THE INFLUENCE OF EMULSIONS OF OILS AND FATS UPON THE ACTION OF BACTERIAL TOXINS

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Investigators now are generally agreed that certain bacterial toxins, when absorbed into the circulation, are capable of producing fatty degeneration in tissues. In this way the heart, kidneys, liver, adrenals, and central nervous system may be profoundly affected.

Numerous workers¹⁻¹¹ have reported that diphtheria toxin, in particular, may cause widespread damage to cells of the body in this way. While investigating this problem in the summer of 1929 I examined several hundreds of hearts taken from guinea-pigs, rabbits, and cats which had succumbed to the lethal effects of diphtheria toxin. The regularity with which fat is laid down in cells in response to injury by this 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. The original idea was that the fat deposited in the cell might act as a protection against further damage to the cell. A few experiments, using various oils and fats, quickly showed 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, however, led to a series of experiments, using oil emulsions in a very fine state of division, and it is a brief outline of the results of these experiments which is embodied in this communication.

SCOPE OF INVESTIGATION

The object of this work was to determine whether very finely divided 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, and those selected as being the most suitable were olive oil, liquid paraffin, and the cream of cow's milk. Their influence upon the lethal effects produced by the toxins of *B. diphtheriae*, *B. tetani*, *Cl. welchii*, and the *Cl. oedematis maligni* (Koch) was studied.

The oils and fats were made into finely divided emulsions by means of mechanical emulsifiers, and, in certain experiments, an emulsifying agent was added to make the emulsions more stable. Aqueous solutions of the toxins were mixed with the emulsions immediately prior to injection into the animals. The toxins were kindly supplied by Dr. O'Brien of the Wellcome Laboratories, and the emulsions were made by Mr. E. Saville Peck of Cambridge. All the toxins were standardized, using standard animals. An emulsion containing 50 per cent. olive oil and gum acacia as an emulsifying agent was used in the first experiments.

EFFECT OF OLIVE OIL EMULSION

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. of the toxin in an aqueous solution, without the addition of the emulsion. All the animals which had been injected with the toxin-emulsion mixture survived, and were fit and well twelve months later. Other experiments, conducted on similar lines, but using 6, 12, and 24 m.l.d., yielded similar results. The volume of the emulsion contained in each injection was usually thirty to fifty times that of the aqueous solution of the toxin, and the total volume injected rarely exceeded 0.5 c.cm.

Three further series of experiments were conducted in a similar way, using the toxins of *B. tetani*, *Cl. welchii*, and the *Cl. oedematis maligni* (Koch) respectively. In every case the results agreed with those using diphtheria toxin, thus proving that if lethal doses of these toxins are mixed with finely divided emulsions of olive oil before injecting them subcutaneously into animals, then such animals do not succumb to the lethal effects of the toxins.

INFLUENCE OF EACH CONSTITUENT OF THE EMULSION

Experiments were then performed to determine the cause of this protection, and the relative importance of the constituents of the 50 per cent. emulsion. The emulsion contained olive oil, gum acacia, and water, and one might conclude that either the oil or the gum may have modified the action of the toxins, or even destroyed them in some way. This might explain the protective action of the emulsion.

OLIVE OIL

The influence of the oil alone was first determined by omitting acacia from the emulsions. This necessitated making a simple emulsion of olive oil with water by means of a mechanical emulsifier. These were mixed with the toxins and injected subcutaneously soon after they had been prepared. The results obtained were very variable. In some experiments the mortality rate of the emulsion-toxin animals was as high as 100 per cent., while in others all the animals survived. This discrepancy was found to be due to the degree of division and stability of the emulsions. The protection afforded appears to be proportional to the degree of fineness of the emulsion. Coarse emulsions afforded no protection against lethal effects of the toxins.

GUM ACACIA

A solution of gum acacia was made so that the concentration of the gum within it was equal to that contained in the 50 per cent. olive oil emulsion. This gum solution was mixed with aqueous solutions of superlethal doses of the toxins immediately prior to injection into animals. Using guinea-pigs, it was found that with 4 m.l.d. of diphtheria toxin all the animals died irrespective of whether they had received the gum acacia or not. Further experiments, using the toxins of *B. tetani*, *Cl. welchii*, and the *Cl. oedematis maligni* (Koch) instead of diphtheria toxin, yielded similar results. It is obvious from this that gum acacia does not modify the lethal effects of these toxins, but simply assists in binding the emulsion together in a more stable form. The protective action of the emulsion is therefore concerned with the fineness of the olive oil globules, while the gum acacia renders the protection more secure by making the emulsion more permanent.

DISTRIBUTION OF TOXIN IN EMULSION

This question was investigated further in another series of experiments. A simple emulsion of olive oil was made in as fine a state of division as possible, using a mechanical emulsifier. The emulsion was mixed with a superlethal dose of diphtheria toxin (8 m.l.d.), and injected subcutaneously into a number of guinea-pigs. A suitable number of control animals were injected subcutaneously with 8 m.l.d. of the toxin alone. A portion of the toxin-emulsion mixture was next centrifugalized at 2,500 revolutions per minute for two hours, when the constituent parts of the emulsion separated into two layers. Two further groups of guinea-pigs were now injected with the aqueous layer and the oily layer respectively. The results were very clearly cut. All the guinea-pigs which had been injected with the toxin-emulsion mixture survived, whereas all the controls died within three days. The group which had received the aqueous layer after centrifugalization of the toxin-emulsion mixture also died within three days, showing a mortality rate of 100 per cent., while those in the group which had been injected with the oily layer all survived.

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 both have been previously

mixed so as to form a very finely divided emulsion. 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. This experiment was repeated using a 50 per cent. emulsion of olive oil containing gum acacia, but the emulsion was found to be so stable that centrifuging it for two hours at 2,500 revolutions per minute failed to cause anything but a very slight separation of the constituents. All the groups of guinea-pigs survived except the control animals, which were injected with toxin alone. The difference between this result and that of the previous experiment is well marked, and is probably due to the greater stability of the toxin-emulsion mixture containing gum acacia, so that the "layers" obtained from it were in reality emulsions.

EXPERIMENTS WITH LIQUID PARAFFIN

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 from ten to fifteen minutes, unless an emulsifying 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 large doses (12 m.l.d.) of the toxins of *B. diphtheriae*, *B. tetani*, *Cl. welchii*, and *Cl. oedematis maligni* (Koch). Emulsions of liquid paraffin without the addition of an emulsifying agent are too unstable, and easily separate out into their constituent parts. The protection conferred by such emulsions was found to be of a very low order and very erratic.

CREAM OF COW'S MILK

A few experiments were conducted using this form of natural animal fat 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. The results showed that this fat, when used in such a manner, afforded no protection whatever against these toxins, even when only 1.5 m.l.d. were used. In later experiments the cream was passed through a mechanical emulsifier before the addition of the toxin. The results obtained using this fat emulsion were very erratic, and very similar to those already described for liquid paraffin without the addition of an emulsifying agent.

PATHOLOGY

The pathological changes in the heart in toxæmia were studied during the course of this investigation. Necropsies were carried out on all the animals which had died from the lethal effects of the toxins used. In addition, a large number of the animals which had been injected with the toxin-emulsion mixtures, and had survived the lethal effects of the toxin, were killed and examined at varying periods, from four months to two 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. formol saline, and (c) Carnoy's solution. Sections from the two latter were stained with haematoxylin and eosin, and haematoxylin and van Gieson's stain. In many cases the liver, kidneys, and other organs were also examined microscopically.

The effects of diphtheria toxin on the heart have led to no detailed agreement among workers, though all are agreed that the heart can be profoundly affected. Several hundreds of sections were examined by me to determine if differences existed between the tissues of the control

animals and those which had been injected with the toxin mixed with the oil emulsions.

The results show quite definitely the following features:

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 had lived for a similar length of time after having been injected with the toxin alone. These findings were very constant. A large number of animals were killed and examined four months after the injection of the toxin-emulsion mixtures.

In all cases the myocardium was practically free from degeneration or fibrosis, and only very minor degrees of fatty changes were seen in a few animals. Speaking generally, the hearts showed no difference from normal healthy hearts. Another series of animals which was alive and well twenty-four months after the administration of the toxin-emulsion mixture was killed and examined. The results are in complete agreement with those just described as occurring in animals after four months.

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

DISCUSSION

It is well known that fine suspensions of charcoal or kaolin have the property of adsorbing ferments and certain toxins both inside the body and *in vitro*. Finely divided oil globules in emulsions carry a negative charge, and may adsorb toxins in the same way as these colloidal suspensions. The amount of toxin adsorbed depends upon the surface area of the particles. This is dependent upon the degree of fineness of the particles in suspension, or, in the case of emulsions, upon the fineness of the oil globules. That the adsorption phenomenon of these emulsions is of the reversible type is shown by the experiments which demonstrated the recovery of the toxin in the aqueous layer after centrifugalization of the toxin-emulsion mixtures.

The pathological findings in this investigation 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 outlined here 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 to be very promising.

Walsh and Frazer¹² have, quite independently and unknown to me, been investigating a somewhat similar problem, and some of their results are in agreement with those outlined in this communication.

CONCLUSIONS

1. Finely divided emulsions of olive oil, when mixed with superlethal doses of diphtheria toxin and injected subcutaneously, protect animals from the lethal effects of the toxin.

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

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

4. Emulsions of liquid paraffin with a suitable emulsifying agent behave in a similar manner, 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 used in this investigation are more soluble in water than in oils.

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

8. Diphtheria toxin, when mixed with stable, finely divided emulsions of olive oil before being injected subcutaneously into animals does not produce the myocardial degeneration which is a constant feature of the control animals.

This investigation was commenced in September, 1929, at the Pharmacological Laboratory, Cambridge, and my sincerest thanks are due to the late Professor W. E. Dixon for his interest and encouragement, and to Dr. Clifford Hoyle for his valuable suggestions and criticisms at the outset. I am also indebted to Mr. E. Saville Peck of Cambridge for his generous assistance in the preparation of the emulsions.

This communication is only an abstract of the results of this investigation, and a more detailed account will appear at an early date in the *Journal of Hygiene*.

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Clinical Memoranda

A CONCEALED INCISION FOR INTERVAL APPENDICECTOMY

There is a certain class of abdominal case in which it is important to avoid anything that might contribute to the production of surgical neurasthenia. The sight of a long scar may constantly remind the patient of her operation. She begins to feel symptoms, her friends make suggestions, and sooner or later a diagnosis of "adhesions" or other trouble is arrived at. In some cases the patient becomes an invalid, and may suffer for years as the result of a simple laparotomy. Quite apart from general considerations, it is an accepted surgical principle that in operating the surgeon should aim at restoring the part to its original anatomical condi-

tion. If the scar can be completely hidden, this ideal is achieved.

Last year I was asked to perform an interval appendicectomy in the case of a nervous, highly strung young woman of 19. It occurred to me that a modification of Pfannenstiel's median inferior incision might be employed. Since then I have used this incision in about a dozen cases with entire success.

A curved skin incision is made round the mons veneris, with its convexity upwards, so that the upper edge of the pubic hair will cover the scar later. The incision is deepened through the fat, until the linea alba and rectus sheaths are exposed on either side. After undercutting, the upper flap can be pulled up sufficiently to allow of a 4 to 5 inch vertical incision through the right rectus sheath. The muscle is displaced outwards, and the peritoneal incision can then be made of sufficient length to allow of the introduction of the half hand. I have not yet found any difficulty in bringing the caecum and appendix out of the wound. The pelvic organs can be examined with great ease.

The photograph shows the incision a week after operation. More room can be obtained, if required, by prolonging the ends of the incision along the folds of the groin. A second photograph (not shown), taken from the same patient three months later, shows the line of incision completely covered.

The concealed incision is not a suitable one for every case. Suspected adhesions or the presence of any acute or subacute inflammatory condition are definite contraindications. The operation does not take any longer than removal of the appendix by the ordinary routes, and the patient has less pain and discomfort afterwards. The patient can be moved out of bed on the third or fourth day, without any risk of damage to the wound.

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MESENTERIC CYST

The first case of mesenteric cyst, in over twenty-five years of practice, to come under my notice is perhaps deserving of mention.

On Christmas morning, 1933, I was called to see Mr. S., aged 27 years. He stated that he had been leading an athletic healthy life. Three weeks previously he had received a severe blow in the abdomen. For a day or two following he experienced some soreness in the region of the navel. About 7.30 p.m. on Christmas Eve, following a fast sprint of one hundred yards, severe pain was suddenly felt in the region of the umbilicus. The pain, which continued all night, was somewhat relieved by the patient adopting a squatting posture.

I first saw him at 11 a.m. on December 25th. He was "doubled up" in agonizing pain. Palpation of the abdomen revealed a swelling in the region of the umbilicus. The tumour was movable in a plane from the right hypochondrium to the left iliac fossa.

No definite diagnosis was made. The physical signs indicated the presence of a mesenteric cyst. Immediate operation was advised, and the patient removed to a private hospital. An adequate abdominal incision was made over the region of the tumour. When the peritoneal cavity was opened a considerable quantity of milky fluid escaped. The abdominal swelling, now exposed, was obviously a mesenteric cyst. It was about as large as a cricket ball. A haemorrhagic area, involving the mesentery, was noted on the anterior surface of the cyst. It is probable this was caused by the blow three weeks before. The mesentery was incised and the cyst enucleated. The abdomen was closed without drainage. The patient made an uneventful recovery.

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