AN EXPERIMENTAL INVESTIGATION ON THE INFLUENCE OF EMULSIONS OF OILS AND FATS ON THE LETHAL EFFECTS OF BACTERIAL TOXINS

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CONTENTS

I. Introduction

It is now generally accepted that certain bacterial toxins are able to give rise to fatty degeneration in tissues and may bring about marked changes of this nature in the heart, liver, kidneys and other organs. When a cell is damaged a state of cloudy swelling or of parenchymatous degeneration generally occurs. The fat is distributed throughout the cell as fine globules and the condition is known as fatty degeneration. Numerous workers (see References) are agreed that many toxins, particularly diphtheria toxin, may cause a widespread damage to cells of the body in this way. The processes involved in the deposition of this fat have led to much discussion and speculation, but do not concern us here.

The regularity with which fat is laid down in cells in response to injury by diphtheria toxin led me to carry out a number of experiments with a view to finding whether fats exert any protective influence against the destructive effects of toxins. My original idea was that the fat laid down might act as a protection against further damage to the cell. A few experiments quickly proved that this supposition was ill-founded and that oils or fats in such coarse globules, as are found in cells during fatty degeneration, have no influence whatever on the lethal effects of the toxins. This led to a series of experiments

https://doi.org/10.1017/S0022172400034586 Published online by Cambridge University Press

with oil emulsions in a very fine state of division, and it is the results of these experiments which are embodied in this communication.

The object of this investigation was to determine whether emulsions of oils and fats have any modifying effect upon the action of bacterial toxins. Oils and fats derived from vegetable, mineral and animal sources were used. These were made up in the form of emulsions and mixed with aqueous solutions of lethal doses of toxins which were injected subcutaneously into animals. The results obtained using diphtheria toxin and emulsions of olive oil will be described first.

Preparation of the olive oil emulsion, 50 *per cent.* (*with gum acacia*). Mix together in a mortar 3 oz. of olive oil, $\frac{3}{4}$ oz. of gum acacia, and $1\frac{1}{2}$ oz. of water, until a thick cream is formed. Sufficient water to make 6 oz. of emulsion is added and the whole put through a mechanical emulsifier. This emulsion mixes well with water and retains its characters even when mixed with an equal amount of water. The emulsion was mixed with aqueous solutions of the toxin immediately prior to injection into the animals.

The toxins used in this investigation were kindly supplied by Dr O'Brien of the Wellcome Laboratories and the emulsions were made by Mr E. S. Peck, of Cambridge. A preliminary determination of the minimum lethal doses of the toxins used was carried out, using standard guinea-pigs.

II. EXPERIMENTAL

(*a*) *The influence of olive oil emulsion*, 50 *per cent., with gum acacia*

For the purposes of the first experiment twelve guinea-pigs were injected subcutaneously with a mixture of 4 M.L.D. of diphtheria toxin in the emulsion. Four guinea-pigs were used as controls and injected with 4 M.L.D. in an aqueous solution, without the addition of the emulsion. The controls ate very little and died within 2 days, whereas the others consumed their food as usual and their appetites were not seriously affected. The results are embodied in Table I, which shows that all the animals which had been injected with the toxin-emulsion mixture survived. These animals were fit and well 12 months later. This result is so decisive that a second experiment was conducted on similar lines, using a much greater lethal dose of the toxin, 12 M.L.D. The result is tabulated in Table II and is as clearly defined as that of the previous experiment. Here again the mortality rate of the control animals is 100 per cent., whereas all the animals which had been given the toxin mixed with the emulsion survived. The result of a further experiment, using thirteen guinea-pigs and increasing the lethal amount of the toxin to 24 M.L.D., was similar. In spite of the enormous increase in the amount of toxin injected, the mortality rates remain in agreement with those of the two previous experiments. Greater doses of toxin were not tried, but it is conceivable that similar results might have been obtained. Similar results were obtained, using the toxins of *B. tetani*, *B. welchii*, and the *Clostridium oedematis-maligni* (Koch) (Tables III–V). The

volume of emulsion contained in each injection was usually 30–50 times that of the aqueous solution of the toxin and the total volume injected into any one animal rarely exceeded 0·5 c.c. These experiments clearly prove that if lethal doses of these toxins are mixed with an emulsion of olive oil before injecting them into animals, then such animals do not succumb to the effects of the toxin.

Table I. *The influence of olive oil emulsion,* 50 *per cent., with gum acacia, upon the resistance of guinea-pigs to diphtheria toxin.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life	Mortality rate
1	310	4	Survived	Nil
2	300	4	,,	
3	300	4	,,	
4	290	4	,,	
5	320	4	,,	
6	310	4	,,	
7	327	4	,,	
8	312	4	,,	
9	357	4	,,	
10	337	4	,,	
11	347	4	,,	
12	310	4	,,	
A	300	4	24 hours	100 %
B	295	4	36 ,,	
C	300	4	36 ,,	
D	290	4	23 ,,	

* Nos. 1–12: emulsion animals. A–D: controls.

Table II. *The influence of olive oil emulsion,* 50 *per cent., with gum acacia, upon the resistance of guinea-pigs to diphtheria toxin.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life	Mortality rate
1	342	12	Survived	Nil
2	380	12	,,	
3	327	12	,,	
4	317	12	,,	
5	347	12	,,	
6	325	12	,,	
7	375	12	,,	
8	361	12	,,	
A	370	12	12 hours	100 %
B	295	12	23 ,,	
C	360	12	21 ,,	
D	300	12	22 ,,	

* Nos. 1–8: emulsion animals. A–D: controls.

Further experiments were performed with a view to determining the cause of this protection against the lethal effects of the toxin and the relative importance of the constituents of the 50 per cent. emulsion. The emulsion used in these early experiments contained olive oil, gum acacia, and water, and it was thought that either the oil or the gum may have modified the action of the toxin or even destroyed it in some way. The influence of the oil was investigated

by omitting the gum acacia from the emulsions. This necessitated the making of a simple emulsion of the olive oil and water by mixing them (*a*) with a pestle in a mortar and (*b*) passing through a mechanical emulsifier. The mixing was continued until the oil was in a very fine state of division, the mixture being injected subcutaneously soon after it had been prepared and mixed with the toxin. The proportions of oil and water were kept the same as in the 50 per cent. emulsion which contained gum acacia. The results of experiments with this emulsion were very variable. In some experiments nearly all the animals succumbed, while in others a large percentage survived. This is well shown in the two experiments about to be described.

(*b*) *The influence of olive oil emulsion, 50 per cent., without gum acacia*

Twelve guinea-pigs were divided into two groups and the animals of each group were given a subcutaneous injection of the emulsion of olive oil containing diphtheria toxin. The amount of toxin contained in the emulsion was varied for each group. Each animal of the first group was given 2 M.L.D., while those of the second received 4 M.L.D. An equal number of control animals were injected with the same amounts of toxin without olive oil emulsion. The results are arranged in Table VI, which shows that the mortality rate in both groups is very high. Only two animals out of twelve survived the lethal effects of the toxin and each of these had only received 2 M.L.D. The difference between this result and those previously obtained, using the 50 per cent. emulsion with gum acacia, is so striking that the last experiment was repeated. The results are embodied in Table VII. The mortality rate here is not so great as that shown in Table VI and the explanation was not at once apparent. The only difference between these two experiments was found to be in the time taken in making the emulsion. This apparently affected the fineness of the emulsion and it was found that the finer the emulsion then the lower the mortality rate. Coarse emulsions afforded no protection whatever against the lethal effects of the toxin. This was confirmed by a number of repeat experiments which were designed to elucidate this problem. The protection afforded appears to be proportional to the degree of fineness attained in the emulsion.

(*c*) *The influence of gum acacia*

$\frac{3}{4}$ oz. of gum acacia was dissolved in 6 oz. of water so as to make the concentration of the gum equal to that in the 50 per cent. olive oil emulsion. This gum solution was mixed with aqueous solutions of the toxin immediately prior to injection into animals.

Eight guinea-pigs were each injected subcutaneously with 4 M.L.D. of diphtheria toxin contained in the gum solution. Four control animals were injected with 4 M.L.D. of the toxin in saline without the addition of the gum solution. Turning to Table VIII, it will be seen that all the animals died irrespectively of whether they had received gum acacia or not. This decisive result proves that gum acacia alone does not influence the resistance of guinea-pigs to diphtheria toxin.

Table III. *The influence of olive oil emulsion,* 50 *per cent., with gum acacia, upon the resistance of guinea-pigs to tetanus toxin.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life	Mortality rate
1	300	4	Survived	Nil
2	310	4	,,	
3	365	4	,,	
4	370	4	,,	
5	390	4	,,	
6	350	4	,,	
A	375	4	2 days	100 %
B	300	4	2 ,,	
C	369	4	2·5 ,,	
D	340	4	3 ,,	
7	330	12	Survived	Nil
8	360	12	,,	
9	350	12	,,	
10	360	12	,,	
11	340	12	,,	
12	345	12	,,	
E	360	12	2 days	100 %
F	375	12	2 ,,	
G	375	12	2·5 ,,	
H	340	12	2 ,,	

Table IV. *The influence of olive oil emulsion,* 50 *per cent., with gum acacia, upon the resistance of guinea-pigs to the toxin of* G. welchii.

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life	Mortality rate
1	340	4	Survived	Nil
2	280	4	,,	
3	290	4	,,	
4	310	4	,,	
5	320	4	,,	
6	300	4	,,	
A	310	4	3 days	100 %
B	315	4	3 ,,	
C	300	4	3·5 ,,	
D	300	4	2·5 ,,	
7	280	12	Survived	Nil
8	275	12	,,	
9	290	12	,,	
10	270	12	,,	
11	300	12	,,	
12	290	12	,,	
E	280	12	2·5 days	100 %
F	270	12	3 ,,	
G	260	12	2 ,,	
H	295	12	2 ,,	

* Nos. 1–12: emulsion animals. A–H: controls.

https://doi.org/10.1017/S0022172400034586 Published online by Cambridge University Press

Table V. *The influence of olive oil emulsion, 50 per cent., with gum acacia, upon the resistance of guinea-pigs to the toxin of* Clostridium oedematis-maligni (*Koch*).

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life	Mortality rate
1	260	4	Survived	Nil
2	250	4	,,	
3	275	4	,,	
4	260	4	,,	
5	265	4	,,	
6	270	4	,,	
A	280	4	2·5 days	100 %
B	270	4	2·5 ,,	
C	270	4	3 ,,	
D	250	4	3 ,,	
7	290	12	Survived	Nil
8	295	12	,,	
9	260	12	,,	
10	275	12	,,	
11	275	12	,,	
12	280	12	,,	
E	250	12	2·5 days	100 %
F	270	12	2 ,,	
G	270	12	2 ,,	
H	270	12	2 ,,	

* Nos. 1–12: emulsion animals. A–H: controls.

Table VI. *The influence of olive oil emulsion upon the resistance of guinea-pigs to diphtheria toxin.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life days	Mortality rate
1	285	2	4	66·6 %
2	300	2	3	
3	290	2	2	
4	295	2	Survived	
5	300	2	,,	
6	300	2	3	
A	320	2	3	100 %
B	300	2	3·5	
C	295	2	3	
D	270	2	2	
E	280	2	2	
F	300	2	2·5	
7	275	4	3	100 %
8	290	4	3	
9	280	4	2	
10	285	4	4	
11	310	4	1·5	
12	305	4	5	
G	300	4	2	100 %
H	320	4	3	
J	275	4	2	
K	270	4	1·5	
L	275	4	2	
M	300	4	2	

* Nos. 1–12: olive oil animals. A–M: controls.

https://doi.org/10.1017/S0022172400034586 Published online by Cambridge University Press

Table VII. *The influence of olive oil emulsion upon the resistance of guinea-pigs to diphtheria toxin.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life days	Mortality rate
1	305	2	Survived	33·3 %
2	320	2	3	
3	300	2	Survived	
4	350	2	,,	
5	341	2	3	
6	335	2	Survived	
A	310	2	2	100 %
B	325	2	2	
C	290	2	3	
D	349	2	2	
E	325	2	1	
F	305	2	2	
7	282	4	3	50 %
8	300	4	3	
9	312	4	Survived	
10	290	4	,,	
11	270	4	3	
12	278	4	Survived	
G	310	4	2	100 %
H	300	4	3	
J	250	4	2	
K	285	4	1·5	
L	300	4	2	
M	292	4	2	

* Nos. 1–12: olive oil animals. A–M: controls.

Table VIII. *The influence of gum acacia upon the resistance of guinea-pigs to diphtheria toxin.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life hours	Mortality rate
1	315	4	36	100 %
2	345	4	35	
3	290	4	34	
4	300	4	36	
5	340	4	30	
6	325	4	33	
7	300	4	30	
8	330	4	30	
A	300	4	24	100 %
B	350	4	36	
C	340	4	35	
D	310	4	23	

* Nos. 1–8: gum acacia animals. A–D: controls.

The well-marked difference in the protection afforded by the 50 per cent. olive oil emulsion containing gum acacia and similar emulsions made without the addition of the gum, is striking. It has already been proved that the gum by itself exerts no protective action whatever, and was added to the emulsion simply to assist in binding the emulsion together into a more stable form. That

the stability of the emulsion is an important factor is shown by the result obtained from the experiments using the simple aqueous olive oil emulsion mixtures. It will, therefore, be seen that the protective action is concerned with the olive oil while the gum acacia makes the protection more secure by making the emulsion more permanent.

(d) Centrifugalisation of emulsion-toxin mixtures

This question was investigated further in another series of experiments. Simple emulsions of olive oil were made in as fine a state of division as possible, using a mechanical emulsifier, and mixed with superlethal doses of diphtheria toxin (4, 8 and 12 M.L.D.). Three groups of six guinea-pigs were given, subcutaneously, a superlethal dose of the toxin mixed with the emulsion. The animals of the first group were given 4 M.L.D. per animal, those of the second 8 M.L.D., and those of the third group 12 M.L.D. A portion of each of the toxin-emulsion mixtures used was then centrifugalised at 2500 revolutions per minute for 2 hours, when the constituent parts of the emulsions separated out into two layers. A further three groups of six guinea-pigs were weighed and given subcutaneous injections of the aqueous layers calculated on a basis of 4, 8, and 12 M.L.D. respectively. In a similar way, three additional groups were injected with the oily layers. The results were very clear and showed that all the animals survived in the first three groups, which had been given the toxin-emulsion mixture. The second three groups, which had received the aqueous layer after centrifugalisation, all died. The third three groups, which had been injected with the oily layer, all survived. Table IX shows the results obtained using 4 M.L.D.

It is apparent from these results that when the emulsion breaks down the toxin has a greater solubility or affinity for water than for oil even when they have been previously mixed so as to form a very finely divided emulsion. A survey of the literature shows that little or nothing has been done to determine the partition coefficients of toxins in oil and water, but it would appear, from these experiments, that diphtheria toxin is insoluble or only very slightly soluble in olive oil.

The last experiment was repeated, using a 50 per cent. emulsion of olive oil containing gum acacia. Superlethal doses of diphtheria toxin (4, 8 and 12 M.L.D.) were used and nine groups of six guinea-pigs. The results obtained showed that all the animals of the first three groups which had been injected with the emulsion-toxin mixture survived. The second three groups, representing the aqueous layer, survived, while the last three groups, which had been injected with the oily layer, also survived. This is well exemplified in Table X, showing the results using 4 M.L.D. The difference between this result and that of the previous experiment is well marked and is capable of a very simple explanation. The emulsion-toxin mixture containing gum acacia was found to be so stable that centrifuging it for 3 hours at 2500 revolutions per minute failed to cause anything but a very slight separation of the con-

Table IX. *The influence of olive oil emulsion upon the resistance of guinea-pigs to diphtheria toxin. The simple toxin-olive oil emulsion mixture was centrifugalised.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Material injected	Duration of life	Mortality rate
1	280	4	Toxin-olive oil emulsion mixture (before centrifugalisation)	Survived	Nil
2	260	4		,,	
3	275	4		,,	
4	230	4		,,	
5	245	4		,,	
6	270	4		,,	
7	255	≡4	Aqueous layer (after centrifugalisation)	36 hours	100 %
8	225	≡4		48 ,,	
9	225	≡4		32 ,,	
10	290	≡4		36 ,,	
11	270	≡4		18 ,,	
12	250	≡4		16 ,,	
13	260	≡4	Oily layer (after centrifugalisation)	Survived	Nil
14	245	≡4		,,	
15	280	≡4		,,	
16	270	≡4		,,	
17	290	≡4		,,	
18	230	≡4		,,	
A	245	4	Toxin only	36 hours	100 %
B	270	4		30 ,,	
C	300	4		36 ,,	

Table X. *The influence of olive oil emulsion,* 50 *per cent., with gum acacia, upon the resistance of guinea-pigs to diphtheria toxin. Emulsion-toxin mixtures centrifugalised.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Material injected	Duration of life	Mortality rate
1	300	4	Toxin-olive oil emulsion mixture (before centrifugalisation)	Survived	Nil
2	310	4		,,	
3	285	4		,,	
4	280	4		,,	
5	300	4		,,	
6	305	4		,,	
7	280	≡4	Aqueous layer (after centrifugalisation)	Survived	Nil
8	300	≡4		,,	
9	265	≡4		,,	
10	255	≡4		,,	
11	265	≡4		,,	
12	255	≡4		,,	
13	315	≡4	Oily layer (after centrifugalisation)	Survived	Nil
14	290	≡4		,,	
15	275	≡4		,,	
16	290	≡4		,,	
17	300	≡4		,,	
18	300	≡4		,,	
A	300	4	Toxin only	24 hours	100 %
B	295	4		36 ,,	
C	290	4		24 ,,	

* Nos. 1–18: emulsion animals. A–C: toxin controls.

https://doi.org/10.1017/S0022172400034586 Published online by Cambridge University Press

stituents, so that the "layers" which were injected into the animals were in reality emulsions, hence the protection and the low mortality rate of the animals, especially those which had received the aqueous layer. Similar results were obtained using the toxins of *B. tetani*, *B. welchii*, and the *Clostridium oedematis-maligni* (Koch).

(*e*) *Liquid paraffin emulsions*

A long series of experiments using liquid paraffin instead of olive oil was conducted. This oil was chosen so that a comparison might be made between oils of vegetable and mineral origin. It might be mentioned here that some difficulty was experienced in preparing simple emulsions with liquid paraffin; the oil persistently separated out after standing 10–15 min. unless a binding agent, such as gum acacia, was added. The results obtained using this oil show that, when gum acacia is added, the emulsion is able to confer upon the animals a protection against the lethal effects of enormous doses of the toxins of *B. diphtheriae*, *B. tetani*, *B. welchii* and the *Clostridium oedematis-maligni*. This protection, like that afforded by olive oil, is absolute up to the limits set in these experiments (12 M.L.D.). Emulsions of liquid paraffin, without the addition of a binding agent, are very unstable, and easily separate out into an aqueous and an oily layer. The protection conferred by such emulsions was found to be of a very low order and very erratic. In some experiments these emulsions protected 80 per cent. of the animals against 4 M.L.D., in other experiments, where a similar emulsion was used, no protection whatever was given against 4 M.L.D. and all the animals died. A 60 per cent. mortality rate with 3 M.L.D. was recorded in one experiment. It might be suggested that the oil and the aqueous portions of these emulsions separated out inside the tissues of those animals which died after the injection of this simple emulsion. It has been shown already that the toxin would remain with the aqueous portion and so be more readily absorbed from the tissues causing death in exactly the same way as in the control animals.

(*f*) *Cream from cows' milk*

It was thought desirable to try a few experiments using natural animal fats instead of vegetable or mineral oils. Milk cream is easy to obtain and is liquid at ordinary temperatures. In the earlier experiments the toxin was added to the cream and mixed thoroughly by shaking immediately prior to injection. It was found that this fat afforded no protection whatever against these toxins when used in this manner, even when only 1·5 M.L.D. were used (Table XI).

In later experiments the cream was passed through a mechanical emulsifier before the addition of the toxin. This produced a fine emulsion-toxin mixture, and the results obtained using this fat emulsion were very erratic and very similar to those already described for liquid paraffin without the addition of a binding agent.

Table XI. *The influence of milk cream emulsion upon the resistance of guinea-pigs to diphtheria toxin.*

No.*	Wt. g.	Amount of toxin injected M.L.D.	Duration of life days	Mortality rate
1	390	1·5	3	100 %
2	380	1·5	3	
3	320	1·5	2	
4	350	1·5	2	
A	375	1·5	3	100 %
B	350	1·5	2	
C	390	1·5	2	
5	360	3	3	100 %
6	375	3	2	
7	360	3	2	
8	390	3	2	
D	400	3	2	100 %
E	350	3	2	
F	300	3	2	

* Nos. 1–8: milk cream animals. A–F: controls:

III. Pathology

The pathological changes in the heart in toxaemia were studied during the course of this investigation. Autopsies were carried out on all the animals which had died from the lethal effects of the toxins used irrespective of whether they had been injected with oil emulsions or not. In addition, a large number of the animals which had been injected with the emulsion-toxin mixtures and had survived the lethal effects of the toxin were killed and examined at varying periods, from 4 months to 2 years after the experiments.

The hearts were removed within a few hours of death and portions fixed in (*a*) osmic acid, (*b*) 10 per cent. formal-saline and (*c*) Carnoy's solution. Sections from the two latter were stained both with haematoxylin and eosin, and haematoxylin and Van Giesen's stain. The sections were cut so that it was possible to examine both ventricles, both auricles and the intraventricular septum. Formal-saline and Carnoy's solution were used as fixatives so that a comparison might be made of them. In many cases the liver, kidneys and other organs were also examined microscopically.

Effects of diphtheria toxin

Studies of the effects of diphtheria toxin on the heart have led to no detailed agreement among workers (see References), though all agree that the heart can be profoundly affected.

The following is a typical protocol of an autopsy held on a control guinea-pig in which death occurred 3–4 days after the injection of the toxin.

The heart is paler than usual and both ventricles and auricles are dilated. Small petechial haemorrhages are occasionally seen beneath the pericardium.

On section the myocardium is soft and tears very easily. Anti-mortem thrombi are often present in both ventricles. All the blood vessels show marked congestion. The valves and coronary arteries appear normal. On microscopic examination the fibres of the myocardium are generally swollen and homogeneous, some however are atrophic and fragmented. Many fibres have undergone hyaline degeneration with loss of striation. The nuclei are pyknotic and have lost their staining reaction. Advanced focal necrosis was rarely seen. Red blood corpuscles lie freely between the muscle fibres indicating small haemorrhages into the myocardium. In many places separation of the muscle fibres was seen. The vessels are dilated, especially the veins. The blood in the vessels shows an increase in leucocytes mainly of the lymphocyte variety. The tissues around the vessels show an infiltration of leucocytes.

Sections stained with osmic acid always show small globules of fat in the muscle fibres. They are especially prevalent adjacent to the pericardial and endocardial surfaces.

Other organs are also congested and often oedematous. The liver generally shows decided fatty degeneration and the kidneys invariably exhibit clouding swelling. Throughout the submucous region of the small and large intestines small petechial haemorrhages are often scattered. Free blood is sometimes present in the lumen of the large intestine and rectum and small haemorrhages are not uncommon beneath the mucous membrane.

The following is a typical protocol of an autopsy held on a control guinea-pig which died 15–16 days after the injection of the toxin.

The pleural and pericardial sacs contain an excess amount of pale yellowish fluid. The pericardium shows nothing abnormal. The heart is enlarged and dilated; the blood vessels are congested. On section the myocardium is pale, soft and friable.

On microscopic examination the muscle fibres show degeneration with loss of striation and small areas of early necrosis. Many fibres stain poorly with eosin and the nuclei are pyknotic. Red blood corpuscles lie freely between the muscle fibres. Fibroblastic proliferation is present and fibrillae can be seen extending into the small necrotic areas.

The blood vessels are congested and very large numbers of lymphocytes are present in the surrounding tissues. The vessels are not dilated. Sections stained with osmic acid show extensive areas of fatty degeneration of the muscle fibres. These areas are most marked in the endocardial region of the left ventricle and in the papillary muscles.

Several hundreds of sections were examined to determine if differences existed between the tissues of the control animals and those which had been given the toxin mixed with the oil emulsions.

When death occurred in an animal which had been injected with a diphtheria toxin-emulsion mixture the post-mortem findings were almost identical

with those of the control animals which lived for the same length of time after being injected with the toxin alone. These findings were constant both in the case of the olive oil emulsions and the liquid paraffin emulsions.

A large number of animals which had been given emulsions of these oils mixed with superlethal doses of diphtheria toxin, and had survived, were examined. These were killed 4 months after the injection of the toxin-emulsion mixtures. In all cases the myocardium was practically free from fatty degeneration or fibrosis. Only very minor degrees of fatty degeneration were seen in a few animals; speaking generally the hearts showed no difference from normal healthy hearts. Another series of animals which had lived as long as 18 and 24 months after the administration of the toxin-emulsion mixture were killed and examined. The results were in complete agreement with those just described as occurring in animals after 4 months.

All the animals died which had been given superlethal doses of diphtheria toxin mixed with the cream of cow's milk. An examination of the hearts, kidneys and livers of these animals showed no differences from the control animals of the same experiments. They all showed the lesions due to diphtheria toxin which have been described already in detail.

No cardiac lesions were found in any of the animals which had succumbed to the lethal effects of the toxins of *B. tetani*, *B. welchii*, or the *Clostridium oedematis-maligni* (Koch), which could definitely be described as being due to the effects of these toxins.

It is obvious from these results that when diphtheria toxin is injected subcutaneously mixed with a very finely divided emulsion of olive oil or liquid paraffin it is unable to produce its lethal effects or to produce the pathological changes in the heart or other organs, which are seen in the control animals which have received the toxin alone. The toxins of *B. tetani*, *B. welchii* and *Clostridium oedematis-maligni* (Koch), when injected subcutaneously, do not produce pathological lesions in the heart.

IV. Discussion

It is well known that certain colloidal suspensions, like those of kaolin, charcoal, etc., are able to adsorb toxins and other substances not only *in vitro* but inside the body itself. Fine oil globules in emulsions of the oil in water type carry a negative charge and it is possible that they may adsorb toxins in the same way as these colloidal suspensions. The amount of toxin adsorbed will depend upon the surface area of the particles which is dependent upon the fineness of the particles of the emulsion. That this adsorption phenomenon is of the reversible type is shown by the experiments which demonstrated the recovery of the toxin in the aqueous layer after centrifugalisation of the toxin-emulsion mixtures. Milk cream may not form a very stable emulsion when in a fine state of division; this would account for the lack of protection afforded by this oil.

The pathological findings offer strong evidence in favour of the suggestion that either the toxins are first adsorbed by the oil particles and very slowly absorbed from the tissues and later destroyed in the body or that the toxins are destroyed by the emulsions. The experimental evidence already given would seem to favour the first suggestion.

Further experiments are at present in progress with a view to determining whether an active immunity can be produced in animals in response to the injection of these toxins mixed with oil emulsions and whether the serum of such animals is able to confer a passive immunity upon other animals, against the specific toxin used. A few preliminary experiments, on these lines, have just been completed and appear promising.

Walsh and Fraser (1934) have, quite independently and unknown to me, been investigating a somewhat similar problem. They injected large doses of toxins with cod-liver oil and olive oil emulsions subcutaneously into rabbits and noted the absence of toxic symptoms. Their results are in agreement with those described in this communication. They also record that large doses of tuberculin B.E., when administered mixed with olive oil emulsion to a patient suffering from pulmonary tuberculosis, do not give the general reaction which much smaller doses of B.E. cause when the tuberculin is given alone.

V. CONCLUSIONS

1. Olive oil emulsions, in a fine state of division, when mixed with superlethal doses of diphtheria toxin, protect animals from the lethal effects of the toxin when injected subcutaneously.

2. Olive oil emulsions also protect against the effects of superlethal doses of the toxins of *B. tetani*, *B. welchii*, and *Clostridium oedematis-maligni* (Koch).

3. The addition of a suitable emulsifying agent to give the emulsion greater stability, makes the protection against the toxins absolutely secure.

4. Emulsions of liquid paraffin have a similar protective action against lethal doses of these toxins, but the cream of cow's milk affords no protection.

5. Coarse emulsions of these oils do not exhibit this protective action.

6. The toxins of *B. diphtheriae*, *B. tetani*, *B. welchii* and *Clostridium oedematis-maligni* are more soluble, or have a greater affinity for water than oils.

7. Solutions of gum acacia do not influence the lethal effects of these toxins.

8. Diphtheria toxin when mixed with stable and finely divided emulsions of olive oil or liquid paraffin, before being injected subcutaneously, does not produce myocardial degeneration as observed in control animals.

The work on which this communication is based was begun in September, 1929, at the Pharmacological Laboratory, Cambridge, and my sincerest thanks are due to the late Prof. W. E. Dixon for the opportunity to conduct my experiments and for his interest, encouragement and criticisms. I am also indebted to Mr E. S. Peck, of Cambridge, for his generous assistance in the pre-

paration of the emulsions, and to Dr Clifford Hoyle for his valuable suggestions and criticisms at the outset of this investigation.

Some of the results contained in this communication were embodied in my thesis for the Ph.D. degree of the University of Cambridge. This thesis was accepted by the Faculty Board of Medicine of the University in October, 1932.

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(*MS. received for publication* 24. III. 1934.—Ed.)