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THE ACTION OF *VIBRION SEPTIQUE* AND *B. WELCHII* TOXIN ON ISOLATED ORGANS.

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THE ACTION OF *VIBRION SEPTIQUE* AND *B. WELCHII* TOXIN ON ISOLATED ORGANS.

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DALE (in a note attached to a paper by Robertson, 1919) demonstrated the effect of *Vibrion septique* toxin on the rabbit's blood-pressure, and showed that its action could be blocked by antitoxin. W. Straub (1919) and Lautenschlager (1920), working with "gasœdema" toxin, stated that it had an action like that of digitalis on the isolated frog's heart and that this action was blocked by antitoxin.

This communication deals with the specific action of the toxins of *V. septique* and *B. welchii* on smooth muscle from various animals, and on the rabbit's auricle. We have also made some experiments on these tissues with the toxins of the diphtheria bacillus, *Streptococcus scarlatinæ*, and Shiga's bacillus of dysentery, but up to the present we have failed to demonstrate any specific action which can be neutralized by the appropriate antitoxic serum. Possibly these toxins have no direct effect on smooth muscle, or it may be that the effect cannot be demonstrated because they act so slowly. The toxins of *V. septique* and *B. welchii* will kill an animal in a few minutes, whereas the other toxins mentioned may take several days to produce their specific effects. Diphtheria toxin, for example, will not kill an animal in under 10 hours however large a dose is given (Glenny, 1925).

(1) *The Toxins Used.*

The toxins used were precipitates obtained by saturating a bacterial-free filtrate of a 40-hour broth culture with ammonium sulphate. Mr. Dalling kindly prepared these for us. A solution containing 50 mgm. per c.c. was made up each day and kept in the cold until required for use. It was found that the toxin did not deteriorate appreciably under these conditions.

(2) *Method.*

Strips of rabbit's uterus have been used for most of our experiments. A large number of pieces of approximately the same sensitivity can be obtained from one animal, and the tissue will remain active for several days if kept on ice. The strips are suspended in a bath containing 15 c.c. of Ringer's solution kept at 37° C. through which air or oxygen is bubbling. The effects are slightly different according as to whether air or oxygen is used. The contractions of the muscle are recorded on a smoked drum.

The effect of the toxin is most easily observed if the spontaneous movements of the muscle are allowed to commence before the toxin is put into the bath; these spontaneous movements usually begin to appear after the muscle has been in the bath of Ringer's solution for about half an hour.

The experiments on the rabbit's auricle were done by the method of Clark (1921), using borate Ringer (Trevan, Boock, Gaddum and Burn) (1928).

FIG. 1.

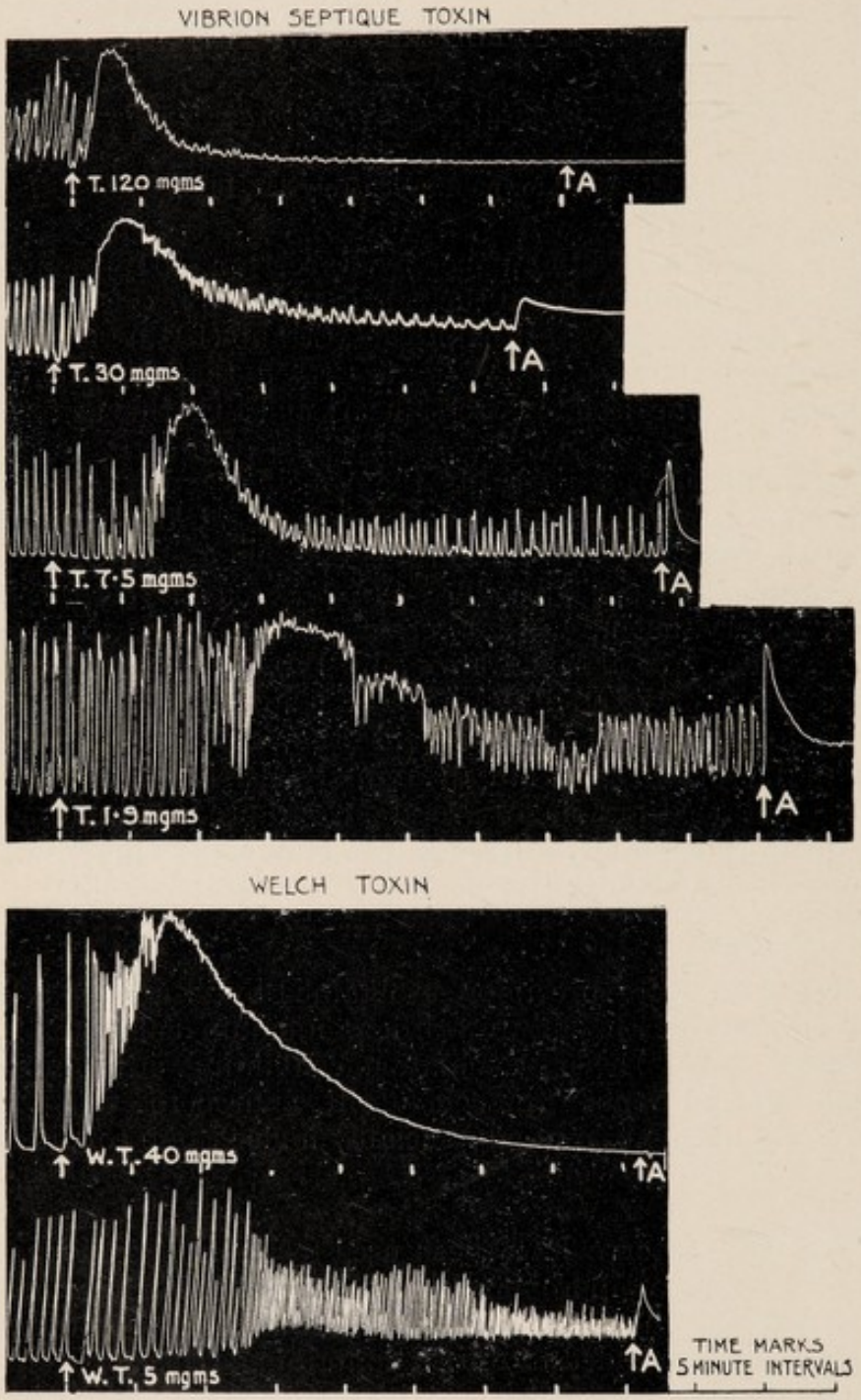

FIG. 1.—The specific effect of various doses of *V. septique* and *B. welchii* toxins on the rabbit's uterus. At A 0·3 c.c. 1/10,000 adrenalin. Time-marker 5-minute intervals. Air is bubbled slowly through the bath; rate about 6 bubbles per second; volume of bath 15 c.c.

(3) *Specific Action of Toxin.*

Fig. 1 shows the effect of different doses of the Septique and Welch toxins on the rabbit's uterus. Air was bubbled slowly through the Ringer's solution.

Small doses of Septique toxin cause a spasmodic contraction of the muscle and a diminution of the size of the spontaneous contractions. Larger doses cause a complete cessation of spontaneous movements. At A in each case a dose of 0·03 mgm. of adrenalin was administered. It will be seen that with increasing doses of toxin the normal contraction to adrenalin is diminished, and after 1·20 mgm. it has entirely disappeared. If the toxin is put into the bath before the spontaneous movements of the uterus appear, there is no spasmodic contraction such as is shown in the tracings in Fig. 1. The spontaneous movements, which occur in a normal strip of uterus after it has been left in the bath for a short time, do not appear in a strip to which toxin has been added; the response to adrenalin is diminished or absent altogether.

The action of the Welch toxin is much the same as that of the Septique, except that with small doses of Welch toxin sometimes there is no spasmodic contraction of the muscle (Fig. 1). The spontaneous movements become smaller and more frequent, and the effect of adrenalin is diminished. The effects of both the Septique and Welch toxins are prevented by mixing the toxin with the specific antisera (see later).

(4) *Effect of Rate of Bubbling.*

The effect of the Septique toxin is dependent on whether oxygen or air is used for oxygenating the Ringer solution, and on the rate at which either gas is bubbled through it. The toxin is destroyed by the bubbling, as is shown in the following experiment:

15 c.c. of Ringer's solution containing 7·5 mgm. of Septique toxin were placed in each of 4 tubes immersed in a bath at 37° C. Oxygen was bubbled through the first, air through the second, and hydrogen through the third. In the fourth there was no bubbling at all. In each of the first three the rate was kept at about 2 bubbles a second and the size of the bubbles was as near as possible the same. At the end of three-quarters of an hour the contents of the four tubes were transferred to baths containing pieces of the same uterus. The effect produced on the uterus by the contents of the fourth tube was much greater than that obtained with the first three. These three all produced similar effects. The rate of disappearance therefore is, as nearly as can be judged, the same in the first three cases, and since incubation at 37° does not destroy the toxin, its disappearance can only be due to its removal on the surface of the bubbles, and its irreversible coagulation or other destruction therein. This may be a similar phenomenon to the irreversible coagulation produced by bubbling a gas through a solution of albumen. Shaking a "solution" of an enzyme will destroy the activity. If the bubbling of air is very rapid this quantity of toxin (7·5 mgm.) disappears completely in 5 minutes.

This phenomenon makes it necessary to control the bubbling rate very strictly if the action of small quantities of toxin is to be demonstrated. For instance, to obtain the effect shown for 1·9 mgm. of toxin in Fig. 1 the rate of bubbling should be as slow as 2 to 6 bubbles per second.

There is a further effect associated with the oxygenation of the Ringer's

solution. If oxygen is used instead of air the spontaneous movements reappear in about half an hour after the spasmodic contraction produced by a small dose of Septique toxin (see Fig. 4). The same dose of toxin, when air is used at the same rate, stops the spontaneous movements entirely. This effect is not due to a more rapid removal of toxin by the oxygen from the Ringer's solution as is shown by the experiment described in the last paragraph, but is due to some difference in the response of the tissue in the presence of excess of oxygen.

(5) *The Effect of Washing Out the Bath with Fresh Ringer's Solution after the Addition of* V. septique *Toxin.*

Washing out the bath with fresh Ringer's solution will sometimes restore the condition of the uterus completely if this is done immediately the toxic action appears. If the washing out is done later, *e.g.* 20 minutes after the toxin has been added to the bath, some return of the normal rhythm occurs; the adrenalin effect, however, is not completely restored. The addition of a large dose of serum at this period will usually restore the condition completely (see later). Washing out during the latent period will not prevent the appearance of a contraction, although the muscle returns to its normal condition after a slight effect has been produced.

When the muscle is restored to its previous condition by washing out after the action of the toxin, a second dose identical with the first produces a very similar effect. The desensitization described later is not seen in the same degree.

In view of the work of Sir Thomas Lewis and his colleagues on the resemblance between the effect of the injection of histamine into the skin and the response to noxious stimuli, we thought that the contraction of the muscle might be due to histamine liberated from it in the early stages of the action of the toxin. We tried the action of the Septique toxin on the rat's uterus, and although histamine relaxes the muscle in this animal, the contraction after the toxin was of the same character as in the rabbit.

Histamine is therefore probably not a factor in this action.

(6) *Unspecific Action.*

Boiling a solution of Septique toxin for 5 minutes destroys all action of the toxin on the uterus if the dose of toxin is less than 100 mgm. With doses of over 100 mgm. of this particular toxin, boiled for 5 minutes, a temporary increase of tone with slowing of the spontaneous movements occurs. Boiling a solution of Welch toxin for 2 hours destroys all action for doses of 40 mgm. and under. There appears to be more "non-specific" substance in this toxin than in the case of our Septique toxin. Fig. 2 shows these "non-specific" effects produced by large doses of boiled toxins. They are clearly different from the effects of the specific constituents, and are not prevented by the specific antisera. Probably they are *not* due to small amounts of the "specific" substance undamaged by the boiling but to constituents from the broth, such as peptone.

(7) *Latent Period of Action.*

Fig. 1 shows also that the latent period of the action of Septique toxins varies very considerably with the dose used. A small dose (1·9 mgm. in 15 c.c.) has a latent period of approximately 15 minutes, whilst a dose of 120 mgm. has a latent period of about 1 minute. A similar effect is shown by simpler drugs. For example, the action of adrenalin on the uterus in a bath of the same size has the following latent periods:

FIG. 2.

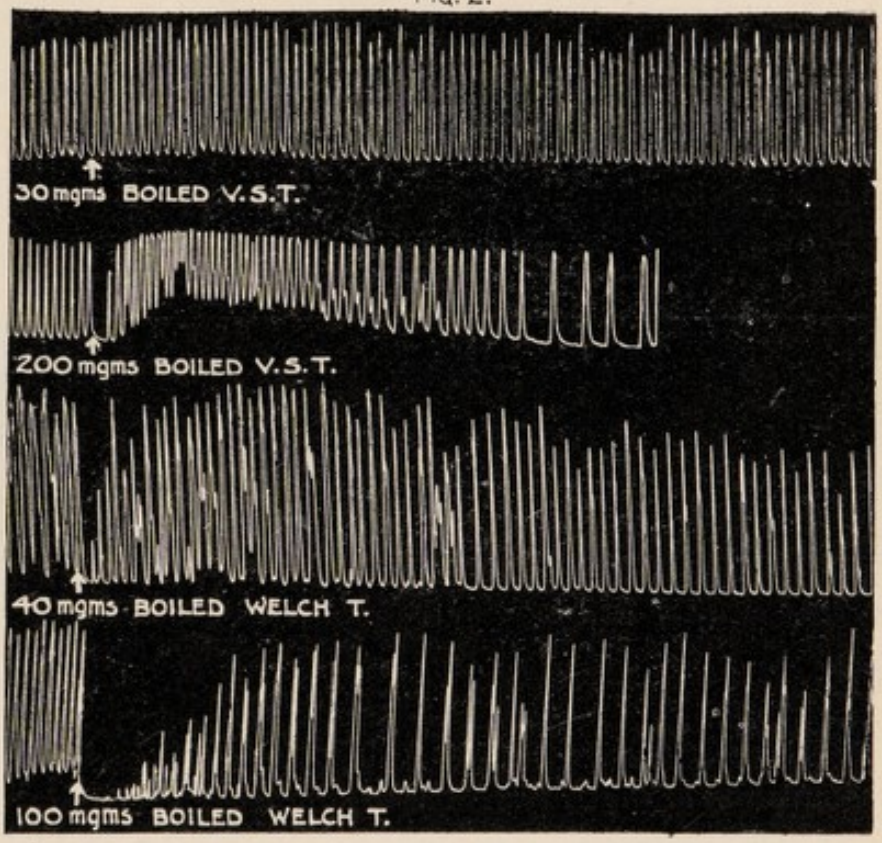

Fig. 2.—Unspecific effects produced by large doses of boiled toxins. Air is bubbled slowly through the bath.

Adrenalin dose.		Latent period.
1/1000 mgm.	.	6·2 secs.
1/100 ,,	.	2·5 ,,
1/10 ,,	.	1·9 ,,

It is possible that the shorter latent period of the action of adrenalin is due to its molecule being smaller than that of the toxin; this would allow it to diffuse into the muscle at a greater speed.

(8) *Effect of Toxin on the Small Intestine and on the Auricle.*

The effect of the Septique toxin on strips of isolated rabbits' *small intestine* suspended in oxygenated Ringer's solution is very similar to the effect on the

uterus. The muscle contracts and the spontaneous movements cease; the action is prevented by the previous addition of an adequate dose of the appropriate serum. Isolated rabbits' *auricles* were suspended in oxygenated Ringer's solution, and the contractions registered on a smoked drum. The effect of the Septique toxin on such a preparation is shown in Fig. 3. There is sometimes a temporary increase in the rate of beat, and, very occasionally (once in 20 experiments), in its size, after which the beat slowly diminishes and finally ceases. Large doses of adrenalin (at A in the chart) cause a slight reappearance of the beat. Washing out with Ringer's solution does not produce a return of the rhythm. A mixture of toxin with an adequate amount of specific serum has no action on the auricle. Lautenschlager claimed that the action of his toxin on the frog's heart was exactly similar to that of the digitalis group, but this is not true for the action of Septique toxin on a rabbit's auricle. The rabbit's auricle *always* shows an increased height of contraction as the first result of the administration of the drugs of the digitalis series.

FIG. 3.

FIG. 3.—Effect of 30 mgm. of *V. septique* toxin on an isolated rabbit's auricle in a bath containing 60 c.c. of Ringer's solution. At A 0·5 mgm. adrenalin. Rapid bubbling with oxygen is used.

(9) *The Concentration of the Toxin Necessary for Various Effects.*

The concentration to produce an effect on the isolated auricle appeared to be about the same as that necessary to produce an action on the intestine or the uterus. An accurate comparison has not yet been attempted. We have found, with Lautenschlager, that when a post-mortem examination is made of an animal which has recently died of the toxin, the ventricles have ceased beating whereas the auricles continue to beat. Probably the ventricle is slightly more sensitive than the other tissues, although no experiments have yet been done with isolated strips of ventricle.

Table I shows a comparison of the concentration of toxin used in the bath with the concentration in the serum of rabbits injected with the toxin intravenously. The latter figures were calculated on a basis of the blood volume being $\frac{1}{15}$ of the body-weight and the blood consisting of $\frac{2}{3}$ serum and $\frac{1}{3}$ corpuscles. Every kilo of rabbit would therefore contain $\frac{1000}{15} \times \frac{2}{3} = 44$ c.c. of serum.

It will be seen that the minimum concentrations are very similar in the two cases.

TABLE I.—*Minimal Effective Concentration of* V. septique *Toxin when Injected into Rabbits Intravenously and when added to a Bath containing an Isolated Strip of Uterus.*

Rabbits Intravenously.			*Isolated Uterus.*		
Dose mgm. per kilo.	Concentration mgm. per c.c. serum.	Result and death time (2 animals at each dose).	Dose in 15 c.c. bath mgm.	Concentration mgm. per c.c. Ringer.	Latent period of effect.
18	0·41	Both died in 3 and 10 min. respectively	7·5	0·5	8 min.
10	0·23	Both died in 4 and 8 min. respectively	3·7	0·25	10 ,,
5	0·11	Both died in 35 and 40 min. respectively	1·9	0·12	14 ,,
2·5	0·057	Both died overnight.	0·9	0·06	20 ,,
1·2	0·027	Both lived.	0·45	0·03	No effect.
·6	0·014	,,			
·3	0·007	,,			

Intravenous injection of a solution of toxin into mice weight 15–25 gm. each:

1·6 mgm.	DD	.	0·40 mgm.	LL
1·2 ,,	DD	.	0·32 ,,	LL
0·8 ,,	DD	.	0·24 ,,	LL
0·56 ,,	DL			

The mouse M.L.D. of the Welch toxin we have been using is about 1·0 mgm. for a 20-gm. mouse.

D indicates a death. L indicates a survival.

(10) *Desensitization to* V. septique *Toxin.*

When oxygen is used for aerating the bath the effect of the toxin on the muscle disappears in about half an hour (this is referred to earlier).

The following experiment (Fig. 4) shows the effect of subsequent doses after this recovery has taken place.

When two similar strips of uterus, hereafter called A and B (see Fig. 4), are suspended in baths containing 15 c.c. of Ringer's solution, and 7·5 mgm. of toxin is added to both baths, a contraction with cessation of the spontaneous movements occurs in both strips A and B. In half an hour both strips have regained their normal rhythm. Another dose of 15 mgm. of toxin added to the bath containing the strip of uterus A then produces no effect on the muscle. After a further interval of 1 hour a 40-mgm. dose put into both baths produces a slight effect on the strip in bath B, but this was not so great as it would have been if no previous dose had been given. No effect at all appears in the case of strip A. If a dose of 40 mgm. of Welch toxin was added to the bath containing the desensitized uterus no effect was produced, whereas a dose of adrenalin produced the usual effect. If air was substituted for the oxygen used for the bubbling during this experiment the spontaneous contractions

ceased. (If the bath containing the desensitized uterus was washed out with fresh Ringer no difference in the desensitization was seen.)

A strip of uterus left in a bath of oxygenated Ringer's solution for a corresponding period without addition of toxin did not show this desensitization.

Successively increasing doses of the Septique toxin therefore produced desensitization to both Welch and Septique toxins. The phenomenon was only obtainable when the oxygen tension of the fluid was high. Formolized toxin, and toxin destroyed by bubbling, failed to produce the desensitization.

While this effect is in some respects similar to the immunity produced in the whole animal as the result of repeated injections of toxin, it has the following points of difference:

(1) The desensitization depends on the tissue, whereas the uterus of an immunized animal did not appear to be any less sensitive than that of a normal animal (see later).

FIG. 4.

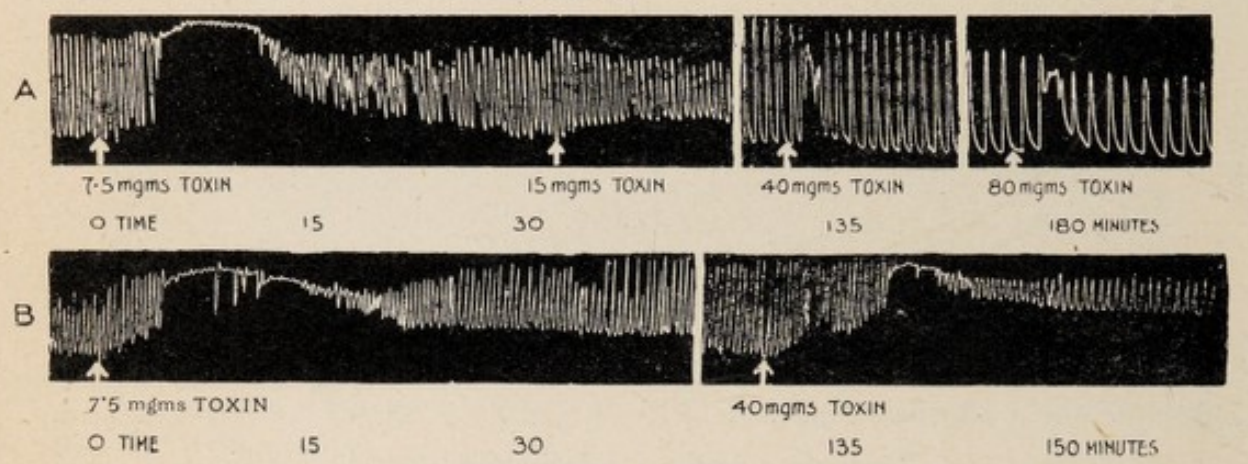

FIG. 4.—Desensitization to *V. septique* toxin. The effect produced by the initial dose is not repeated by the later and larger doses. Rapid bubbling with oxygen is used. (The white lines indicate that the tracing is interrupted.)

(2) It is not specific in that a muscle desensitized to Septique is also desensitized to Welch toxin.

(3) It did not appear to be produced by doses of formolized toxin.

(4) The time taken to produce the effect was very much shorter than in the case of the whole animal.

Other drugs will produce a desensitization to subsequent and similar doses, *e. g.* pituitary acting on the blood-pressure of an animal, for here also a second dose fails to produce an effect if it follows closely on the first.

(11) *Effect of Antitoxin.*

If the Septique toxin is mixed with an adequate amount of the appropriate anti-serum, and the mixture allowed to stand for periods varying from 1 minute to 1 hour before use, the uterus fails to contract. Doses of toxin amounting to 40 times the minimal effective dose* can be completely neutralized in this

* A minimal effective dose is the dose of toxin alone which will just produce an effect on the uterus.

manner. If larger doses are used the "non-specific" effect previously mentioned is obtained.

A. *Titration.*—The titration of a serum for antitoxic value can be carried out as follows: Doses of 75 mgm. of our toxin (about 40 times the minimal effective dose) are mixed with varying quantities of serum, and the volume made up to 3 c.c. This mixture is warmed to 37°, and added to the bath containing a piece of uterus showing spontaneous contractions. Air is used for aerating the solution during this experiment. The amount of serum is varied until two mixtures differing by 20–25% are found, one of which produces a toxic effect and the other does not. Fig. 5 shows the result of this experiment, and

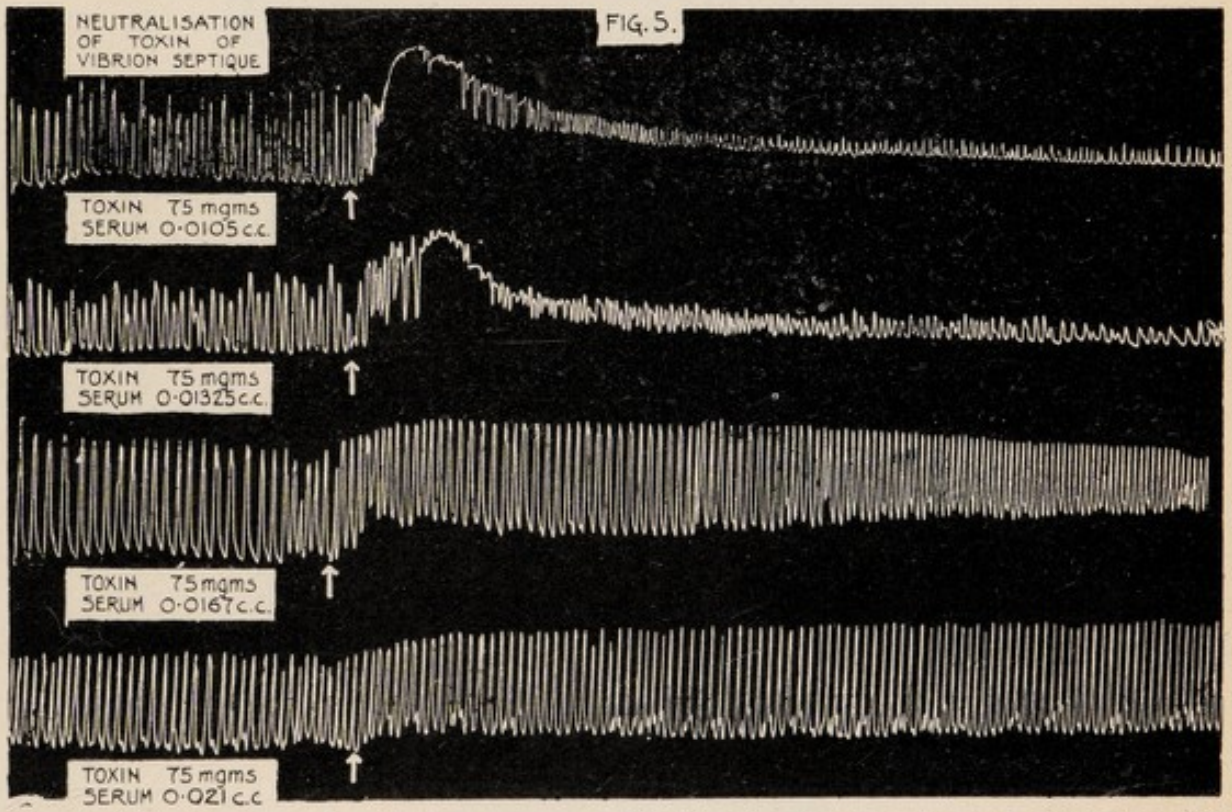

Fig. 5.—The titration of a Septique serum and toxin (the amounts of serum differ by 26 %). Air was bubbled rapidly through the bath; rate of bubbling is not controlled.

Table II gives the comparison with the determination of the L_+ dose obtained by injecting toxin-antitoxin mixtures intravenously into mice.

Table II.—*Toxin* NN_{11} *Titrated Against Serum* 2441.

L + on Mice (by Intravenous Injection).

Mixture injected.	Deaths out of 10 mice used for each dose.	Proportion of serum to 1 mgm. of toxin.
8 mgm. toxin + 0·0012 c.c. serum	10	0·00015
,, ,, + 0·0015 ,, ,,	10	0·00019
,, ,, + 0·0020 ,, ,,	4	0·00025
,, ,, + 0·0025 ,, ,,	0	0·00031
,, ,, + 0·0030 ,, ,,	0	0·00037
,, ,, + 0·0035 ,, ,,	0	0·00044

On Isolated Rabbit's Uterus.

Mixture used.	Result.	Proportion of serum to 1 mgm. of toxin.
75 mgm. toxin + 0·0105 c.c. serum .	Large effect .	0·00014
,, ,, + 0·0132 ,, ,, .	,, ,, .	0·00018
,, ,, + 0·0167 ,, ,, .	Small ,, .	0·00022
,, ,, + 0·021 ,, ,, .	No ,, .	0·00028

FIG. 6.

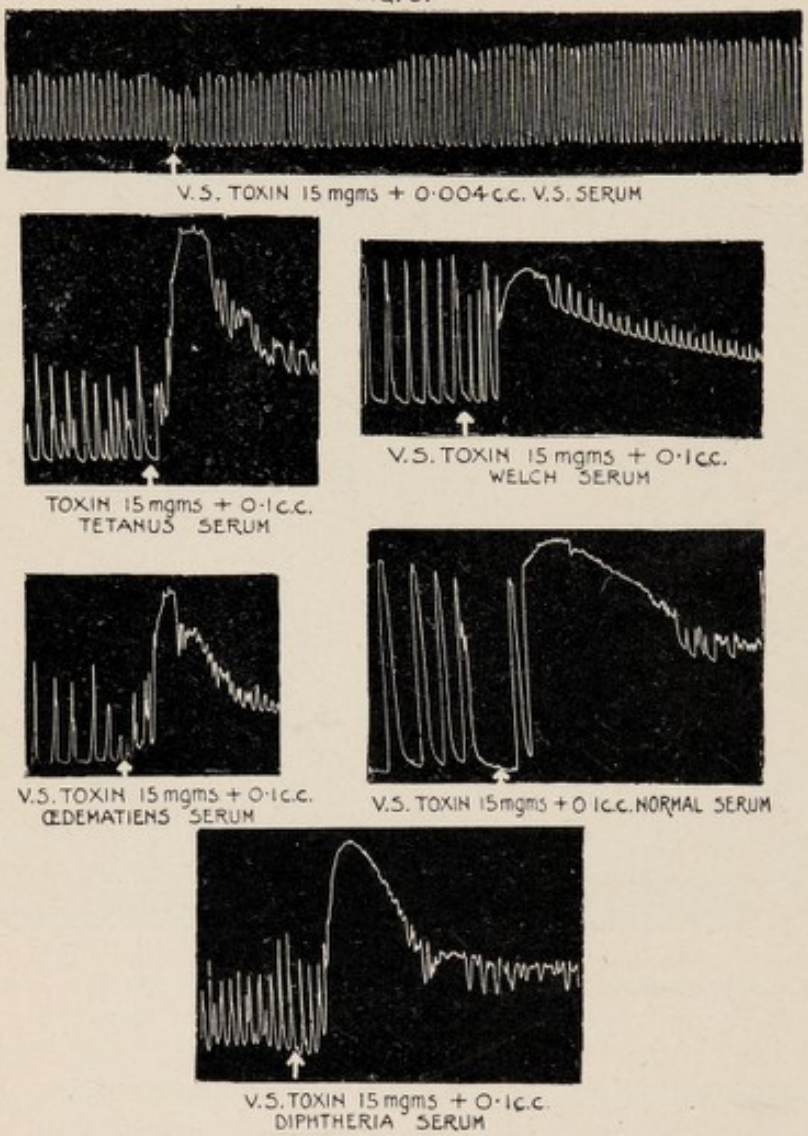

FIG. 6.—The specific effect of *V. septique* serum. The blocking of the toxic effect produced by ·004 c.c. of *V. septique* serum is not obtainable with ·1 c.c. of tetanus, Welch, *œdematiens*, diphtheria, or normal sera. Air was bubbled rapidly through the bath; rate not controlled.

It is clear that if uteri from different rabbits vary in their sensitivity to the toxin, then the apparent neutralization value of the serum will vary. Such variations of neutralizing value as we have observed with different rabbits are not greater than 25%. It is possible that these differences are due to differences in the rate of bubbling. The error produced could be entirely avoided by putting up simultaneous titrations of a standard serum and of the unknown

serum on pieces of uterus from the same rabbit, and assigning a neutralizing value in terms of the dose of the unknown which produces the same effect as another dose of the standard.

Fig. 6 shows the specificity of the reaction. Whereas 0·004 c.c. of Septique antitoxin neutralizes the specific toxin, 25 times as much anti-Welch, tetanus, *œdematiens*, or diphtheria antitoxins or normal serum fails to neutralize the effect. It was also shown that 15 c.c. of normal rabbit's serum had no effect in neutralizing the specific toxin.

We have worked out a neutralization curve for Septique toxin, the results of which are given in Fig. 7. The amounts of toxin left unneutralized in mixtures of 75 mgm. of toxin with various quantities of serum were estimated by comparing the effects produced by such mixtures with those produced by

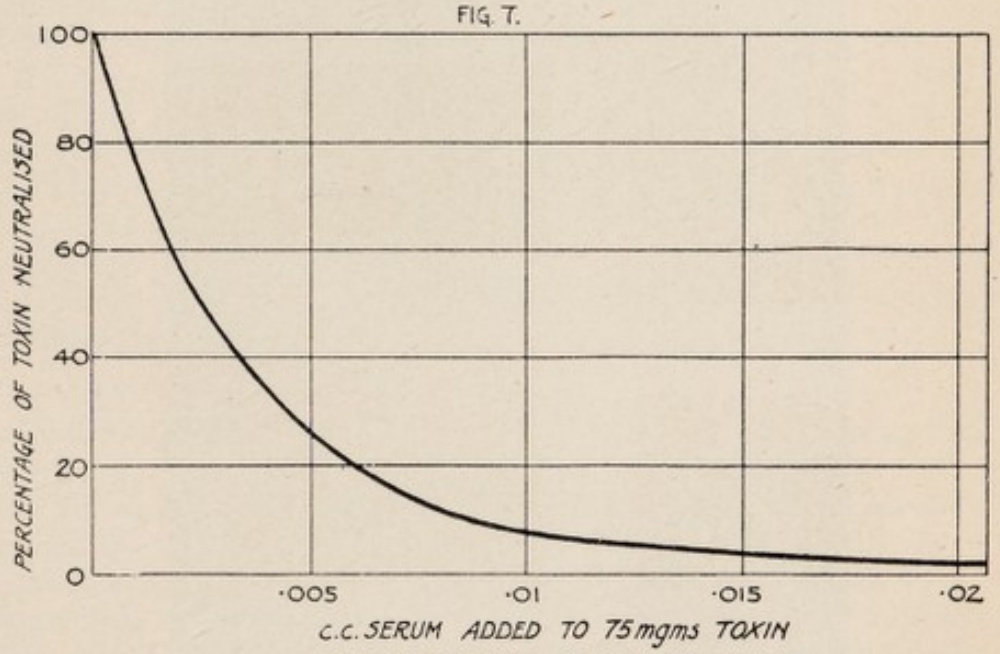

Fig. 7.—Neutralization curve for a *V. septique* toxin and serum. The ordinates are the quantities of free toxin estimated as a percentage of the total toxin used (*i.e.* 75 mgm.). The abscissæ are the quantities of serum measured in c.c. added to 75 mgm. of toxin.

small doses of toxin alone on similar strips of the same uterus. Where the amount of serum was so small that more than a maximal contracting dose of toxin was present in the mixture, a fraction of the mixture only was added to the bath. The estimated amounts of toxin left unneutralized, expressed as a percentage of the whole quantity of toxin used, are plotted as ordinates in the curve shown in Fig. 7 against the corresponding quantity of serum as abscissæ. The length of the vertical lines is an estimate of the error of the determination. The shape of this curve suggests that there is a large amount of toxoid present in the toxin (*cf.* neutralization of diphtheria toxin, Glenny, Pope and Waddington, 1925).

B. *Effect of adding serum after toxin.*—We have done some experiments to show the effect of putting the toxin and the serum into the bath separately at various intervals of time (Fig. 8). The dose of Septique toxin used in these experiments was 15 mgm. It was found that 0·004 c.c. of serum mixed with this amount of toxin before addition to the bath completely blocked the toxic

effect (Fig. 8, No. 1); 0·004 c.c. is therefore called the serum "equivalent" for this dose of toxin. Fig. 8, No. 2, shows the effect of 15 mgm. of toxin alone.

If 1 "equivalent" of the serum is put in 1 minute after the toxin (No. 3), *i.e.* during the latent period of action of the latter, a toxic effect appears. It is not so great, however, as when no serum is added after the toxin (No. 2). If 3 "equivalents" of serum (No. 4) are added at this time, *i.e.* 1 minute after the toxin, the toxic effect is blocked completely; no trace of effect

FIG. 8.

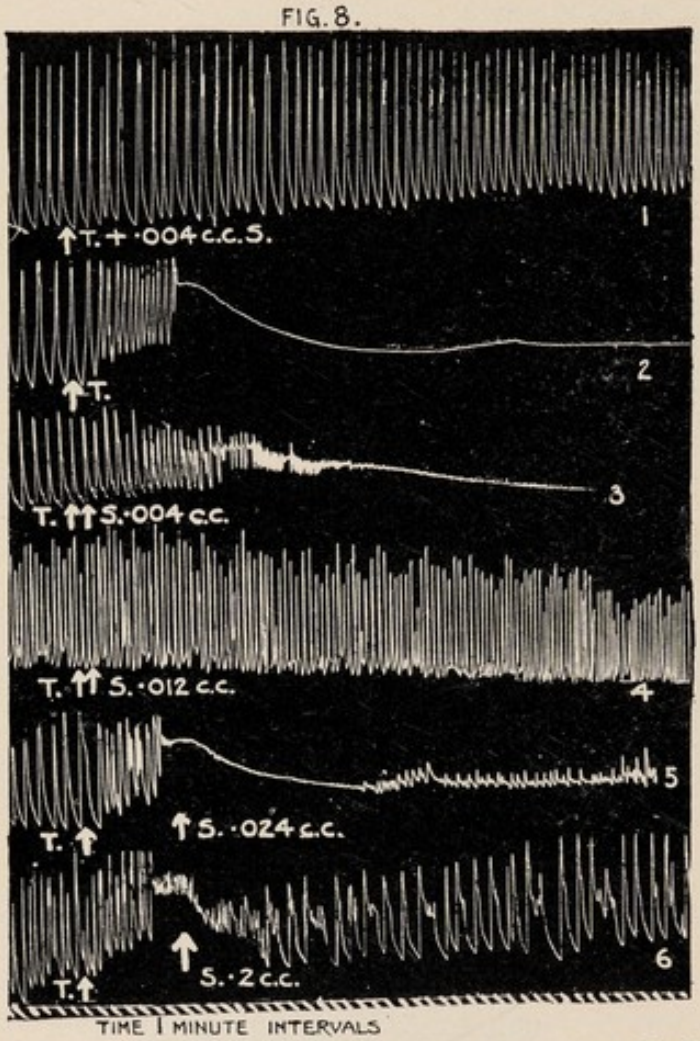

FIG. 8.—At T (in all tracings) 15 mgm. of *V. septique* toxin is added. No. 1: 15 mgm. toxin + 1 "equivalent" of serum mixed together for one minute before adding to bath. No. 2: 15 mgm. toxin alone. No. 3: 15 mgm. toxin, then 1 "equivalent" of serum one minute afterwards. No. 4: 15 mgm. toxin, then 3 "equivalents" of serum one minute afterwards. No. 5: 15 mgm. toxin, then 6 "equivalents" of serum 7½ minutes afterwards. No. 6: 15 mgm. toxin, then 50 "equivalents" of serum 7½ minutes afterwards. Time-marker 1-minute intervals. Air was bubbled slowly through the bath 2–3 bubbles per second.

appears. The action of the serum is much more complete than the simple renewal of the Ringer's solution (*cf.* Section 5).

It was found that a larger amount of serum added to the bath 7½ minutes after the toxin, when the action of the latter appeared to have reached its maximum, would produce a reversal of the effect of the toxin and an apparently normal rhythm would recur (No. 6); 50 equivalents of the serum (No. 6) would do this in 2 or 3 minutes, whereas 6 equivalents (No. 5) produced only

a slight return of the rhythm in 20 minutes. This reversal of the effect of the toxin has been produced by adding serum 30 minutes after the toxin.

In view of the fact that the toxin can be washed out with Ringer's solution (Section 5) and that serum restores the normal rhythm after the toxic effect is produced, it would seem that the "affinity" of the toxin for the tissue is not much greater than its "affinity" for the serum. The toxin does not become firmly fixed on to the tissue. However, very much more serum is necessary to reverse the toxic effect (Fig. 8, No. 6) than would be necessary to block it if the serum were mixed with the toxin beforehand (No. 1). This difficulty of "catching up" a dose of toxin with serum may be largely due to the serum being able to penetrate the tissue only at an extremely slow rate compared with that of the toxin. Presumably the antitoxin "molecule" is larger than the toxin "molecule" and penetrates more slowly.

We have also tried putting the serum into the bath 10 minutes before the toxin. In this case the action of the toxin is slightly greater than would be the case if the same quantities of toxin and serum had been mixed before being put into the bath. The slightly greater action of the toxin in this case is presumably due to an appreciable decrease of its rate of "combination" with the serum in dilute solution, so that free toxin is allowed to penetrate into the tissue. Further evidence of this phenomenon was obtained by adding a dose of toxin with 1 "equivalent" of serum to the bath simultaneously, without previous mixing, when a slight toxic effect was obtained.

(12) *The Titration of Welch Antitoxin.*

We have titrated three sera for their content of Welch antitoxin, using a method similar to that described for Septique in paragraph 11.

Table III shows a summary of the results with all the Welch and Septique sera titrated by this method and by intravenous injection into mice.

A titration of a Welch serum is shown in Fig. 9. It will be seen from the figure that the diminution in the response to adrenalin can be used as an indication of the toxic effect produced; this method is useful in the case of uteri which do not give regular spontaneous movements.

The titration of Welch toxin and serum by this method is not quite so accurate as the titration of Septique toxin and serum. For instance, when this titration was done on the uterus, the results for which are shown in Fig. 9, a mixture of 40 mgm. of toxin with 0·004 c.c. of the serum (No. 2782) gave a toxic effect, whilst a mixture of 40 mgm. of toxin with 0·006 c.c. of the serum showed no toxic effect; 40 mgm. of toxin with 0·005 c.c. of serum sometimes showed no toxic effect, and sometimes produced an effect nearly as great as that of 40 mgm. + ·004 of serum. Two other uteri out of the 10 which we used for this titration gave the neutralizing value for the serum between 0·003 and 0·004 c.c. for 40 mgm. of toxin; the other 8 uteri gave a similar result to that shown in the figure.

The reasons for this variation appear to be as follows:

(1) It is not possible to use a large number of minimal effective doses "for the test-dose"* of toxin in the titration. Our Welch toxin

* A "test-dose" is the dose of toxin which is mixed with varying quantities of serum in making the titrations for antitoxic value—*e. g.* for the titration shown in Fig. 9 the test-dose of Welch toxin is 40 mgm.

contains a relatively larger quantity of "non-specific" material than our Septique toxin (para. 6).

(2) The uteri from different animals appear to vary more in their sensitivity to Welch toxin than they do to Septique toxin.

The maximum "test-dose" of Welch toxin which it was found possible to use in the titration was 40 mgm. The minimum effective dose of Welch toxin has not been determined for a large series of rabbits, but 5 mgm. produced a distinct effect in the case of some 8 animals, whereas the uteri from two other animals failed to react to 10 mgm. of toxin.

TABLE III.—*Summary of Results with Toxin and Sera.*

Toxin.	Serum.	c.c. of serum to 1 mgm. toxin. On mice.	On isolated rabbit's uterus.
Septique NN_{10}	2441	0·00019 +	0·00021 +
	anti VST	0·00062 —	0·00052 —
,, NN_{11}	2441	0·00019 +	0·00023 +
	anti VST	0·00025 +—	0·00028 —
		0·00031 —	
,, ,,	2639	0·00025 +	0·0004 +
	anti VST	0·00075 --	0·00053 —
,, ,,	2756	0·0011 +	0·0015 +
	anti VST	0·0015 —	0·0018 —
Welch L_1	2782	0·00009 +	0·0001 +
	anti Welch	0·00012 —	0·00015 —
,,	2748	0·00009 +	0·00012 +
	anti Welch	0·00018 +—	0·0002 —
		0·00045 —	
,,	2756	0·001 +	0·0012 +
	anti VST	0·003 —	0·0025 —

\+ indicates death of both mice (two used at each dose); toxic reaction of uterus.
+ — ,, ,, one out of two mice.
— ,, both mice live; no action on uterus.

The test-dose of Welch toxin is therefore equivalent to about 8 minimal effective doses in the case of the more sensitive uteri; this figure compares unfavourably with the Septique test-dose, which is equivalent to about 40 minimal effective doses for Septique toxin. It would appear that this method would not be satisfactory for the routine estimation of Welch sera unless a Welch toxin with a relatively smaller quantity of "non-specific" material was available; or else a standard serum would have to be put up on each uterus used and the unknown serum compared with it.

When we were titrating this serum (No. 2782) for Welch antitoxin, we found that if a fast stream of small bubbles was used for aerating the bath a toxic effect on the muscle was produced, although the amount of serum used might be greatly in excess (up to five times the amount) of that required to neutralize the toxin if slow bubbling was employed. It would appear that the serum

was being removed and destroyed in the film of the bubbles at a faster rate than the toxin, the "combination" between the two being split up and the mixture becoming more toxic. This effect has not yet been investigated with other sera. It was only possible to obtain consistent results with this Welch serum by using a slow stream of large bubbles of air; the rate was kept between 1 and 2 bubbles a second.

(13) *Specificity of the Welch and Septique Sera in this Reaction.*

It was found that 0·1 c.c. of Welch serum 2782 would not protect against 3·7 mgm. of Septique toxin, whereas 0·006 c.c. of this serum protected against 40 mgm. of Welch toxin.

Similarly 3 c.c. of a specific Septique rabbit serum would not protect

FIG. 9.

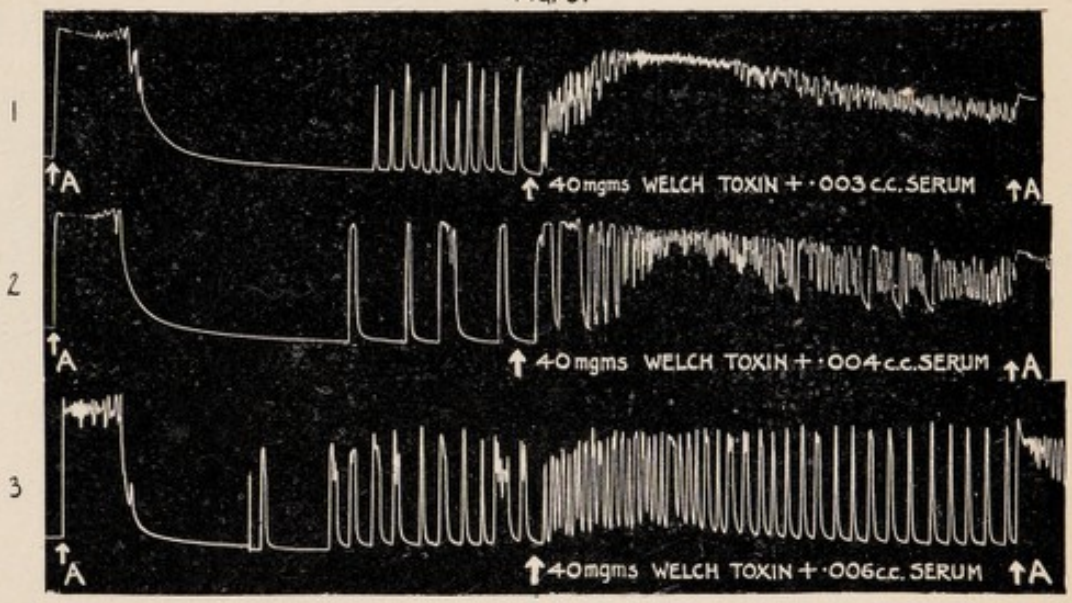

FIG. 9.—The titration of a Welch toxin and serum. (1) 40 mgm. Welch toxin + 0·003 c.c. Welch serum; (2) 40 mgm. Welch toxin + 0·004 c.c. Welch serum; (3) 40 mgm. Welch toxin + 0·006 c.c. Welch serum. At A in each case 0·02 mgm. adrenalin. Air was bubbled slowly through the bath; rate 1–2 bubbles per second.

against 10 mgm. of Welch toxin, whereas 1 c.c. protected against 30 mgm. of Septique toxin.

(All the Septique horse-sera available contained Welch antitoxin when tested on mice intravenously and on the isolated uterus.)

(14) *Experiments on Immunized Animals.*

We immunized 3 rabbits with Septique toxin-antitoxin mixtures. The first had 3 injections of 2 c.c. each, the second 3 weeks after the first, and the third 1 week after the second; the serum of this rabbit was titrated against Septique toxin by intravenous injection into mice and the following results obtained:

1·2	mgm.	toxin +	0·3	c.c. serum	LD
0·8	,,	,,	,,	,,	LL
0·4	,,	,,	,,	,,	,,

(The letter D indicates 1 mouse which died. L = lived.)

Another rabbit had 4 injections; the fourth dose of 3 c.c. was injected 7 days after the third.

The serum gave the following results:

8	mgm.	toxin	+ 0·3 c.c.	serum	DD
4	,,	,,	,,	,,	,,
1·6	,,	,,	,,	,,	LL
1·2	,,	,,	,,	,,	,,

The third had 2 further injections of 5 and 10 c.c. respectively at further 7-day intervals, making a total of 6 injections.

The results with the serum were:

4	mgm.	toxin	+ 0·3 c.c.	serum	DD
1·6	,,	,,	,,	,,	LL
1·2	,,	,,	,,	,,	,,

All rabbits were killed 7 days after the last injection, and the uteri were tested in the bath with small doses of Septique toxin.

The minimum dose of Septique toxin necessary to give an effect on the uteri of these rabbits in the bath was found to be the same as that for the normal rabbits done at the same time. At the time these experiments were done the effect of the bubbling in removing the toxin was not appreciated, and small differences might not have been noticed. With this reservation an isolated uterus of an immune animal would not appear to be less sensitive to the toxin than that of a normal animal.

Another rabbit had 15 injections of T.A. mixture. The serum gave the following results on mice:

0·5	c.c.	serum	+ 2 mgm.	toxin	LL
0·1	,,	,,	,,	,,	,,
0·075	,,	,,	,,	,,	LD
0·06	,,	,,	,,	,,	,,
0·05	,,	,,	,,	,,	DD
0·04	,,	,,	,,	,,	,,

The auricle from this rabbit when washed free of serum reacted to the septique toxin in the same way as a normal rabbit's auricle under the same conditions. Strips of small intestine from this animal reacted to the same doses of toxin as those from a normal animal. It would appear that these tissues of an immune animal are not protected against the toxin when they are separated from the serum.

Mr. Mason kindly consented to immunize these rabbits and titrate the sera for us. Our thanks are due to Mr. Dalling, Mr. Mason and Mr. Glenny for advice and materials used in these experiments.

SUMMARY.

1. The actions of *V. septique* and *B. welchii* toxins on involuntary muscle *in vitro* are described.

2. The toxin of *V. septique* is shown to be destroyed by bubbling air oxygen or hydrogen through its solution.

3. The action of *V. septique* toxin is shown to be reversible by washing with Ringer's solution and by adding a large amount of serum.

4. The concentration of toxin producing effects on smooth muscle in aerated Ringer's solution is shown to be of the same order as that in the blood of a rabbit receiving an average lethal dose.

5. A small dose of *V. septique* toxin added to a bath of oxygenated Ringer's solution containing a piece of uterus renders the tissue insensitive to the action of larger doses of either *V. septique* or *B. welchii* toxin.

6. The important part of the action of the two toxins is specific in that they are neutralized by the appropriate antisera and not by other antisera. There is a trace of non-specific toxic material in both toxins.

7. By using this effect titrations of antitoxin potency of sera have been carried out. Differences of 20% of the sera in a neutralizing mixture can be determined. The end-point approximates closely to that given by the determination of L_+ dose in mice.

8. Rabbits were immunized against *V. septique* toxin. The uteri from these rabbits reacted to the toxin in the same way as normal uteri. The minimum dose was about the same.

REFERENCES.

Clark, A. J.—(1921) *J. Pharmacol.*, **18**, 423.
Glenny, A. T.—(1925) *J. Path & Bact.*, **28**, 241.
Idem, Pope, C. G., and Waddington, H.—(1925) *Ibid.*, **28**, 279.
Lautenschlager, I. L.—(1920) *Arch. f. exp. Path.*, **85**, 1.
Robertson, M.—Note by H. H. Dale.—(1919) *J. Path. & Bact.*, **23**, 153.
Straub, W.—(1919) *München. med. Wchnschr.*, **66**, 89.
Trevan, J. W., Boock, E., Burn, J. H., and Gaddum, J. H.—(1928) *Quart. J. Pharm.*, **1**, 6.

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