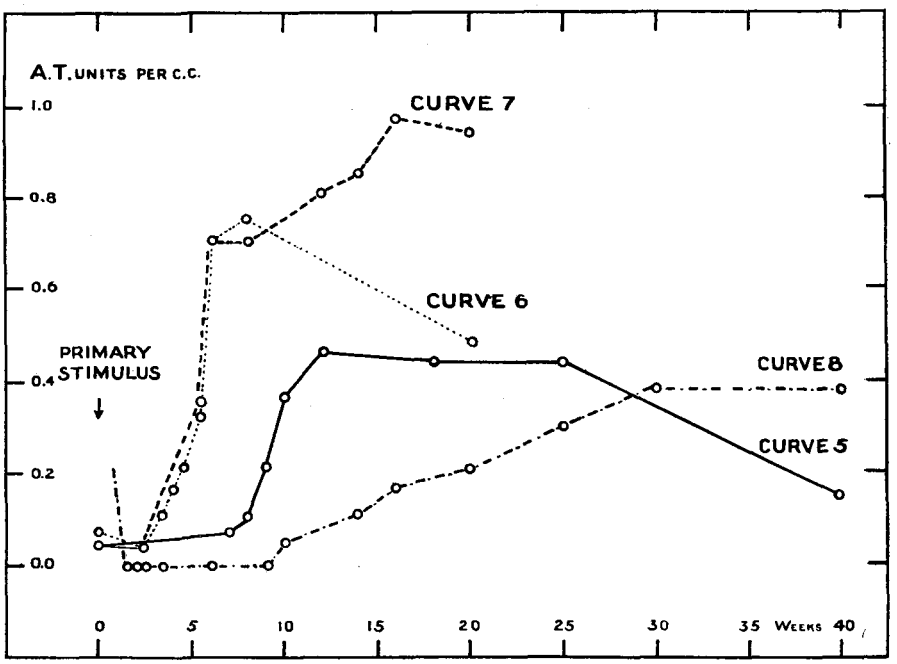
(19) *The amount of toxin injected must be large in proportion to the antitoxin present in order that immunity may result.*

No definite conclusion can be drawn from Table XXIII, except that the dose of toxin should be high in proportion to the antitoxin present in order that immunity may result. In other words, it would appear that a local reaction must be produced by the toxin. In the first two cases in the table,

no local reaction occurred, and little or no antitoxin resulted. Guinea-pig *C* 6.9 A appears exceptional in that a high degree of immunity resulted from an injection causing only a small local reaction. In this case, the original antitoxic value of the guinea-pig was low. Two guinea-pigs *OO* 12.7 C and *OO* 12.7 D are interesting in that they were from the same litter and injected at the same time when the antitoxic content of the blood was the same. The higher immunity (0·25 unit compared with 0·11) is shown in the guinea-pig which had received the larger toxin dose.

(20) *Comparatively little immunity is produced during the first four weeks after the injection.*

Table XXIII shows the long delay, which may vary from three to nine weeks after the injection, before the appearance of antitoxin. The average latent period appears to be about five weeks. Curves 5 and 6 depicting



CURVES 5, 6, 7 and 8. Showing results of injection of toxin into guinea-pigs *C* 6.9 A, *N* 21.7 C, *A* 18.7 B, *OO* 12.7 A, passively immune by maternal transmission.

	Curve 5	Curve 6	Curve 7	Curve 8
Latent period	7 weeks	2½–3½ weeks	2½–5½ weeks	9 weeks
Maximum height	12th week	8th week	16th week	30 „

C 6.9 A and *N* 21.7 C respectively are given as typical of this immunity curve. A comparison of these curves with Curves 1–4 will show the resemblance of all immunity curves resulting from a single injection of toxin under cover of antitoxin. Two atypical curves are also given; Curve 7 shows that in the

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case of *A* 18.7 B, the usual rapid rise was followed by a long gradual rise; Curve 8 shows an unusual reaction of guinea-pig *OO* 12.7 A, from the ninth to the thirtieth week a gradual increase occurred following a prolonged latent period.

(21) *Summary of Part I. The primary stimulus in guinea-pigs, rabbits, goats and horses is followed by a latent period of about three weeks, and the maximum immunity is reached in about eight weeks.*

Table XXIV summarises the latent period and times of maximum height of immunity for the animals dealt with in Part I. It will be seen that the latent period may be as short as two weeks or as long as nine weeks, but in the majority of instances, it is of three weeks duration. The maximum immunity is reached in one instance in five weeks, usually in from eight to twelve weeks, and in an exceptional instance not until thirty weeks have elapsed.

Table XXIV.

Summary of latent periods and times of maximum height of immunity following the primary stimulus in different animals.

Animal	Nature of stimulus	Reference	Latent period weeks	Maximum height weeks
Guinea-pig	Toxin-antitoxin mixture	Curve 1	3	8–12
„	„ „	Table IX	over 4	over 8
Rabbit	„ „	„ XIV	about 2	„ 6
„	„ „	„ XVI	2–3	5
Goat	„ „	Curve 2	3	7
Horse	„ „	„ 3	3	10
Guinea-pig	Toxin subsequent to antitoxic horse serum	„ 4	4	8–12
„	Toxin in animals passively immune by maternal transmission ...	„ 5	under 7	12
„	„ „ ...	„ 6	$2\frac{1}{2}$–$3\frac{1}{2}$	8
„	„ „ ...	„ 7	$2\frac{1}{2}$–$5\frac{1}{2}$	16
„	„ „ ...	„ 8	9	30

PART II.

INJECTION OF TOXIN INTO ACTIVELY IMMUNE ANIMALS

Guinea-pigs.

(22) *An injection of toxin into an actively immune guinea-pig produces far greater immunity than an injection into a passively immune guinea-pig.*

The highest immunity, recorded in Part I of this paper, produced by an injection of toxin into a passively immune guinea-pig was 1·5 units of antitoxin per 1 c.c. blood. From subsequent tables it will be seen that an injection of toxin into an actively immune guinea-pig produces far greater immunity; as much as 80 units per c.c. of blood may be produced. Reference

Table XXV. Results of injection of toxin into eight guinea-pigs actively immunised by an injection of a toxin-antitoxin mixture.

Guinea-pig	*Z* 7.11	*C* 3.7	*T* 3.7	*K* 3.7	*B* 7.11	*X* 3.7	*R* 3.7	*KK* 7.11
Primary stimulus (injection of toxin antitoxin mixture)								
Toxin	0·36 c.c.	0·36 c.c.	0·36 c.c.	0·36 c.c.	0·36 c.c.	0·36 c.c.	0·36 c.c.	0·36 c.c.
Antitoxin	1·2 units	1·2 units	1·2 units	1·3 units	1·5 units	1·5 units	1·5 units	1·5 units
Local reaction	Nil	Trace	SS	Trace	Nil	Nil	Nil	Nil
Change in weight	+15	+10	0	+10	+40	+35	+20	+15
Highest A.T. value	0·25 unit	0·10 unit	0·20 unit	0·11 unit	0·09 unit	0·25 unit	0·07 unit	0·07 unit
Secondary stimulus. Injection of toxin								
Interval between the primary and secondary stimulus	144 days	93 days	105 days	106 days	145 days	93 days	105 days	130 days
Volume of toxin injected	2·0 c.c.	2·0 c.c.	2·0 c.c.	2·0 c.c.	2·0 c.c.	2·0 c.c.	2·0 c.c.	2·0 c.c.
Local reaction	VLS	VLS	VLS	VLS	VLS	VLS	VLS	VLS
Change in weight	−5	−55	−50	−20	0	+10	−30	−40
Antitoxin produced								
5 days after toxin injection	—	*< 1·7 units	0·7 unit	1·1 units	—	—	0·7 unit	—
6 „ „	—	—	—	—	—	—	—	—
7 „ „	—	> 1·7 „	7·5 units	9·0 „	> 10 units	> 1·7 units	2·0 units	—
8 „ „	> 20 units	—	—	—	—	—	—	—
9 „ „	—	—	22·0 „	15·0 „	> 20 „	—	3·0 „	—
10 „ „	70 „	> 10 „	—	—	—	8·3 „	—	—
11 „ „	—	—	—	—	< 25 „	—	—	—
12 „ „	75 „	—	28·0 „	16·0 „	—	8·7 „	> 3·5 „	—
13 „ „	—	—	—	—	—	—	—	—
14 „ „	80 „	> 20 „	28 „	14 „	—	—	< 6·0 „	0·7 unit
15 „ „	—	—	—	—	—	—	—	—
16 „ „	—	—	22 „	—	—	—	—	—
17 „ „	—	> 40 „	—	—	—	—	—	—
18 „ „	—	—	—	—	—	—	—	—
19 „ „	—	< 62 „	—	11 „	—	—	—	—
20 „ „	—	—	21 „	—	—	—	> 3·5 „	—
23 „ „	—	—	20 „	—	—	—	—	—
26 „ „	—	—	18 „	8 „	—	—	—	—
36 „ „	—	—	12 „	6 „	—	—	—	—
47 „ „	—	—	7·5 „	4·5 „	—	—	—	—
61 „ „	—	—	7·0 „	3·2 „	—	—	—	—
83 „ „	—	—	3·3 „	1·7 „	—	—	—	—
293 „ „	—	—	0·2 unit	—	—	—	—	—

* N.B. >, < indicate "greater than," "less than" respectively.

has already been made to a group of guinea-pigs injected with one test dose of toxin together with varying excess of antitoxin, and subsequently injected with 2 c.c. of toxin (see Table X). The antitoxic value of the blood of these guinea-pigs was tested after the injection of toxin; the results are recorded in Table XXV and summarised in Table XXVI.

Table XXVI.

Summary of results of injection of toxin into eight guinea-pigs actively immunised by an injection of a toxin-antitoxin mixture.

	Primary stimulus		Secondary stimulus		
Guinea-pig	Antitoxin in original mixture. Units	Antitoxic value produced by mixture. Unit per c.c. of blood	Result of injection of 2 c.c. toxin	Antitoxic value produced by toxin. Units per c.c. of blood	Approximate time taken to reach highest value
Z 7.11	1·2	0·25	VLS – 5	80	14 days
C 3.7	1·2	0·10	VLS – 55	40–60	over 5 „
T 3.7	1·2	0·20	VLS – 50	28	12 „
K 3.7	1·3	0·11	VLS – 20	16	12 „
B 7.11	1·5	0·09	VLS 0	20–25	about 10 „
X 3.7	1·5	0·25	VLS + 10	8·7	12 „
R 3.7	1·5	0·07	VLS – 30	3·5–6	over 9 „
KK 7.11	1·5	0·07	VLS – 40	0·7	about 14 „

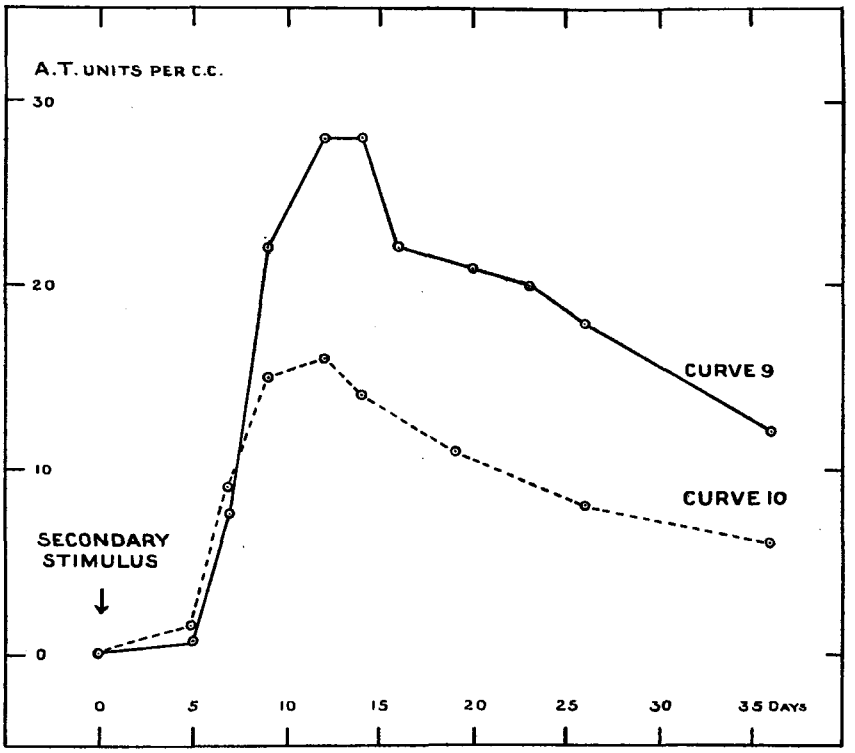
In addition to the animals recorded in these tables, four other guinea-pigs that had received toxin-antitoxin mixtures, causing local reaction, were injected with toxin 12 weeks after the mixture injection. From 20–60 units of antitoxin per c.c. of blood resulted.

(23) *A marked increase of antitoxin occurs within a few days of an injection of toxin into an actively immune guinea-pig and the maximum is reached in about* 12 *days.*

In passively immune guinea-pigs little or no antitoxin was produced until three or four weeks after injection; eight weeks at least elapsed before the maximum was reached. In the actively immune guinea-pigs recorded in Tables XXV and XXVI an increase in antitoxic value occurred within five days after injection and the maximum was reached about 12 days after. In two cases, where sufficient determinations were made, curves have been plotted. It has been necessary to alter considerably the scale of these curves from that of curves 1–8. The period covered by the earlier curves was 12 weeks or more and in no case was a higher antitoxic value than 1 unit depicted. In the curves here shown a period of only five weeks is covered and an antitoxic value of 28 units recorded.

Curve 9 represents guinea-pig *T* 3.7 and Curve 10 guinea-pig *K* 3.7.

The marked difference between the immunity response to the primary and to the secondary stimulus is shown in Curve 11 giving the antitoxic value of guinea-pig *K* 3.7 during its course of treatment.



CURVES 9 and 10. Injection of toxin into actively immune guinea-pigs *T* 3.7 and *K* 3.7 respectively.

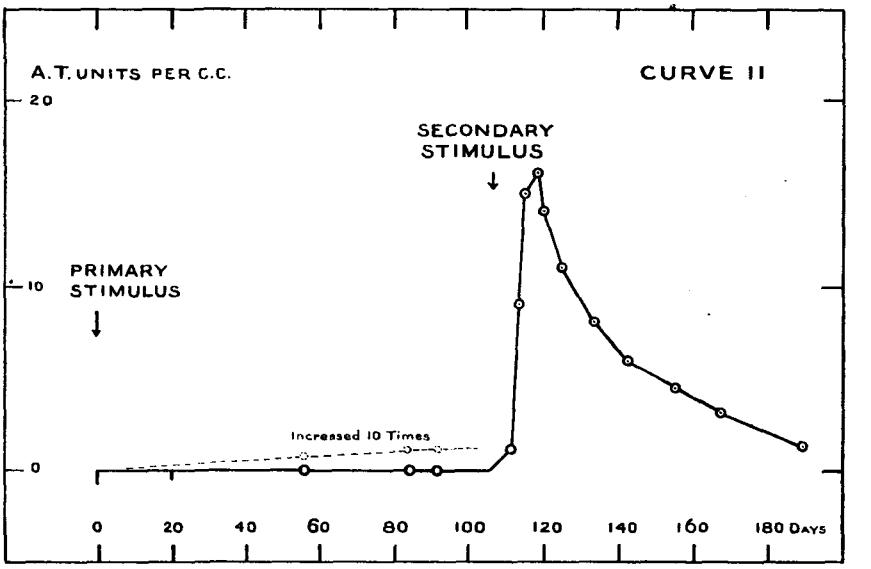
	Curve 9	Curve 10
Latent period	About 5 days	Under 5 days
Maximum height	12 days	12 days

Comparing the two sections of the curve it is seen that the latent period following the injection into the normal animal is approximately five weeks while that following the second injection when the animal is already actively immune is less than five days. The highest value is reached in the one case in 12 weeks and in the other case in 12 days; the highest value after the first injection is 0·11 unit per c.c. and after the second injection 16 units.

(24) *The degree of immunity produced depends partly upon the constitution of the original immunising mixture.*

Neither by tests of antitoxic value nor by the reactions produced by the toxin injection could much distinction be made between the immunity produced in normal guinea-pigs by the injection of toxin-antitoxin mixtures containing 1·2 units of antitoxin and those containing 1·5 units (see Tables X and XI). The antitoxin produced, however, by the injection of 2 c.c. of toxin at least 13 weeks later, differentiates the groups strongly. The average production of antitoxin as a result of the subsequent injection of 2 c.c. of

toxin in the two sets of guinea-pigs, the one of which received a preliminary L0 mixture, while the other received an over-neutralised mixture, is greater in the former than in the latter set.



CURVE 11. Showing the antitoxic value of guinea-pig *K* 3.7 after an injection of a toxin-antitoxin mixture followed 15 weeks later by an injection of toxin.
In the dotted line after the primary stimulus the ordinates are increased tenfold in order to make apparent the shape of the curve.

	Response to primary stimulus	Response to secondary stimulus
Latent period	Under 8 weeks	Under 5 days
Maximum height	About 12 „	12 days
Maximum unitage	0·11 unit	16 units

The three guinea-pigs originally injected with mixtures containing 1·2 units of antitoxin, produced, as a result of the injection of 2 c.c. of toxin, 28, 40–60, 80 (average 50) units of antitoxin per 1 c.c. of blood. Four guinea-pigs of the other group originally injected with mixtures containing 1·5 units antitoxin eventually produced as a result of the toxin injection 0·7, 4–6, 8·7 and 20–25 (average 10) units of antitoxin.

(25) *Relatively high immunity similarly results from the injection of toxin-antitoxin mixtures into actively immune guinea-pigs.*

High immunity may result from a L0 mixture in place of an injection of toxin in actively immune guinea-pigs. Table XXVII gives the results from injecting L0 mixtures into guinea-pigs that had previously received toxin-antitoxin mixtures.

In Table XXVII the five guinea-pigs are arranged in order of magnitude of immunity response to the secondary stimulus; this order is the same as

that of the time interval elapsing between the primary and the secondary stimulus and of the antitoxic values of the guinea-pigs at the time of the second injection. In Part I it has been shown that the maximum antitoxic value is reached about 12 weeks after the primary stimulus; it would appear therefore that the first two guinea-pigs in the table had already achieved their

Table XXVII.

Results of injecting toxin-antitoxin mixture into five guinea-pigs actively immunised by a previous injection of a toxin-antitoxin mixture.

	Primary stimulus				Secondary stimulus		
Guinea-pig	Antitoxin in mixture with 1 test dose of toxin	Result	Interval weeks	A.T. value at time of second injection	Antitoxin in mixture with 1 test dose of toxin units	Result	Highest value reached units
R 26. 8	less than 1·2 units	LS −21	13	0·60 unit	1·3	nil + 15	20–25
H 15. 8	more than 1·2 „	nil − 7	13	0·35 „	1·3	nil + 5	20
K 5. 9	less than 1·2 „	LS −33	11	0·16 „	1·3	nil + 40	1·4
L 22. 8	1·1 „	VLS − 1	9	less than 0·07 „	1·3	nil + 50	1·0
O 21. 8	1·3 „	nil − 2	9	„ 0·07 „	1·3	nil + 5	0·7

maximum response to the primary stimulus before the injection of the secondary stimulus. The remaining three animals gave only a poor response to the secondary stimulus received before the maximum effect of the primary stimulus was reached. The conclusion is that an injection given on a rising antitoxin curve is less effective than an injection given when the maximum height has been reached.

(26) *Two injections of a neutral mixture into normal guinea-pigs do not produce any higher immunity than a single injection unless sufficient time elapses between the two injections.*

Three guinea-pigs (of the group marked 7.11 of which *Z*, *B*, *KK* in Table XXV also formed a part) that had received toxin-antitoxin mixtures containing 1·5 units of antitoxin were injected five months later with a similar mixture. These animals produced 0·7, 0·7 and 1·7 units of antitoxin per c.c. of blood. Another set of the same group received a second injection of the mixture seven days after the first injection. Their antitoxic values later were 0·0, 0·11 and 0·14 unit per c.c.; this level is no higher than that reached by animals that received one injection only.

It is of interest here to summarise the results obtained from this group of guinea-pigs.

Table XXVIII.

Summary of results obtained from a single group of guinea-pigs (marked 7.11).

1st injection	Interval	2nd injection	Antitoxin produced
One L0 mixture	—	—	A.T. of the order of 0·1 unit
L0 mixture	7 days	L0 mixture	„ „ 0·1 „
„	Several months	„	„ „ 1·0 „
„	„	Toxin (causing large local reaction)	„ „ 10·0 units

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From this table it is seen that if a similar L0 mixture is repeated with an interval of one week only, no higher immunity is ultimately reached than when one injection only is given, whereas if an interval of several months elapses between the two injections the resulting immunity is of a much higher order, being ten times the amount in the particular experiment. This shows that a second injection given during the latent period following the first injection acts as a primary and not as a secondary stimulus.

(27) *If a long interval of time (a year or more) elapse before the second injection, high immunity is likewise produced.*

The cases recorded so far were of those with an interval of a few months only between the two injections, that is to say, the second injection was given before there was any appreciable drop in the immunity curve resulting from the first injection. An experiment was made to see whether the same effect would be produced on a dropping curve.

Guinea-pig 223 *LA* 1. Immunised by a series of injections, in Oct. 1911; 14 units per c.c. resulted. In August 1913 when the antitoxic value had fallen to 0·11 unit per c.c., 2 c.c. of toxin were injected causing very large local reaction; over 20 units A.T. resulted.

A number of other actively immune guinea-pigs were injected but were not individually tested. The pooled serum from these guinea-pigs bled ten days after the injection of toxin contained 30 units per c.c. In all cases at least nine months, and in the majority of cases, over a year had elapsed since the previous injection.

Rabbits.

(28) *The injection of toxin into actively immune rabbits gives results similar to those obtained with guinea-pigs.*

Six of the rabbits previously mentioned (Tables XIV and XV) that had received toxin-antitoxin mixtures were injected 9 to 16 weeks later with 2 c.c. toxin; all developed high immunity within ten days as recorded in Table XXIX.

Table XXIX.

Results of injecting toxin into six rabbits actively immunised by an injection of a toxin-antitoxin mixture.

Rabbit	Change in weight 5 days after the injection of 2 c.c. toxin	Maximum antitoxic value reached	Maximum height reached in days after toxin injection
1	−340 grms.	65 units	10
2	+ 2 „	50 „	8
3	−130 „	22 „	8
9	−135 „	28 „	9
11 } pooled	−365 „	50 „	10
12 } pooled	− 85 „		

Table XXX records in detail the results given by two of these rabbits. The antitoxic values are plotted in Curves 12 and 13.

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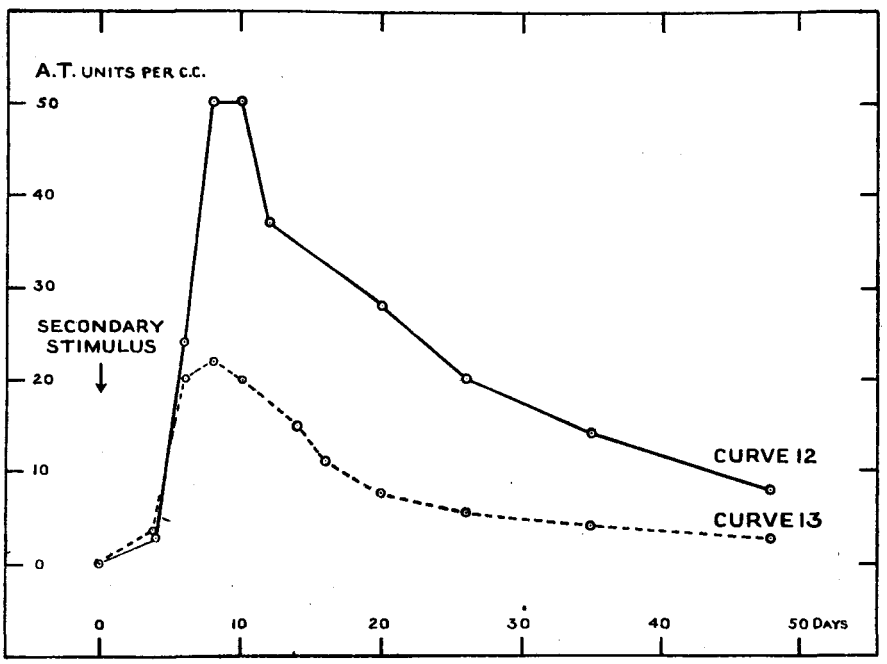
Table XXX.

Results of injecting toxin into two rabbits actively immunised by an injection of a toxin-antitoxin mixture.

Primary stimulus. Injection of toxin-antitoxin mixture	Rabbit 2	Rabbit 3
Toxin	0·36 c.c.	0·36 c.c.
Antitoxin	1 unit	1 unit
Local reaction...	4 × 4 sq. cm.	7 × 6 sq. cm.
Change in weight	−75 grms.	−110 grms.
Highest A.T. value	0·12 unit per c.c.	0·10 unit per c.c.
Secondary stimulus. Injection of toxin		
Interval between primary and secondary stimulus	13 weeks	13 weeks
Volume of toxin injected	2 c.c.	2 c.c.
Local reaction...	4 × 6 sq. cm.	3 × 3 sq. cm.
Change in weight	+10 grms.	−130 grms.
Antitoxin produced		
4 days after toxin injection	2·8 units	3·2 units
6 „ „	24 „	20 „
8 „ „	50 „	22 „
10 „ „	50 „	20 „
12 „ „	37 „	—
14 „ „	—	15 „
16 „ „	—	11 „
18 „ „	—	—
20 „ „	28 „	7·5 „
26 „ „	20 „	5·5 „
35 „ „	14 „	4·0 „
48 „ „	8 „	2·8 „



CURVES 12 and 13. Injection of toxin into actively immune rabbits 2 and 3 respectively.

	Curve 12	Curve 13
Latent period	Under 4 days	Under 4 days
Maximum height	8 days	8 days

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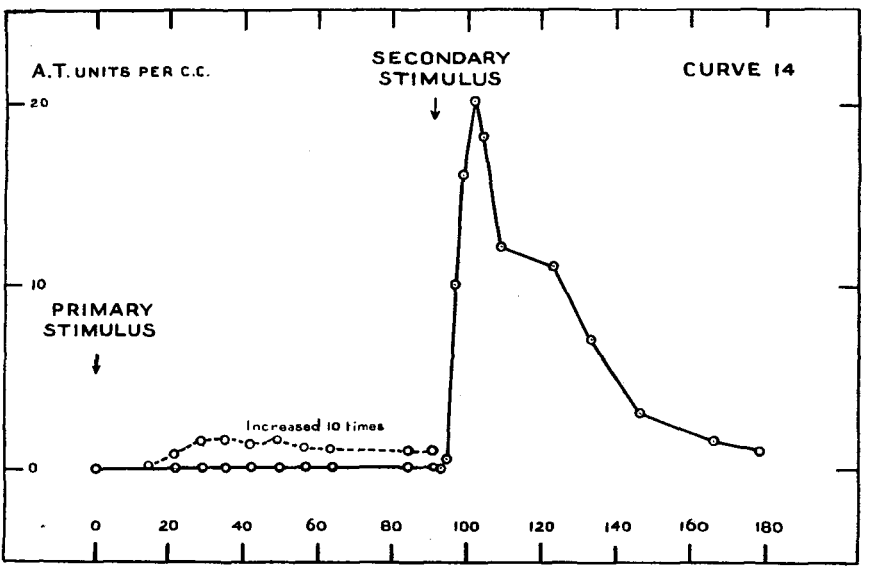
Table XXXI.

Results of injecting toxin into splenectomised rabbit number 16 actively immunised by an injection of a toxin-antitoxin mixture.

Primary stimulus		
Toxin		0·36 c.c.
Antitoxin		1 unit
Highest value		0·16 unit per c.c.
Secondary stimulus		
Interval between primary and secondary stimulus		13 weeks
Volume of toxin injected		2 c.c.
Antitoxic value		
Before injection		0·10 units per c.c.
2 days after		0·04 „
4 „		0·5 „
6 „		10·0 „
8 „		16·0 „
11 „		20 „
13 „		18 „
18 „		12 „
32 „		11 „
42 „		7 „
56 „		3 „
75 „		1·5 „
88 „		1·0 „



CURVE 14. Showing the antitoxic value of a splenectomised rabbit after an injection of a toxin-antitoxin mixture followed 13 weeks later by an injection of toxin.
In the dotted line after the primary stimulus the ordinates are increased tenfold in order to make apparent the shape of the curve.

	Response to primary stimulus	Response to secondary stimulus
Latent period	3 weeks	4 days
Maximum height	5 „	11 „
Maximum unitage	0·16 unit per c.c.	20 units per c.c.

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A splenectomised rabbit (number 16) already mentioned in section 13, was injected with 2 c.c. of toxin 13 weeks after the original injection of a toxin-antitoxin mixture. The results are recorded in Table XXXI and Curve 14.

These results conform to those of normal rabbits and clearly show that the spleen plays no essential part in the production of immunity.

The test two days after the secondary stimulus shows the phenomenon of the negative phase.

Sheep and Goats.

(29) *A rapid formation of antitoxin occurs after the injection of toxin into actively immune sheep and goats.*

Goat number 12 recorded in Table XVII was injected with 2 c.c. of toxin ten weeks after the first injection of a toxin-antitoxin mixture. The result of the second injection is recorded in Table XXXII and Curve 15.

Table XXXII.

Results of injecting toxin into a goat actively immune from an injection of a toxin-antitoxin mixture.

Primary stimulus		
Toxin	3·8 c.c.	
Antitoxin	10 units	
Highest value	0·04 unit per c.c.	
Secondary stimulus		
Interval between primary and secondary stimulus	10 weeks	
Volume of toxin injected	2 c.c.	
Antitoxic value		
Before injection	0·02 units per c.c.	
3 days after	0·02	„
5 „	1·7	„
7 „	12·5	„
9 „	21·5	„
12 „	20	„
14 „	19	„
16 „	19	„
19 „	13·5	„
27 „	12·5	„
36 „	6	„
43 „	4	„
99 „	2	„
162 „	1	„

The sheep and goat already mentioned in Table XIX were subsequently injected with a second toxin-antitoxin mixture. Tests were made to determine whether a rapid production of antitoxin occurred but the curves were not followed out in detail.

Goat 1. Injected 0·36 c.c. toxin + 1 unit A.T. Nine weeks later no antitoxin detectable in serum. Injected 0·38 c.c. + 1 unit A.T. Nine days later, 0·5 unit. Fourteen days later, 0·65 unit.

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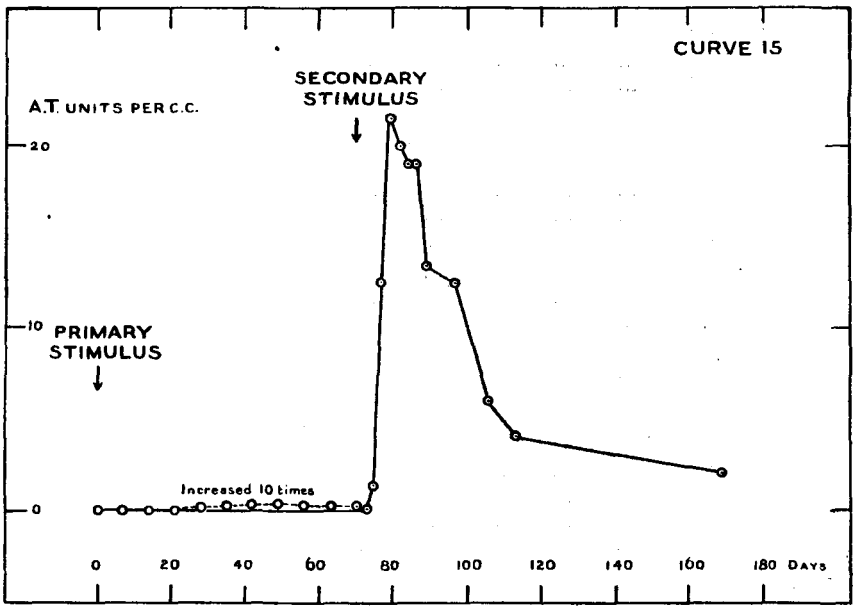
Sheep 4. Injected 0·38 c.c. toxin + 1 unit A.T. Nine weeks later no antitoxin detectable in serum. Injected 0·38 c.c. toxin + 1 unit A.T. Fourteen days later, 0·7 unit. Twenty-one days later, 0·7 unit. Thirty-five days later, 0·15 unit.



CURVE 15. Showing the antitoxic value of a goat after an injection of a toxin-antitoxin mixture followed 10 weeks later by an injection of toxin.

In the dotted line after the primary stimulus the ordinates are increased tenfold in order to make apparent the shape of the curve.

	Response to primary stimulus	Response to secondary stimulus
Latent period	3 weeks	Between 3 and 5 days
Maximum height	7 „	9 days
Maximum unitage	0·04 unit per c.c.	21·5 units per c.c.

In these animals the second injection nine weeks after the first acted as a secondary stimulus; it must be concluded therefore that active immunity resulted from the primary stimulus although the amount of circulating antitoxin was too small to be detected. The total quantity of toxin injected compared with the size of the animals was very small, being only just above the L + dose for a guinea-pig.

A cow was similarly treated, but only $3\frac{1}{2}$ weeks elapsed between the two injections; no antitoxin was subsequently detected.

Horses.

(30) *The second injection of a toxin-antitoxin mixture causes a rapid production of antitoxin in horses possessing no normal antitoxin.*

Horse 27 *Y* previously mentioned in Table XVIII was injected with ten times the L + mixture for a guinea-pig $14\frac{1}{2}$ weeks after the first injection.

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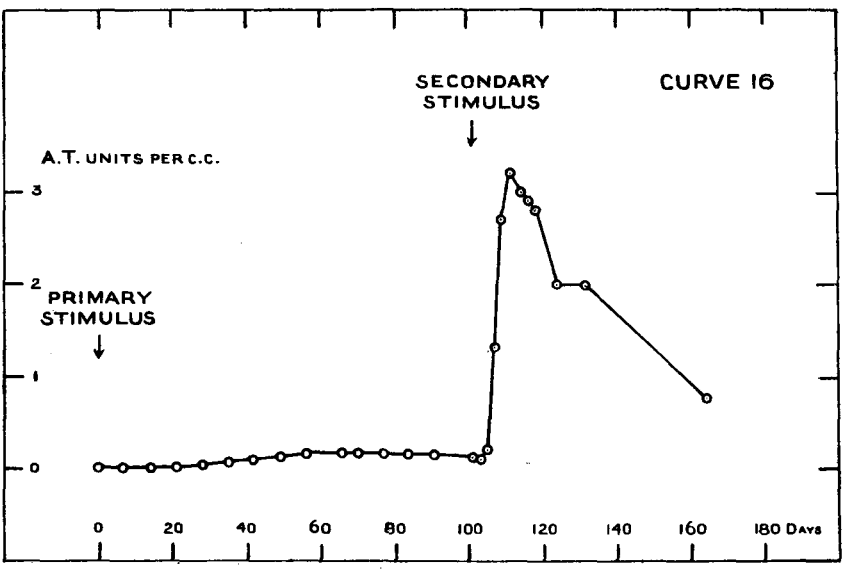
A rapid production of antitoxin occurred as recorded in Table XXXIII and Curve 16.

Table XXXIII.

Results of injection of a toxin-antitoxin mixture into a horse actively immunised by a previous injection of a toxin-antitoxin mixture.

Primary stimulus		
Toxin	... ...	3·6 c.c.
Antitoxin	... ...	10 units
Antitoxin produced	... ...	0·16 unit per c.c.
Secondary stimulus		
Interval between primary and secondary stimulus		14½ weeks
Toxin	... ...	3·6 c.c.
Antitoxin	... ...	10 units
Antitoxin produced		
Before second injection	... ...	0·11 units per c.c.
2 days after	... ...	0·10 „
4 „	... ...	0·2 „
6 „	... ...	1·3 „
8 „	... ...	2·7 „
10 „	... ...	3·2 „
13 „	... ...	3·0 „
15 „	... ...	2·9 „
17 „	... ...	2·8 „
23 „	... ...	2·0 „
31 „	... ...	2·0 „
63 „	... ...	0·75 „



CURVE 16. Showing the antitoxic value of a horse, originally possessing no normal antitoxin, after an injection of a toxin-antitoxin mixture, followed 14½ weeks later by a similar mixture

	Response to Primary stimulus	Response to secondary stimulus
Latent period	3 weeks	Between 2 and 4 days
Maximum height	9½ „	10 days
Maximum unitage	0·16 unit per c.c.	3·2 units per c.c.

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The curve for this horse is particularly interesting because both primary and secondary stimulus consisted of toxin-antitoxin mixtures of the same constitution.

(31) *The response to a second injection rapidly following the primary stimulus in a horse possessing no normal antitoxin is of an intermediate character between the usual responses following the primary and the secondary stimulus.*

A horse with no normal antitoxin (*H* 47) previously mentioned in Table XIX, was injected with a toxin-antitoxin mixture containing five L + doses for a guinea-pig three weeks after the initial injection of a mixture containing one L + dose. The antitoxin response is recorded in Table XXXIV and Curve 17.

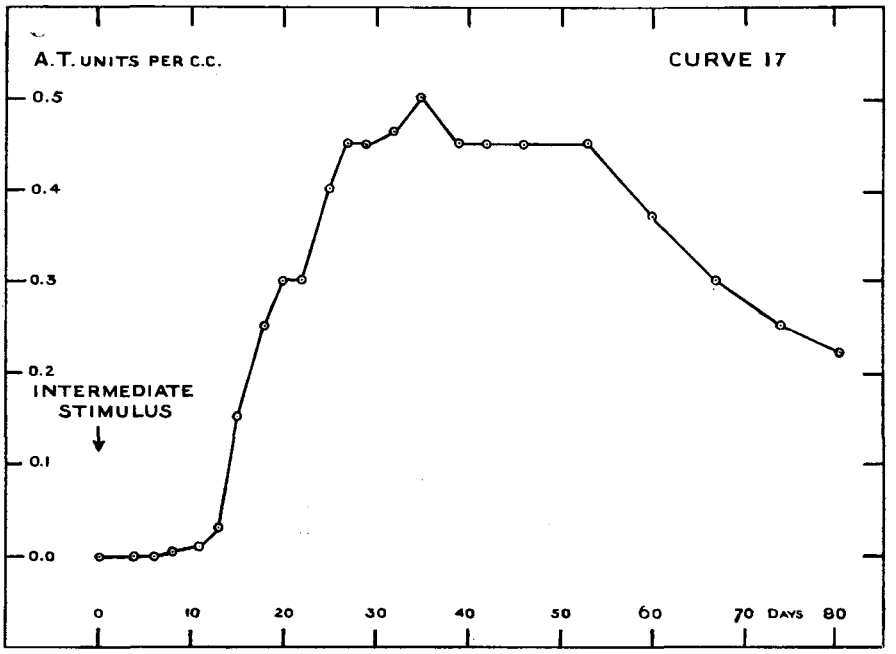
Table XXXIV.

Results of injection of a toxin-antitoxin mixture into a horse three weeks after the initial injection of a similar mixture.

Primary stimulus	
Toxin	0·36 c.c.
Antitoxin	1 unit
Antitoxin produced	Not detectable
Intermediate stimulus	
Interval between primary and intermediate stimulus	3 weeks
Toxin	1·8 c.c.
Antitoxin	5 units
Antitoxin produced	
4 days after second injection	0·00 unit per c.c.
6 „ „	0·00 „
8 „ „	0·005 „
11 „ „	0·01 „
13 „ „	0·03 „
15 „ „	0·15 „
18 „ „	0·25 „
20 „ „	0·3 „
22 „ „	0·3 „
25 „ „	0·4 „
27 „ „	0·45 „
29 „ „	0·45 „
32 „ „	0·46 „
35 „ „	0·50 „
39 „ „	0·45 „
42 „ „	0·45 „
46 „ „	0·45 „
53 „ „	0·45 „
60 „ „	0·37 „
67 „ „	0·30 „
74 „ „	0·25 „
81 „ „	0·22 „

The second injection in this horse was given when the animal was partially immunised and can be termed an intermediate stimulus. The latent period of eight days lies between the usual three weeks for a primary stimulus and

three days for secondary stimulus; similarly the maximum height was reached in 35 days, a period intermediate between ten days and eight weeks.



CURVE 17. Showing the antitoxic value of a horse, originally possessing no normal antitoxin, after a second injection of a toxin-antitoxin mixture three weeks after the first injection.

Latent period: 8 days. Maximum height: 35 days.

(32) *Summary of Part II. The secondary stimulus in guinea-pigs, rabbits, goats, sheep and horses is followed by a latent period of about four days and the maximum immunity is reached in about ten days.*

Table XXXV summarises the latent period and times of maximum height of immunity for the animals dealt with in Part II. It will be seen that after a secondary stimulus the latent period is never more than five days and the maximum height of immunity is reached in from eight to twelve days.

Table XXXV.

Summary of latent periods and times of maximum heights of immunity following the secondary stimulus in different animals.

Animal	Nature of stimulus	Reference	Latent period	Maximum height
Guinea-pig	Toxin	Curve 9	about 5 days	12 days
„	„	„ 10	under 5 „	12 „
Rabbit	„	„ 12	„ 4 „	8 „
„	„	„ 13	„ 4 „	8 „
„	„	„ 14	4 days	11 „
Goat	„	„ 15	3 to 5 days	9 „
Horse	Toxin-antitoxin mixture	„ 16	2 to 4 „	10 „

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PART III.

INJECTION OF TOXIN INTO ANIMALS POSSESSING NORMAL ANTITOXIN.

Horses.

(33) *The injection of toxin into horses possessing normal antitoxin produces a rapid increase in antitoxin.*

The effect of a single injection of toxin into a horse (*H* 32 v) naturally immune is recorded in Table XXXVI and Curve 18.

Table XXXVI.

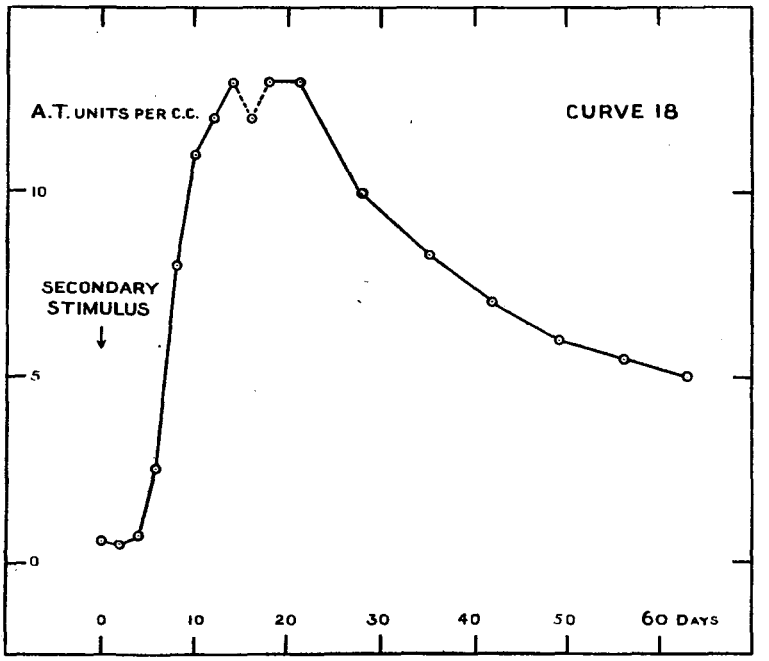
Results of injecting toxin into a horse naturally immune.

Primary stimulus. Probable infection in normal life	
Antitoxin produced	0·6 unit per c.c.
Secondary stimulus	
Interval between primary and secondary stimulus	Unknown
Toxin	10 c.c.
Local reaction	20 sq. in. lasting 3 days
Temperature reaction	Nil
Antitoxin produced	
2 days after injection	0·5 units per c.c.
4 „ „	0·65 „
6 „ „	2·5 „
8 „ „	8 „
10 „ „	11 „
12 „ „	12 „
14 „ „	13 „
16 „ „	12 „
18 „ „	13 „
21 „ „	13 „
28 „ „	10 „
35 „ „	8·3 „
42 „ „	7 „
49 „ „	6 „
56 „ „	5·5 „
63 „ „	5 „

The similarity of the curve of antitoxin produced in this horse to that obtained after a secondary stimulus in an animal actively immunised by a primary stimulus is strongly suggestive evidence that the antitoxin found in normal horses has been formed by a process of active immunisation.

A number of horses with varying normal antitoxin content were injected with 1 c.c. of weak toxin (*Y* 29: m.l.d. = 0·04 c.c.) and their blood tested nine or ten days later. In one case 20 units of antitoxin per c.c. of serum were produced after this single injection of less than one L + dose for a guinea-pig. The total serum content of a horse may be taken as 25 litres; 500,000 units were therefore produced, in other words a single injection of toxin stimulated the production of as much antitoxin as would neutralise

500,000 times the amount of toxin injected. The summary of results given in Table XXXVII shows that the majority of horses had developed considerable immunity within ten days after the injection.



CURVE 18. Showing the antitoxic value after an injection of toxin into a naturally immune horse. Latent period: 4 days. Maximum height: 14 days.

Table XXXVII.

Results of injecting toxin into 103 horses possessing normal antitoxin.

Increase of antitoxic value within ten days	Number of horses
Less than 0·5 unit per c.c.	23
Between 0·5 and 1·0 unit per c.c.	7
„ 1·0 „ 5·0 units per c.c.	55
Over 5·0 units per c.c.	18

A group of horses injected with 1 c.c. of the same weak toxin were examined more closely and are recorded in Table XXXVIII.

It is of interest to note that in four cases a fall in antitoxic value was recorded in the first three days after injection. No such alteration in normal antitoxic content has yet been noted during such a short time in untreated horses. The quantity of toxin injected (under one test dose) would, by neutralisation, only reduce the antitoxic value by 1/25,000 unit and this reduction would be undetected. It follows therefore that the phenomenon cannot be due only to neutralisation by the toxin, but must be regarded as an instance of the negative phase.

The above results are shown graphically in Curves 19–23.

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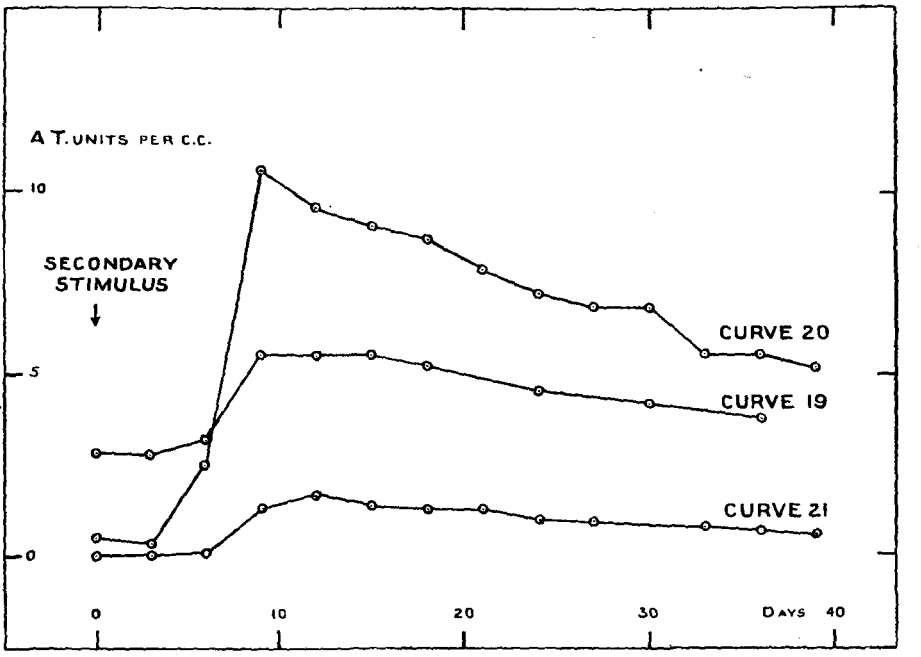
Table XXXVIII.

Results of injecting toxin into five horses possessing normal antitoxin.

	Horse 6 *N*	Horse 56 *C*	Horse 27 *S*	Horse 69	Horse 63
Normal antitoxic value in units per c.c.	2·8	0·5	0·05	0·05*	0·02
Volume of toxin injected	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Local reaction	4 sq. in.	6 sq. in.	32 sq. in.	192 sq. in.	225 sq. in.
Duration of local reaction	2 days	2 days	5 days	7 days	9 days
Rise in temperature ...	nil	nil	1° F.	1·8° F.	3·4° F.
	A.T. value produced in units per c.c.				
Days after injection					
3	2·75	0·35	0·03	under 0·02	under 0·02
6	3·2	2·5	0·08	0·03	0·02
9	5·5	10·5	1·3	0·03	0·02
12	5·5	9·5	1·7	0·04	0·02
15	5·5	9·0	1·4	0·03	0·04
18	5·2	8·7	1·3	0·06	0·07
21	—	7·8	1·3	0·12	0·10
24	4·5	7·2	1·0	0·12	0·07
27	—	6·8	0·9	0·12	0·07
30	4·2	6·8	—	0·10	—
33	—	5·5	0·8	0·10	0·05
36	3·75	5·5	0·7	0·10	—
39	—	5·2	0·6	0·08	0·05
42	3·2	4·7	0·5	0·08	—

* Probably an over-estimate—strong evidence in favour of normal value being 0·03 unit per c.c.



CURVES 19, 20, 21. Showing the antitoxic value of three naturally immune horses 6 *N*, 56 *C* and 27 *S* after an injection of toxin.

	Curve 19	Curve 20	Curve 21
Latent period	3–6 days	3–6 days	3–6 days
Maximum height	9 „	9 „	12 „

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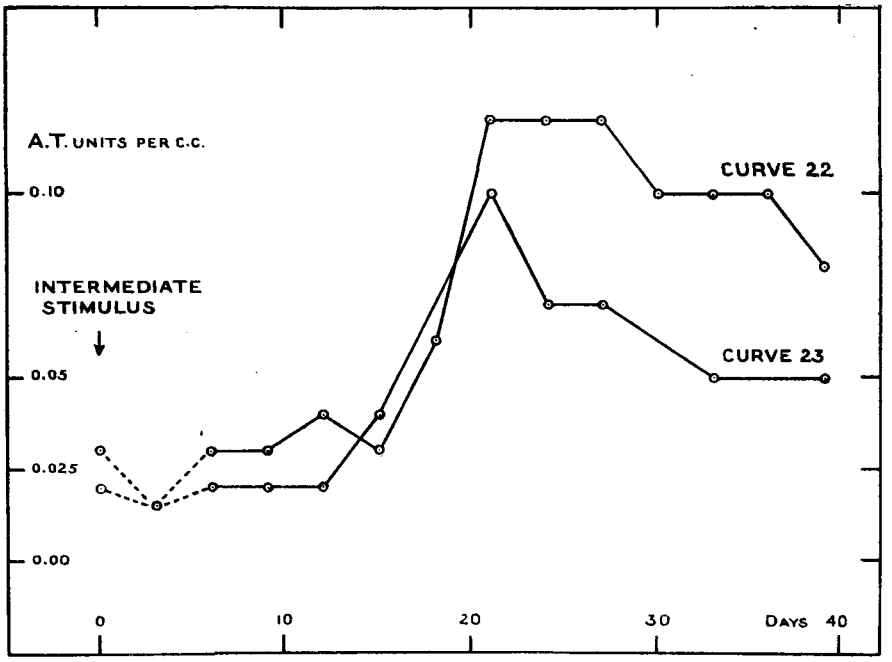

CURVES 22, 23. Showing the antitoxic value of two naturally immune horses 69 and 63 after an injection of toxin.

	Curve 22	Curve 23
Latent period	15–18 days	15 days
Maximum height	21 „	21 „

(34) *The injection of toxin into horses possessing very little immunity acts as an intermediate stimulus.*

The first two horses, 6 *N* and 56 *C*, recorded in Table XXXVIII and Curves 19 and 20, showed by their normal values and by the small size and short duration of their local reactions produced by the toxin that they possessed a high degree of natural immunity. Horse 27 *S*, recorded in Table XXXVIII and Curve 21, had a relatively small antitoxic content, but the small local reaction produced by the toxin showed that the horse possessed a considerable degree of immunity. The injection of toxin into these three horses gave rise to the typical secondary stimulus phenomenon. On the other hand, the last two horses, 69 and 63, recorded in Table XXXVIII and Curves 22 and 23, showed a low normal antitoxin content, and both gave very large local reactions to the toxin. The long latent period and delay in reaching the maximum height are typical of the intermediate stimulus phenomenon.

(35) *A toxin-antitoxin mixture may produce considerable immunity in a naturally immune horse.*

Horse 38 *H*, recorded in Table XXXIX, was injected with a toxin-antitoxin mixture containing five L + doses for a guinea-pig.

Table XXXIX.

Primary stimulus. Probable infection in normal life	
Antitoxin produced	0·45 unit per c.c.
Secondary stimulus	
Interval between primary and secondary stimulus	Unknown
Toxin	1·9 c.c.
Antitoxin	5 units
Antitoxin produced	
3 days after injection	0·8 units per c.c.
6 „ „	1·5 „
9 „ „	6·0 „
12 „ „	6·0 „
15 „ „	6·0 „
18 „ „	5·0 „

Humans.

(36) *The injection of a toxin-antitoxin mixture into a naturally immune human causes a rapid production of antitoxin.*

Table XL records the results of injecting a toxin-antitoxin mixture containing three L0 doses for a guinea-pig into a naturally immune human (A. T. G.).

Table XL.

Primary stimulus. Probable infection in normal life	
Antitoxin produced	0·10 unit per c.c.
Secondary stimulus	
Interval between primary and secondary stimulus	Unknown
Toxin	3 L0 doses
Antitoxin	3 units
Antitoxin produced	
2 days after injection	0·16 unit per c.c.
4 „ „	0·20 „
7 „ „	0·5 „
15 „ „	1·0 „
21 „ „	1·0 „

(37) *Summary of Part III. The injection of toxin into a naturally immune horse and human acts as a secondary stimulus and is followed by a latent period of about four days, and the maximum height of immunity is reached in about ten days.*

Table XLI summarises the latent periods and times of maximum height of immunity for the animals dealt with in Part III. The periods of time recorded correspond closely with those given in Table XXXV.

Table XLI.

Summary of latent periods and times of maximum height of immunity following the secondary stimulus in naturally immune animals.

Animal	Nature of stimulus	Reference	Latent period	Maximum height
Horse	Toxin	Curve 18	4 days	14 days
„	„	„ 19	3–6 „	9 „
„	„	„ 20	3–6 „	9 „
„	„	„ 21	3–6 „	12 „
„	Toxin-antitoxin mixture	Table XXXIX	under 3 „	9 „
Human	„ „	„ XL	„ 2 „	7–15 „

(38) *The injection of toxin into a "partially" immune animal acts as an intermediate stimulus.*

Three instances have been recorded, one in Part II of an artificially immunised horse, and two in Part III of naturally immune horses in which the immunity response was delayed. To this delayed response, we have applied the term "Intermediate Stimulus Phenomenon." A summary of the three horses is given in Table XLII.

Table XLII.

Animal	Nature of stimulus	Reference	Latent period	Maximum height
Horse	Toxin-antitoxin mixture	Curve 17	8 days	35 days
„	Toxin	„ 22	15–18 „	21 „
„	„	„ 23	15 „	21 „

SUMMARY.

(*a*) *Primary Stimulus.* In animals possessing no normal antitoxin a single injection of toxin either "attenuated" or under cover of antitoxin, whether injected previously or at the same time or present in the form of passive immunity maternally transmitted, is followed by a latent period of about three weeks, and the maximum immunity is reached in about eight weeks.

(*b*) *Secondary Stimulus.* In immune animals, whether naturally immune or artificially immunised, a single injection of toxin or of a toxin-antitoxin mixture is followed by a latent period of about four days and the maximum immunity is reached in about ten days; the great and rapid immunity response to the secondary stimulus offers a striking contrast to the small and gradual response to the primary stimulus.

(*c*) *Intermediate Stimulus.* In partially immune animals the response to an injection of toxin is in magnitude and rapidity of a character intermediate between the responses following a primary and a secondary stimulus.