On a New Factor in the Mechanism of Bacterial Infection.

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Introduction.

The observations recorded in this paper were made in the course of investigations on gas gangrene. The work of McIntosh, of Weinberg and Séguin, and of other investigators has shown that the organisms chiefly responsible for the production of gas gangrene are the bacillus of Welch, the *Vibrion septique*, and the *Bacillus œdematiens*. Small amounts of broth cultures of these organisms when injected into animals belonging to a susceptible species, *e.g.*, the mouse or the guinea-pig, produce a violent gas gangrene, and kill the animal within 24 hours.

It is known and was confirmed by us that suspensions in saline of *B. Welchii* and of *Vibrion septique* from a surface culture are practically non-pathogenic; half a cubic centimetre of a dense suspension of these organisms can be injected into a mouse or guinea-pig subcutaneously or intramuscularly without producing gas gangrene and, indeed, without producing any signs of ill-health. The same result is obtained if broth cultures of *B. Welchii* or of *Vibrion septique*, which contain toxins in addition to the bacteria, are centrifuged, and the bacteria, after having been washed free from adherent toxin, are suspended in saline and injected. In the case of *B. œdematiens*, the toxin is so potent that it is not easy to remove the last traces of toxin by washing, and it is necessary to destroy the last traces of toxin by heating the washed bacteria to 80° C. for half-an-hour, when spores are formed. These spores, when suspended in saline and injected, are again non-pathogenic. Very occasionally it does happen that gas gangrene develops after the injection of detoxicated bacteria or their spores, but such an event is quite exceptional and apparently accidental. With the detoxicated *B. Welchii*, for instance, we have observed gas gangrene to occur only once out of a large number of experiments on more than a hundred animals. But if these "detoxicated" bacteria or spores are again mixed with a dose of toxin too small to produce an effect by itself, gas gangrene develops regularly and kills the animal.

Further investigations, which will be published separately, showed that the bulk of the detoxicated bacteria when injected undergoes lysis, while

some of the bacteria are taken up by phagocytes and slowly undergo disintegration within the phagocytes, but that these two processes, by means of which the animal defends itself against the infecting bacteria, do not take place when the bacteria are injected together with toxin. It is clear, therefore, that the toxin paralyses the defensive mechanism of the animal. But it also follows that this defensive mechanism is normally so efficient that the formation of toxin in a concentration sufficient to produce this paralysing action is prevented.

We were thus led up to the problem: Why does gas gangrene ever occur in man? Since the defences of the body against the bacteria of gas gangrene are so efficient, the infection of wounds with these bacteria is not in itself an adequate explanation of the occurrence of gas gangrene. And clinical experience leads up to the same problem. Very many wounds, especially those received on the Western Front, were infected with the bacillus of Welch, but only a very small percentage of those infected wounds developed gas gangrene.

It is clear that a new factor, extrinsic to the infection, enters here, and it was in the search for this factor that the phenomenon recorded in this paper was observed and found to have a more general significance.

The small incidence of gas gangrene in wounds infected with the bacteria of gas gangrene is, of course, well known. The explanation generally given is that, owing to the special conditions obtaining in war wounds, the latter offer an exceptionally favourable nidus for the growth of these bacteria. Thus, interference with the circulation, the presence of large masses of dead and lacerated muscle, the presence of cloth, dirt or foreign bodies generally, have all been suggested as factors capable of eliciting gas gangrene. But clinical experience shows that many wounds, in which these factors are undoubtedly present, do not develop gas gangrene, and that there are no clinical features of a wound pathognomonic of gas gangrene. And experiment completely fails to give any support to the nidus theory. Splinters of wood, pieces of muscle, of paper, wool, cotton-wool, and of khaki cloth, have been soaked in a suspension of *B. Welchii*, and then introduced underneath the skin of a mouse without ever producing gas gangrene. Even the minced muscle from a meat broth culture of *B. Welchii* does not produce gas gangrene if introduced subcutaneously after having been freed from toxin by washing with saline. Nor was it found possible to produce gas gangrene by cutting the femoral artery and injecting a suspension of *B. Welchii* into the leg or into the hæmatoma, or by injecting suspensions of *B. Welchii*, together with staphylococci, streptococci, *B. coli*, *B. proteus*, or *B. sporogenes*. The injection of lactic acid in varying con-

centrations, together with the detoxicated bacteria of gas gangrene, has also failed to produce gas gangrene, even although an extensive sore was frequently produced as the result of the injection of the lactic acid.

The negative results have been reported in detail because they demonstrate clearly how difficult it is to elicit the pathogenic action of the bacteria of gas gangrene or their spores. In this respect they resemble the bacterium of tetanus. We are informed by Dr. Tulloch that, in his experience, it is not possible to induce tetanus regularly and with certainty after infection with the spores by any of the means given in the literature and tested by him. As the negative results of the experiments given above accumulated, the conviction grew that in normal animals, in which the experimental conditions can be controlled and kept constant, an adequate explanation of the relation between these bacteria and the specific disease with which they are associated cannot be deduced from the occasional and apparently accidental production of gas gangrene or of tetanus, as the case may be, which sometimes occurs, but must be based on a definite factor, by which these conditions can be elicited from the bacteria or their spores with certainty and at will. Such a factor was eventually found in injections of small doses of a soluble ionisable calcium salt. The severity of the trauma which can be inflicted on animals infected with the detoxicated bacteria or their spores without eliciting from them the specific disease, stands in striking contrast to the phenomenon which will now be described.

Calcium Salts as an Accessory Factor in Gas Gangrene and Tetanus.

Doses of 2·5 mgrm. of calcium chloride, when injected subcutaneously into mice of 10–15 grm. weight, together with a suspension of a virulent strain of *B. Welchii* or of *Vibrion septique*, will produce a violent gas gangrene in every case. The same dose when injected with a suspension of the tetanus bacillus or its spores will produce tetanus. Larger doses up to 5 mgrm. have the same effect. With mice the dose of calcium chloride cannot safely be increased above 10 mgrm., since with doses of such magnitude the toxic action of calcium salts comes into play. Doses of 10 mgrm. are, as a rule, without any ill effect for normal mice of 15–20 grm. weight. Sometimes, however, even with such a dose the animals are ill a few hours after the injection and die within 24 hours. With smaller doses the effect becomes at first irregular, and if the dose is further diminished fails to appear. The smallest dose with which it has been possible to cause gas gangrene with the spores of *Vibrion septique* has been 0·5 mgrm. of calcium nitrate. For guinea-pigs of 250 grm. weight the minimal dose necessary to produce this effect in every case is larger, namely, 5 mgrm. of calcium chloride. With

B. œdematiens experiments were made only with spores, for the reasons already given, and for these larger doses are necessary to elicit their pathogenic action, namely, 5 mgrm. for a mouse and 10 mgrm. for a guinea-pig. In all our experiments the solutions of calcium salts were used in a strength of 1 per cent. or 2 per cent. It is possible that the dose of calcium salt necessary to produce the effect may vary with the concentration. This point has not yet been tested by us.

For the sake of convenience and brevity it is advisable to designate this new phenomenon by a new name. For reasons which will be given below, and which are based on the mechanism by which the phenomenon is produced, the terms "kataphylaxis," or "defence-rupture," will be used in this paper to describe it. The smallest dose of a calcium salt necessary to elicit the specific disease from the detoxicated bacteria in every case is called the "minimal rupturing dose." Smaller doses which are still capable of eliciting the specific disease, but are not capable of doing so in every case, are called "subminimal rupturing doses." In fixing these doses it is of course assumed that a virulent strain of the specific bacteria is being used. The density of the bacterial suspensions can apparently be varied within wide limits without affecting the results. We have worked, as a rule, with a suspension of such a density that large print is just readable through it in a test-tube. But we have also used denser and more dilute suspensions with practically identical results.

Calcium nitrate and calcium acetate have the same effect as calcium chloride. The insoluble calcium carbonate has no rupturing action. Similar doses of the chlorides of potassium, sodium, ammonium, magnesium, and strontium* have no effect when injected together with a suspension of *B. Welchii.*

B. sporogenes, when injected together with calcium chloride, does not produce gas gangrene, and does not even make the animal ill. It may be recalled, that even in broth cultures this organism is non-pathogenic, if present alone.

The rupturing action of calcium chloride is abolished by sodium citrate. As an illustration the following experiment will be given. A number of mixtures of 2 per cent. solutions of calcium chloride and sodium citrate were made up to a volume of 2 c.c. with water. Two drops of a dense freshly prepared suspension of *B. Welchii* in saline were added to these mixtures;

* [*Note added April* 4.—We have since found that with *Vibrion septique*, the pathogenic properties of which are more readily elicited than those of *B. Welchii*, strontium will produce gas gangrene, if sufficiently large doses (5 mgrm.–10 mgrm.) are given. But, even then, a positive result is not obtained in every animal, as is the case with *B. Welchii.*]

0·5 c.c. of each of these mixtures were injected subcutaneously into batches of three mice. The results were as follows:—

Batch.	$CaCl_2$ solution 2 per cent.	Na citrate solution 2 per cent.	H_2O.	Dose of $CaCl_2$ injected.	Mouse 1.	Mouse 2.	Mouse 3.
	c.c.	c.c.	c.c.	mgrm.			
A	1	—	1	5	+	+	+
B	1	0·5	0·5	5	+	+	+
C	1	1	—	5	0	0	0
D	0·5	—	1·5	2·5	+	+	+
E	0·5	0·5	1	2·5	0	0	0
F	0·5	1·5	—	2·5	0	0	0

In the Tables + means "died," 0 means "alive and well."

All the mice in batches A, B, and D developed gas gangrene and died within 20 hours after the injection, except one animal in batch D, which died within 48 hours. All the animals in batches C, E, and F remained alive and well for three days after the injection.

By means of a similar experiment it has been possible to demonstrate an antagonism between Ca ions and Mg ions with reference to the production of gas gangrene from *B. Welchii*. 0·2 c.c. of a suspension of these bacteria was added to mixtures of equimolecular solutions of $MgCl_2$ and $CaCl_2$ in varying proportions. These mixtures were then injected subcutaneously into batches of four mice each. In each batch two mice received a "rupturing dose" of calcium chloride, *i.e.*, a dose which contained 2·75 mgrm. of $CaCl_2$, two mice received a "subminimal rupturing dose," containing 1·4 mgrm. of $CaCl_2$, *i.e.*, a dose which does not elicit gas gangrene in every case. The results are as follows:—

Batch.	$CaCl_2$ solution M/5.	$MgCl_2$ solution M/5.	H_2O.	Dose of $CaCl_2$ injected.	Result after 3 days.	
					Mouse 1.	Mouse 2.
	c.c.	c.c.	c.c.	mgrm.		
A	0·5	—	1·5	2·8	+	+
B				1·4	+	+
C	0·5	0·5	1	2·8	+	+
D				1·4	0	0
E	0·5	1	0·5	2·8	+	+
F				1·4	0	0
G	0·5	1·5	—	2·8	+	0
H				1·4	0	0

The experiment shows a distinct protective action of the magnesium salt against a subminimal rupturing dose of the calcium salt, when tested with *B. Welchii*.

In all the experiments mentioned so far, the bacteria or their spores have been injected suspended in the various salt solutions. This direct contact between the bacteria and the calcium salts gives the most favourable conditions for the phenomenon which we have described, but it is not essential. It has been possible to produce gas gangrene in mice by injecting the spores of *Vibrion septique* and calcium salts either at the same site at different times, or secondly, at the same time at different sites, the injection of calcium salts either preceding or following that of the spores. Similarly, tetanus has been produced by injecting the spores and the calcium salt at different sites at the same time and also by injecting calcium salts and tetanus spores at the same site but at different times, the injection of spores also either preceding or following that of the calcium salts. In such an experiment, for instance, no tetanus occurred for seven days in mice which had received an injection of tetanus spores. But, when, on the seventh day, calcium chloride was injected tetanus developed on the following day.

These experiments, in which the injections of calcium salts and of bacteria were separated in time or in space, throw light on the mechanism by which the peculiar effect of calcium salts, with which this paper deals, is brought about. A detailed consideration of these experiments will be given below in dealing with the probable explanation of the phenomenon.

On the Etiology of Gas Gangrene and of Tetanus.

We have referred in the introduction to the fact that infection with the bacteria of gas gangrene and of tetanus is not in itself an adequate explanation of the specific diseases produced by these bacteria, and that it was necessary to postulate the existence of an accessory factor. The question which naturally suggests itself is whether the phenomenon which we have described in this paper represents this accessory factor.

It has always been realised that both tetanus and gas gangrene are diseases particularly associated with earth, and, in fact, these diseases can be produced by injecting emulsions of earth into mice or guinea-pigs. The explanation which is generally accepted is that the soil contains the spores of the specific bacteria. No specific action is credited to the earth itself; it is supposed to act as a foreign body like many other things, such as pieces of cloth, or splinters of wood, the presence of which is assumed to create an exceptionally favourable nidus for the growth of the bacteria. As stated in the introduction we have failed to find any experimental evidence which would support the nidus theory. Vaillard,* who investigated in great detail

* Vaillard et Rouget, 'Annales de l'Institut Pasteur,' vol. 6, p. 385 (1892).

the relation of the contamination of the wound with earth to the etiology of tetanus, and whose observations as to the inadequacy of the nidus theory agree in many respects with ours, recognised that this contamination exercises a specific influence. But he ascribed this specific factor to the presence of other micro-organisms in the earth.

Of the various foreign bodies tested by us, emulsions of earth were the only ones which frequently, although not invariably, elicited gas gangrene when injected together with the detoxicated bacteria of gas gangrene or their spores. That the effect of earth is not due to its mechanical action but to a chemical constituent of the earth was demonstrated by the following experiment: A sample of earth was taken which, when autoclaved and made into an emulsion, would elicit gas gangrene from the spores of *Vibrion septique*. The watery extract of such an emulsion, after having been filtered through filter paper, autoclaved and tested for its sterility, was just as capable of eliciting gas gangrene as the original emulsion. It could be shown, moreover, by qualitative chemical tests, that this earth extract contained calcium salts and that it lost its power to elicit gas gangrene when these salts had been removed by precipitation with sodium carbonate. We are not however prepared to state that the calcium salts in the soil are the only chemical constituents which are responsible for this phenomenon, for some extracts of earth which were capable of eliciting gas gangrene from the spores of *Vibrion septique* contained only traces of calcium salts. It seems probable that some other chemical substance present in these extracts, which also forms an insoluble carbonate, is also capable of producing the kata-phylactic phenomenon, and may be even more powerful in this respect than calcium salts. Further investigations on this point are being carried out.

It is of interest to note that samples of earth taken from the surface may fail to show this effect when a sample from the same locality about 6 inches below the surface will give a positive result. It has also been found that samples from different localities differ in their activity, and that samples taken from the same locality and from the same depth may show differences at different times of the year, *i.e.*, according to the treatment, such as "liming," which the soil has received. These differences may not exhibit themselves qualitatively by the presence or absence of the power to elicit gas gangrene or tetanus, but quantitatively by the doses of earth extract or emulsion necessary to produce this effect. These observations account satisfactorily for the curious fact that the occurrence of gas gangrene on the Western Front was very "patchy." It varied with the locality in which the wounds had been received, and was relatively infrequent in certain localities, even although the wounds were infected with the bacteria of gas gangrene.

There can be little doubt that the presence of certain simple chemical constituents of the soil which have the property of producing the kataphylactic phenomenon is responsible for the occurrence of gas gangrene and of tetanus. This statement does not imply that every case of gas gangrene and of tetanus can be accounted for in this way. Experimentally, mice infected with detoxicated *B. Welchii* and exposed to cold have occasionally developed gas gangrene. As a rule, gas gangrene does not develop in mice exposed to cold either before or after the injection of a suspension of *B. Welchii*. But out of 16 mice, two animals, in which the exposure to cold had been exceptionally severe so that the animals were already ill when they received the injection of *B. Welchii*, did develop gas gangrene. The experiments with cold are complicated by the fact that mice frequently develop an enteritis as the result of a severe exposure to cold. The general depression of the vitality of an animal which has received such a severe exposure to cold may be reasonably assumed to involve also the processes of lysis and phagocytosis which constitute the defensive mechanism of the infected animal against the infected bacteria, and thus account for the development of gas gangrene in the two positive experiments. In the human subject, where such a depression may be the result not only of exposure to cold but also to shock, it will probably be responsible also for the development of gas gangrene in some cases. But it cannot be looked upon as the only or even most frequent exciting cause, since gas gangrene develops in men who, apart from the wound, are in good health, are not in a state of shock, and have not been exposed to cold.

There is another way in which tetanus and gas gangrene can be produced experimentally in animals infected with the detoxicated bacteria without making use of the phenomenon of defence rupture. Tulloch* has shown that tetanus spores will produce tetanus in guinea-pigs if they are injected together with a non-lethal dose of the toxin of *B. Welchii*. We have found that gas gangrene can be produced in mice if the detoxicated *B. Welchii* are injected together with diphtheria toxin, which happens to be non-lethal for mice but has a transient local action. The explanation of this fact is probably to be found in the aggressin-like nature of the toxins. We have pointed out in the introduction that the toxin of *B. Welchii* acts at first by paralysing the defensive mechanism of lysis and phagocytosis by which the animal defends itself against infection with the detoxicated *B. Welchii*, and diphtheria toxin has probably a similar effect in mice. It seems possible, therefore, that the presence of a non-specific and non-lethal toxin with an aggressin-like action may have to be considered as a factor in the causation of gas gangrene or of

* Tulloch, 'British Medical Journal,' June 1, 1918.

tetanus. As stated in the introduction, we have not been able to obtain experimental evidence in support of this view in the case of gas gangrene by injecting the bacteria of gas gangrene together with other bacteria which are likely to form concomitant infections in wounds. And even in the production of tetanus from the spores by means of the toxin of *B. Welchii*, it must be remembered that this does not represent the effect produced by a concomitant infection with the bacilli of Welch. For such an infection does not, as we have seen, lead to the production of toxin sufficient to paralyse the defensive mechanism. It still requires to be demonstrated that tetanus will result when the spores of tetanus are injected together with the detoxicated bacteria of Welch.

The preceding considerations emphasise the importance of the contamination of the wound with earth from the point of view of the etiology of gas gangrene and of tetanus. For this contamination carries into the wound not only the infecting bacteria, but also frequently the chemical constituents of the soil capable of producing the phenomenon of defence-rupture. But there is also some experimental evidence to show that tetanus and gas gangrene may sometimes develop even without the intervention of the phenomenon.

On the Mode of Action of Calcium Salts.

Two possible explanations suggest themselves at once: the calcium salts may produce their effect either by making the bacteria more virulent, or by making the animal more sensitive to the action of the bacteria. Both these possibilities were tested experimentally.

In order to see whether there is a direct action on the bacteria, *B. Welchii* freed from toxin were suspended in a solution of calcium chloride and incubated for three hours. The suspension was then centrifuged, and the bacteria after washing with saline suspended in a sodium chloride solution, and the suspension injected into mice. No effect was produced.

The same negative result was obtained when *B. Welchii* were incubated for 20 hours in a broth culture to which some calcium chloride solution had been added. A suspension of *B. Welchii* in saline prepared from such a culture was incapable of producing gas gangrene. It is clear, therefore, that calcium salts do not act directly on the bacteria in such a way as to render them capable of producing gas gangrene by themselves in the absence of a toxin. Nor does the addition of calcium chloride to a broth culture increase the amount of toxin produced. This was demonstrated both for *B. Welchii* and for *Vibrion septique*. It was however noted that the rate of growth of *B. Welchii* and of *Vibrion septique* was much more rapid after the addition of calcium chloride. It was further found that a culture of *B. Welchii* or

Vibrion septique, obtained by inoculating meat broth with fragments of breast muscle from pigeons which had been inoculated four hours previously with a broth culture of these organisms to which calcium chloride had been added, was not more virulent than a culture from a pigeon which had been infected with broth cultures alone of these bacteria.

It is clear, therefore, that the action of calcium chloride is not due to a change in the essential properties of the bacteria so as to make them individually more virulent. This conclusion is confirmed by the observation, to which reference has already been made, that a direct contact between the bacteria and calcium salts is not essential for the production of the kataphylactic effect. There remains the other alternative, that the effect of calcium salts might be to make the animals more sensitive to the action of the bacteria. Now it is known that the bacteria of gas gangrene and of tetanus owe their pathogenic effect to the formation of a toxin. The correctness of the second alternative can therefore be tested by determining whether the injection of calcium chloride diminishes the minimal lethal dose of these toxins. This test was applied to the toxin of *B. Welchii*. It was found that the injection of calcium salts did not make an animal more sensitive to the action of this toxin. What, then, is the explanation of the phenomenon described in this paper if it cannot be attributed either to an increased sensitiveness of the infected animal or to an increased virulence on the part of the infecting bacteria? A partial answer to this question is given by the experiments, in which the injections of calcium salts and of bacteria were separated in time or in space.

Gas gangrene will result if *Vibrion septique* spores are injected into an animal which some hours or days previously has received an injection of calcium salts. This result is obtained most readily if the two materials are injected into the same site, and in that case the interval between the two injections may extend over several days if a large dose of calcium salt has been given. Thus in two different experiments the following results were obtained: 2·5 mgrm. Ca salt given two hours before *Vibrion septique* spores: injected into same site into eight mice. Result.—All mice dead within 24 hours. 10 mgrm. Ca salt given three days before *Vibrion septique* spores: injected into same site into nine mice. Result.—All mice dead within 24 hours. Even if the two materials are inoculated in two different sites, gas gangrene may follow, although not in every case.

The following experiment may be given to illustrate the result of separating the sites and times of injecting the bacteria and the rupturing substance. In this experiment 36 mice, in 12 batches of 3 mice each, received at the same time injections of calcium nitrate in varying doses.

At two different intervals of time—2 and 24 hours afterwards—the mice received injections of a suspension of *Vibrion septique* spores, half the number of animals being injected subcutaneously at the same site (right flank), the other half being injected subcutaneously on the back. The results were as follows:—

Gas Gangrene from *Vibrion septique*. Effect of separating Time and Site of Injections of Bacteria and of Calcium Salt.

Batch.	No. of mouse.	Dose of Ca nitrate.	Interval of time.	Site.	Result after 24 hours.
		mgrm.	hours.		
A	1	2·5	1¼	Same	+
	2				+
	3				+
B	4	2·5	1¼	Different	0
	5				0
	6				0
C	7	2·5	20	Same	+
	8				+
	9				+
D	10	2·5	20	Different	0
	11				0
	12				0
E	13	5	1¼	Same	+
	14				+
	15				+
F	16	5	1¼	Different	0
	17				+
	18				+
G	19	5	20	Same	+
	20				+
	21				+
H	22	5	20	Different	0
	23				0
	24				0
J	25	10	1¼	Same	+
	26				+
	27				+
K	28	10	1¼	Different	+
	29				0
	30				0
L	31	10	20	Same	+
	32				+
M	33	10	20	Different	0
	34				0
Control	35	Spores alone	—	—	0
	36				0

This experiment shows that a minimal rupturing dose will elicit gas gangrene in every case from the spores of *Vibrion septique*, even when the

latter are injected after an interval of 20 hours into the same site. When different sites are used, however, the production of gas gangrene becomes more difficult, and it is necessary to use larger doses of the calcium salt and to inject the spores within a short time after the calcium salts. *Post-mortem* examinations were made of one dead mouse in each batch. All showed the typical lesion of *Vibrion septique* gas gangrene in the right flank, and this was the case even in the three mice where the spores had been injected on the back. Only one of these three mice had the typical lesion also on the back; the two others only showed a slight serous exudate, such as is found in normal mice after the injection of spores. This remarkable fact will be referred to again below.

Similar experiments were carried out with a suspension of *B. Welchii*. With these bacteria the dose of calcium salt has to be increased to 5 mgrm. if an interval of time is allowed to elapse before the injection of the bacteria, and this interval of time within which gas gangrene can be produced is limited to a few hours. After an interval of 24 hours, we have not been able to produce gas gangrene even with a dose of 10 mgrm. of calcium salt. Doses of 10 mgrm. of calcium salt are necessary when different sites are used. Under these conditions, we have observed two different courses which the infection may take. Either, as in the case of *Vibrion septique*, the typical lesion of *B. Welchii* gas gangrene is produced at the site of injection of the calcium salt, and may be completely absent from the site where the bacterial suspension was introduced; or there is no distinct localised gas gangrene lesion, but a *B. Welchii* septicæmia results, in which the hæmolytic action of the infection seems to predominate. For in such animals *B. Welchii* can be seen in films made from the heart blood, the liver and kidney are very pale instead of showing the intense congestion of a typical *B. Welchii* gas gangrene, and the blood is very poor in hæmoglobin.

Experiments with spores of *B. tetani* (Type II of Dr. Tulloch) gave essentially the same results as those with *B. Welchii* and the spores of *Vibrion septique*. As the Table shows, it was possible to elicit tetanus by injecting the spores $2\frac{1}{2}$ hours after the injection of even the minimal rupturing dose of the calcium salt, when the spores were injected into the same site as the calcium salt. But, when different sites were used, tetanus did not occur even with the biggest dose of calcium salt used. Even with an interval of 24 hours tetanus was produced, but then it was delayed and occurred only with larger doses of calcium salt and using the same site. It must be noted, however, that for the experiments with a 24-hours' interval, a different and older preparation of spores was used, which appeared to have lost some of its virulence, since the spores did not

produce tetanus in every case when injected together with a rupturing dose of a calcium salt.

The details of the experiment are given in the following Table. It is perhaps necessary to point out that this experiment is quite different from those mentioned in an earlier part of this paper, in which tetanus spores were injected first and then calcium salts.

Tetanus. Effect of separating Time and Site of Injections of Bacteria and of Calcium Salt.

Batch.	No. of mouse.	Dose of Ca nitrate.	Interval of time.	Site.	Result after 1	2	3	4 days.
		mgrm.	hours.					
A	1	2·5	2½	Same	0	0	0	0
	2				0	+	—	—
B	3	2·5	2½	Different	0	0	0	0
	4				0	0	0	0
C	5	5	2½	Same	0	+	—	—
	6				0	+	—	—
D	7	5	2½	Different	0	0	0	0
	8				0	0	0	0
E	9	10	2½	Same	0	+	—	—
	10				0	+	—	—
F	11	10	2½	Different	0	0	0	0
	12				0	0	0	0
G	13	5	24	Same	0	0	0	+
	14				0	0	0	0
H	15	5	24	Different	0	0	0	0
	16				0	0	0	0
J	17	10	24	Same	0	+	—	—
	18				0	0	+	—
K	19	10	24	Different	0	0	0	0
	20				0	0	0	0
Control	21	Spores alone	—	—	0	0	0	0
	22				0	0	0	0
	23				0	0	0	0
	24				0	0	0	0
	25				0	0	0	0
	26				0	0	0	0

0 means alive and well with absence of symptoms of tetanus. + means died with symptoms of tetanus.

All these experiments point to the conclusion that the injection of calcium salts produces at the site of injection a local change in the tissues of the animal since the specific disease is most easily elicited when the calcium

salt and the bacteria are injected into the same site. It does not appear reasonable to suppose that this local effect is due to the actual presence of calcium salts at the site of injection. Since the effect is produced only by soluble, ionisable calcium salts, it would be necessary to assume that such a salt could remain deposited at the site of injection for a day or even three days. Moreover, if that were so, it ought to be possible to counteract their effect by injecting sodium citrate together with the bacterial suspension. That, however, we have been unable to do. In one experiment, for instance, eight mice received an injection of 2·5 mgrm. of calcium chloride in the right flank. Two hours afterwards four of the mice received a suspension of *Vibrion septique* spores in saline in the right flank, while the other four received a suspension of these spores in 2 per cent. sodium citrate solution, also in the right flank. All the eight mice were dead within 24 hours with the typical local lesion of gas gangrene. Similar experiments have been carried out with the same result. Sodium citrate only protects when it is mixed with the calcium salt before it is injected into the animal.

One must conclude, therefore, that calcium salts produce a local change in the tissues at the site of injection. This conclusion is confirmed by the fact that it is much more difficult to produce gas gangrene and tetanus when the bacteria and the calcium salts are injected at different sites, than when they are injected at different times. The local change produced in the tissues by calcium salts persists for a considerable time, and has, as the ultimate result, the formation in the animal of a place of diminished resistance against the infecting bacteria. The most striking confirmation of this conception is furnished by the result of the experiments in which gas gangrene occurred after injection of the calcium salt and bacterial suspension at different sites. For in these experiments the fact was observed that the typical local lesion of gas gangrene—an intense hæmorrhagic œdema—occurs always at the site of injection of the calcium salt, while the site where the bacteria have been injected does not show, as a rule, any macroscopic evidence of gas gangrene. Films made from the two sites present this difference very strikingly (see fig. 1); the film from the site of injection of *B. Welchii* shows active lysis, as indicated by the presence of bacterial *débris*, and active phagocytosis with intracellular digestion of the engulfed bacteria. Only a few bacteria can still be seen lying free and apparently intact, but many of these have become gram-negative. The picture is practically identical with that given by a normal mouse which has received a suspension of detoxicated bacteria, and which is defending itself successfully against the infection. But the film from the site of injection of the calcium salt presents the picture typical of an animal which has developed gas gangrene by, let us say, the

injection of the bacteria together with toxin. The whole field is covered with densely packed bacteria which are almost all gram-positive. Only very few leucocytes can be seen, and none of these show any phagocytosis.

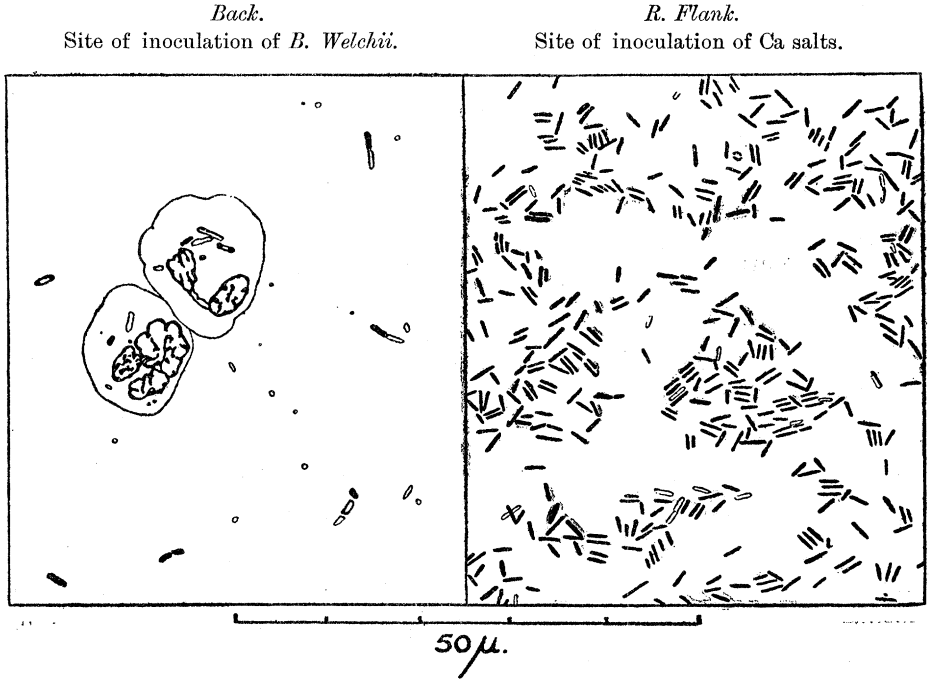

FIG. 1.—Films from the back and right flank of a mouse which developed gas gangrene and died 24 hours after having received first 10 mgrm. of $CaCl_2$ in the right flank, and 2 hours later a suspension of *B. Welchii* in saline on the back. Stained with gram and neutral red. Gram-negative staining is indicated by merely outlining the bacteria.

Both macroscopically and microscopically the picture presented by the right flank is that of an animal which has succumbed to the *B. Welchii* infection; the picture presented by the back that of an animal which is successfully defending itself against such an infection.

This apparently paradoxical result becomes clear in the light of the conception that the calcium salts produce at the site of their injection a local change in the tissues which has as its ultimate effect a local breaking down of the defensive mechanism. This conception of the mechanism is expressed by the terms "defence rupture" or "kataphylaxis," with which we have designated this phenomenon.

When calcium salts and bacteria are injected together it is quite possible that this effect may be assisted by other factors. There may be a direct favouring effect of the calcium salts on the rate of growth of the bacteria,

such as has been observed *in vitro*. It is conceivable too that calcium salts may directly interfere with phagocytosis and lysis, and experiments *in vitro* will have to be carried out in order to test this point. But these factors, even admitting for the sake of the argument that calcium salts did produce these effects *in vivo*, could only be accessory factors under the special experimental conditions which involve the actual presence of calcium salts. When a separation of the sites or times of the injections is made, the actual presence of calcium salts can be excluded, but the phenomenon still makes its appearance.

Summary.

The bacteria of gas gangrene (*B. Welchii, Vibrion septique,* and *B. œdematiens*) and of tetanus, when completely freed from their toxins, either by washing or by heating to 80° C. for half-an-hour, so that spores are formed, do not produce the specific disease when injected into a mouse or a guinea-pig. The normal animal disposes of the bacteria mainly by lysis and partly also by phagocytosis, and this defensive mechanism is so efficient as to render these bacteria non-pathogenic when injected by themselves.

If a small dose of a soluble ionisable calcium salt is injected together with the bacteria or their spores, the specific disease is elicited in a very virulent form. The chlorides of sodium, potassium, ammonium, strontium and magnesium, when injected together with *B. Welchii,* are not capable of producing gas gangrene.

A direct contact between the bacteria and the calcium salt is not essential. The phenomenon will occur if the bacterial suspension and the calcium salt are injected at different times into the same site, or into different sites at the same time or at different times.

From these experiments and other experimental evidence the conclusion is drawn that calcium salts, when injected subcutaneously, produce a local change in the tissues at the site of injection. The effect of this change is to bring about a local breaking down of the defensive mechanism against the bacteria of gas gangrene and tetanus. The terms "kataphylaxis" or "defence rupture" are proposed to designate this new phenomenon.

Sterile watery extracts of earth are capable of producing this phenomenon. They may owe this property in many cases entirely to the presence of calcium salts, but there is evidence that in some cases the extracts of earth owe their rupturing action to the presence of another chemical substance or substances which have not yet been identified.

The bearing of these observations on the etiology of gas gangrene and tetanus is discussed.

The figure is by Mr. W. Pilgrim, the laboratory draughtsman. We wish to express our great indebtedness to Dr. H. Henry, Dr. J. McIntosh, Dr. R. A. O'Brien, Miss Muriel Robertson, and Captain W. J. Tulloch, R.A.M.C., for their readiness in assisting our investigations by supplying us with information and material.

The Distribution of the Serological Types of B. tetani *in Wounds of Men who received Prophylactic Inoculation, and a Study of the Mechanism of Infection in, and Immunity from, Tetanus.*

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(From the Laboratories of the Lister Institute of Preventive Medicine and the Royal Army Medical College.)

Introduction.

The bacteriological investigation of tetanus, the results of which are summarised in the present communication, was undertaken on behalf of the War Office Committee for the Study of Tetanus.

Before the bacteriological examination of a relatively large number of cases of tetanus could be effected, it was found necessary to elaborate a suitable technique,* as the existing methods for the cultivation of *B. tetani* proved to be wholly inadequate.

Apart from its practical application, this preliminary work was of great value, in that it called attention to the important factor of symbiosis in the growth of anaërobic bacteria. The cycle in the development of anaërobic bacteria in cultures of the mixed flora of wound exudates is remarkably constant, and it is not improbable that a similar sequence occurs in the wounds themselves. The first organisms to appear are rapidly growing bacilli typified by *B. Welchii*, followed by the proteolytic group of anaërobes of the *B. sporogenes* Types. On the decline of the proteolytic group, *B. tetani* and other similar organisms appear, which may ultimately predominate in the cultures.

* See p. 539.