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THE FORMATION OF LYSOCITHIN AND OF A MUSCLE-STIMULATING SUBSTANCE BY SNAKE VENOMS

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IT has long been known that lecithin treated with venom becomes powerfully haemolytic through the formation of lysocithin. In a previous paper [Feldberg & Kellaway, 1938] the pharmacological actions of alcoholic extracts of envenomed monkey's liver and of a sample of lecithin treated with cobra venom were examined. These extracts, which were shown to be free from venom, were strongly haemolytic, caused slow delayed contraction of the isolated jejunum of the guinea-pig and produced other venom-like effects if applied to other organs. It appeared at first that all these actions might be properties of a single substance, lysocithin, but when a purer preparation of lysocithin was made by one of us (H. F. H.) it lacked the stimulant action on the gut. The effects obtained with extracts of lecithin treated with venom and of envenomed organs had therefore to be attributed to the formation of at least two active substances, and a re-examination of our new lysocithin preparation was necessary. In addition we have studied the action of venom on lymph and on fresh egg yolk.

METHODS

Lysocithin preparation. Lysocithin was prepared by a method based on the work of Levene, Rolf & Simms [1924] and of King & Dolan [1933]. The yolks of twenty-three fresh eggs (approximately 400 c.c.) were washed with distilled water and emulsified with gentle stirring in 400 c.c. 0·11 *M* Na_2HPO_4 adjusted to *p*H 7·4 with HCl. To this emulsion 51 mg. of the venom of the Australian black snake (*Pseudechis porphyriacus*),

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two drops of toluene and 1 g. of thymol were added. The mixture was incubated at 37° C. for 4 hr. A test sample in an amount corresponding to 0·002 c.c. of the mixture haemolysed 0·4 c.c. of 5% washed rabbit red corpuscles at 22° C. After incubation for another hour the mixture was poured into 2 l. of hot 95% C_2H_5OH and filtered immediately. The precipitate was washed with 4 l. 95% C_2H_5OH and the washings added to the filtrate. An excess of saturated $CdCl_2$ aq. was added and the solution cooled overnight in a refrigerator. Next day the precipitated $CdCl_2$ compound was removed by centrifuging and well washed with acetone. It was dissolved in $CHCl_3$ and enough alcoholic NH_3 added to decompose all the compound. The precipitate was removed from the $CHCl_3$ solution and well washed three times with $CHCl_3$. The united solution and washings were concentrated to dryness and the residue dissolved in hot CH_3OH. The CH_3OH solution was cooled in a refrigerator for 3 days, centrifuged while cold and the precipitate well washed with cold CH_3CH. The CH_3OH solution and washings were united and concentrated to dryness *in vacuo*. The residue was dissolved in a little warm chloroform and an excess of ether added. The mixture was immediately cooled and centrifuged, and the precipitate was washed with ether and dried *in vacuo*.

The methods used for the study of the effects of lysocithin were those described in our earlier papers [1937, 1938].

Results

Effect of cobra venom on lymph in vitro

When lymph is acted upon by cobra venom it acquires muscle-stimulating and haemolytic properties.

Lymph was collected from the thoracic duct of a heparinized dog. To 2 c.c. of lymph 100 μg. of cobra venom were added and the mixture kept at room temperature for 1½ hr. Small samples were removed from time to time and were tested on the guinea-pig's jejunum. Such an assay is illustrated in Fig. 1. The gut had previously been treated with three doses each of 20 μg. of cobra venom. When the third dose of venom was added to the bath no contraction occurred, the muscle having become desensitized to doses of venom of this order. At A, 0·2 c.c. of lymph, to which no venom had been added, was tested, it caused slight relaxation. B and C show the effect of the same amount of lymph after venom had been allowed to act on it for 1 and 25 min. respectively. The contraction at B started after a latent period of 25 sec., that at C after a latent period

of 14 sec. The contractions could not have resulted from the 10 μg. of venom present in 0·2 c.c. of lymph added to the bath, since the gut was insensitive to twice this dose. The stimulating action of the lymph continued to increase during a further period. The tests at D, E and F were made with 0·02 c.c. of lymph containing 1 μg. of venom only. D was tested 35 min., E 45 min. and F 80 min. after the addition of the venom to the lymph. The increase in stimulating activity is indicated not only by an increase in contraction but also by shortening of the latent period, which was 27 sec. at D, 23 sec. at E, and 17 sec. at F. The stimulating action of the lymph was often followed by characteristic changes in the excitability of the muscle to histamine. These changes were the same

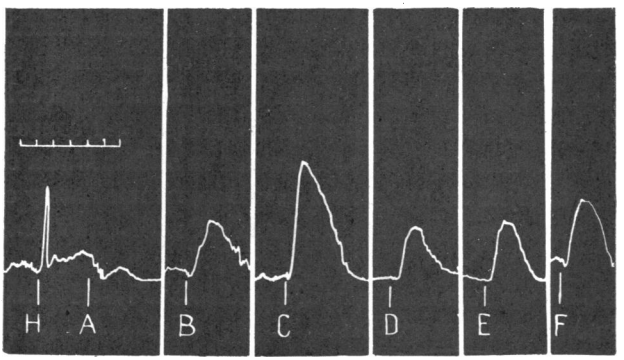

Fig. 1. Responses of the isolated guinea-pig's jejunum in 5 c.c. bath to lymph before (at A) and after treatment with cobra venom (at B to F). At H, 0·04 μg. histamine. Time in minutes. (Details in text.)

as those described in our previous paper [1938] following the addition to the bath of an extract of lecithin treated with cobra venom or of egg yolk treated with venom (*vide infra*).

When lymph on which cobra venom had been allowed to act for 2 hr. was extracted twice with absolute methyl alcohol the extract retained its muscle-stimulating property and caused immediate haemolysis of washed human and sheeps' red blood corpuscles. Similarly prepared extract of normal lymph had no immediate haemolytic property.

In 1936 one of us (K.) and Le Messurier had found that dog's lymph, to which small amounts of copperhead venom had been added, caused contraction of the isolated guinea-pig's uterus though the muscle had been desensitized to the venom. This effect and that which we have just described can probably be attributed to the same substance formed by the action of venom upon lymph.

Effect of cobra venom on egg yolk in vitro

When egg yolk is acted upon by cobra venom it acquires muscle stimulating and haemolytic activities. These can no longer be regarded as properties of a single substance, lysocithin, since they can be segregated in different fractions. The haemolytic property must be attributed to lysocithin. The principle responsible for the muscle stimulating effect is also formed by the action of the venom, but we have hitherto not been able to identify it nor to determine the constituent (or constituents) from which it is derived.

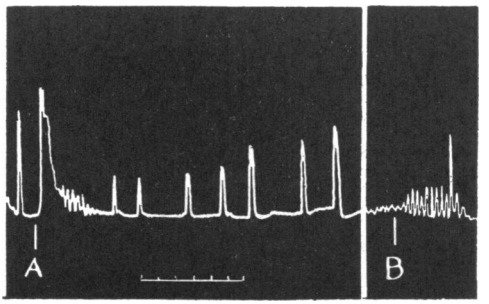

Fig. 2. Responses of the isolated guinea-pig's jejunum to egg yolk treated with cobra venom (at A and B). The rapid responses are to 0·04 μg. histamine. Time in minutes. (Details in text.)

Egg yolk (0·1–0·2 c.c.) has only a feeble and irregular stimulating action on the guinea-pig's gut, but becomes strongly active when venom has been allowed to act upon it. The amounts of venom necessary to produce the effect are small. We found that the addition of 2 μg. of cobra venom to 1 c.c. of egg yolk was sufficient, although the effect became more pronounced when larger amounts of venom were used. Fig. 2 illustrates the effect on the gut of 0·1 c.c. of egg yolk tested 1 hr. after 0·25 μg. of venom had been added to it. The gut had been desensitized to 20 μg. of cobra venom. The contraction at A started after a latent period of 7 sec., and was followed by a period of decreased excitability of the muscle. In some instances this condition was preceded by a short period of increased excitability. If still smaller amounts of venom were added to the yolk, for example 0·5 μg. of venom to 1 c.c., 0·1 c.c. of the mixture caused an increased rhythm for a period of 1–3 min. (Fig. 2 B). In this instance the venom had been allowed to act for 30 min. on the yolk.

For further examination egg yolks treated with cobra venom were extracted with alcohol. For this purpose the yolks of two eggs were incubated for 3 hr. with 12·6 mg. of cobra venom in 30 c.c. of 3% Na_2HPO_4 (*p*H 7·4). The gut-stimulating substance was found to be soluble in absolute ethyl alcohol, and the extracts were powerfully haemolytic on washed sheep red corpuscles. When the alcoholic solution was kept in the refrigerator for 48 hr. a precipitate formed and was removed but the solution still retained most of its gut stimulating and haemolytic properties.

Ether solubility. The alcoholic solution was taken to dryness *in vacuo* and extracted with ether. In the ethereal solution a precipitate formed at once and further precipitation occurred during 48 hr. in the refrigerator. The ether-soluble and insoluble fractions were made up to the same volume and tested for haemolysis and for gut stimulating action. The ether-soluble fraction was about 2½ times more active in stimulating the gut but only half as powerful in causing haemolysis as the ether-insoluble fraction. This indicated that the gut stimulating and haemolytic actions were not properties of a single substance.

Acetone solubility. The ether-soluble fraction when taken to dryness was extracted with acetone, and acetone-soluble and insoluble fractions were obtained. The acetone-soluble fraction was devoid of haemolytic power but was much more active in causing slow contraction of the guinea-pig's jejunum than the acetone-insoluble fraction which was strongly haemolytic. Since lysocithin is known to be insoluble in acetone, the haemolytic power of the acetone-insoluble fraction can be ascribed to lysocithin.

Oleic acid and oleates. The gut-stimulating principle is not only formed by the action of venom on egg yolk and lymph and in perfused organs, but also, as shown in the previous communication, by the action of venom on a preparation of lecithin. It is known that better yields of lysocithin are obtained when venom acts on egg yolk than on lecithin, and in accordance with this fact our egg-yolk extracts had much stronger haemolytic activity than extracts of lecithin treated with venom. The gut-stimulating action of egg-yolk extracts was also much stronger. It appeared therefore that the formation of the gut-stimulating substance was closely associated with lysocithin formation, and it seemed possible that oleic acid or oleates formed in this process might be responsible for the stimulant action on the gut.

We tested oleic acid and sodium oleate in concentrations greater than those which could have been present in our alcoholic extracts of egg yolk and of lecithin treated with cobra venom, and found that they had no

stimulating action upon the gut. An 0·5% emulsion of oleic acid in Tyrode solution was added in amounts of 0·2–0·4 c.c. to the bath and sodium oleate in amounts up to 10 mg.

Pharmacological actions of the lysocithin preparation

Sheeps' red corpuscles. Our lysocithin was tested for its haemolytic power by Miss F. E. Williams and found to be active in concentrations up to 1 : 6400, causing complete haemolysis of an equal volume of a 5% suspension of washed red cells within 40 sec.

Guinea-pig's jejunum. Our lysocithin preparation had no stimulating action on the gut, but had an inhibitory influence if given simultaneously with histamine or with an extract containing the stimulating substance formed by venom. When the lysocithin was washed out the jejunum exhibited a period of decreased excitability. In the experiment of Fig. 3 at A and B, 0·1 and 0·5 mg. of our preparation were added to the bath and left in contact with the muscle for 30 sec. After 0·1 mg. the first response to 0·1 μg. of histamine was diminished, after 0·5 mg. the first two doses of 0·1 μg. of histamine were almost without effect and the muscle regained its original excitability in the course of 10 min. After large doses spontaneous rhythm often disappears during the phase of depressed excitability.

We have shown in numerous experiments reported in the previous and in the present paper that the slow delayed contraction of the jejunum produced by extracts of envenomed liver and of lecithin treated with cobra venom is followed by periods of increased and of decreased excitability. The decreased excitability may have resulted at least in part from the presence of lysocithin in these extracts. This would explain the irregularity with which the phenomenon has been observed. We have further shown that the contraction of the gut produced by different venoms, known to form lysocithin, is followed by a period of decreased excitability, and the formation of this lipin-split-product in the muscle may be responsible for this effect. Apparently it is not a direct effect of venom, since a dose of venom which, when added to the bath for the first time, gives the effect, has no such action if added for the second or third time to the then desensitized muscle. This is shown in the experiment of Fig. 3, in which 20 μg. of cobra venom were added to the bath at C and D. The second dose of venom failed to cause any significant contraction of the gut, and there was no after-decrease in excitability. This cannot be explained by loss of sensitivity of the jejunum to lysocithin, because when this was tested subsequently at E and F it had a stronger depressant

action than before. Desensitization to the phenomenon of decreased excitability may thus be reduced to the more general phenomenon of desensitization against formation of lysocithin.

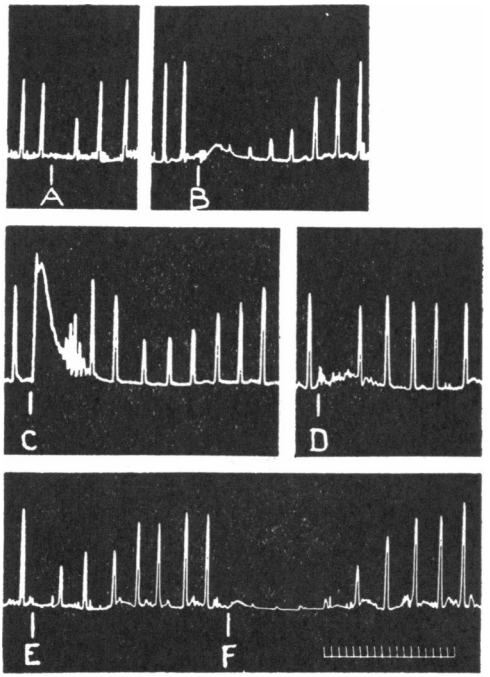

Fig. 3. Responses of the isolated guinea-pig's jejunum to lysocithin (at A, B, E and F) and to cobra venom at C and D. The rapid responses are to 0·1 μg. of histamine. Time in half minutes. (Details in text.)

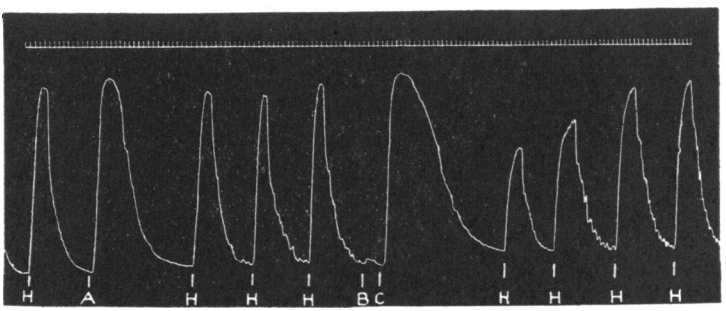

Fig. 4. Responses of the isolated horn of the uterus of a virgin guinea-pig in a 5 c.c. bath to 2 μg. of histamine (at H) and to 5, 1 and 10 mg. of lysocithin (at A, B and C). Time in half minutes.

Guinea-pig's uterus. The contraction of the virgin uterus which we have described as occurring after extracts of "envenomed liver" and of lecithin treated with cobra venom, must in part at least be attributed to lysocithin. Unlike the intestinal muscle the uterus contracts to our lysocithin preparation in doses from 1 to 2 mg., and the contractions produced by larger doses are followed by a period of decreased excitability. These effects are illustrated in Fig. 4.

Rabbit's eye. The dense and persistent opacity of the cornea, the apparent bulging of the eye from its socket and the associated conjunctivitis which we have described as occurring after the injection into the anterior chamber of extracts of "envenomed liver" and of lecithin treated with cobra venom, must be attributed to the presence of lysocithin in these extracts. The full development of these changes within 12 hr. followed the injection into the anterior chamber of 4 mg. of our preparation of lysocithin. As we have shown, cobra venom produces similar changes which it is reasonable to assume are brought about by the formation of lysocithin.

Intravenous injections in guinea-pigs. The symptoms which we have described in guinea-pigs following the intravenous injection of extracts of envenomed liver and of lecithin treated with cobra venom can be reproduced by the injection of our preparation of lysocithin. After a latent interval of a few seconds there were obstruction to respiration, ineffective respiratory efforts, cyanosis, convulsions, coma and death. In half of the injected animals blood-stained fluid poured out from the nostrils. Post-mortem there was in all cases haemorrhagic oedema of the lungs which did not collapse when the thorax was opened. The intravenous injection of 2 mg. of our preparation produced no symptoms, but 5 mg. caused death in from $1\frac{1}{2}$ to 6 min.

The perfused liver of the dog. Liberation of histamine. The swelling and depigmentation of the liver and the appearance in the perfusate of coagulable protein and of histamine which we have described following the intraportal injection of extracts of envenomed liver and of lecithin treated with cobra venom, must be ascribed to the presence of lysocithin. We were able to produce all these effects with our sample of lysocithin.

After the injection of lysocithin the perfusate became turbid and brownish in tint from liver pigments and sometimes contained a little haemolysed blood in addition. We found that in perfusion experiments lasting from 2 to 3 hr., in the course of which two injections of 100 mg. of our lysocithin preparation were given, the swelling of the organ (as esti-

mated by weighing it before and at the end of the experiment after draining about 10–15 c.c. of fluid) amounted to from 20 to 25%.

Fig. 5 illustrates the output of coagulable protein and of histamine from a piece of liver weighing 60·5 g. and containing about 2·4 mg. histamine. The total output of albumen after the first injection of 100 mg. of our lysocithin (in 2 c.c.) was 0·7 g. The histamine output rose steeply and fell rapidly. At its maximum it was 3·2 μg./min. or 0·13% of the total histamine. A second injection of 100 mg. lysocithin given 1½ hr. later also caused a steep rise which in some experiments reached a higher

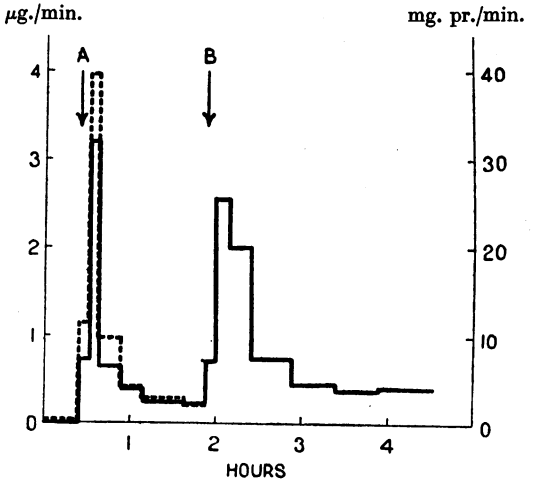

Fig. 5. Output of histamine (continuous line) and of protein (dotted line) from perfused lobe of dog's liver. At A and B, 100 mg. of lysocithin intraportally. Abscissae, time in hours. Ordinates at the left histamine output in μg. per min., at the right protein output in mg. per min.

maximum than after the first injection. But in all cases the total output of histamine after a second was greater than after a first injection and the output per minute remained at a high level for a longer time.

In our previous communication we have drawn attention to the close resemblance between the histamine output caused by extracts of envenomed liver and of lecithin treated with cobra venom and that caused by repeated small doses (0·3 mg.) of cobra venom. With the larger dose of lysocithin used in the present experiments a striking difference makes its appearance. Whereas the output of histamine after lysocithin rises to a maximum very steeply and subsides fairly rapidly, the maximum output after cobra venom is reached more gradually and may persist at

a high level for some hours. In some experiments with bee venom [Feldberg & Kellaway, 1937] a high output lasted from 10 to 15 hr.

There was another difference between the effects of perfusates collected after the intraportal injections of cobra venom and of lysocithin. After cobra venom the perfusates contain large amounts of a slow contracting substance, and the effect can often be elicited with small doses (0·01 c.c.) of these. After lysocithin, though the perfusate was not devoid of such activity, it caused only a moderate effect when doses of 0·2 and 0·4 c.c. were tested. This difference was particularly striking when perfusate after the injection of lysocithin was compared with that obtained when cobra venom was injected into the same liver. Furthermore, when cobra venom was added (50 μg./c.c.) to the perfusate collected after lysocithin and it was tested on a gut which had been desensitized to cobra venom, it was shown to have acquired greatly increased activity and gave strong slow contractions in doses of 0·02–0·04 c.c. Such an experiment on the monkey's liver is described more fully later. We have not ascertained whether the relatively small amounts of slowly contracting substance which appear in the perfusate from the dog's liver after an intraportal injection of lysocithin are liberated or formed.

Monkey's liver. The intraportal injection of our lysocithin preparation caused reticulation of the surface of the liver and depigmentation. There was no perceptible swelling of the organ, and when the liver was weighed at the end of the experiment it had not increased in weight. The venous perfusate became turbid and brownish in colour and contained protein and liver pigments. The perfusate was free from detectable amounts of histamine, which was to be expected, because, as we have shown, the monkey's liver contains less than 1 μg./g. of this substance. No appreciable amount of the principle which causes slow and delayed contraction of the guinea-pig's jejunum could be found in the perfusate. This was not because of any abnormal condition of the liver cells, since perfusate collected after a subsequent injection of a small dose of cobra venom contained the active principle in large amounts. Furthermore, perfusate which, after the injection of lysocithin, was devoid of this activity, rapidly acquired it when treated *in vitro* with a minute amount of venom. These facts are illustrated in Figs. 6 and 7.

Fig. 6 gives the output of protein in mg. per minute after two successive injections each of 100 mg. of our lysocithin (in 2 c.c. of saline) into a lobe weighing 39 g. The output after the first injection reached its peak during the first 15 min. and more quickly still after the second

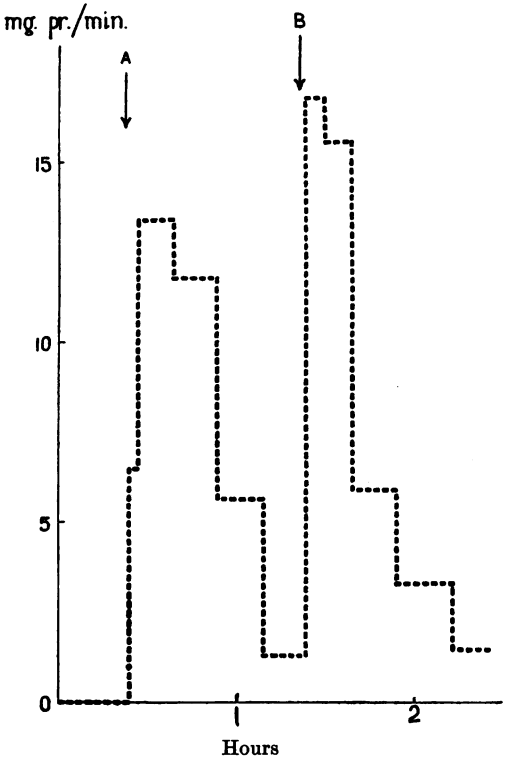

Fig. 6. Output of protein from perfused lobe of monkey's liver. At A and B, 100 mg. of lysocithin intraportally. Abscissae, time in hours, ordinates output of protein in mg. per min.

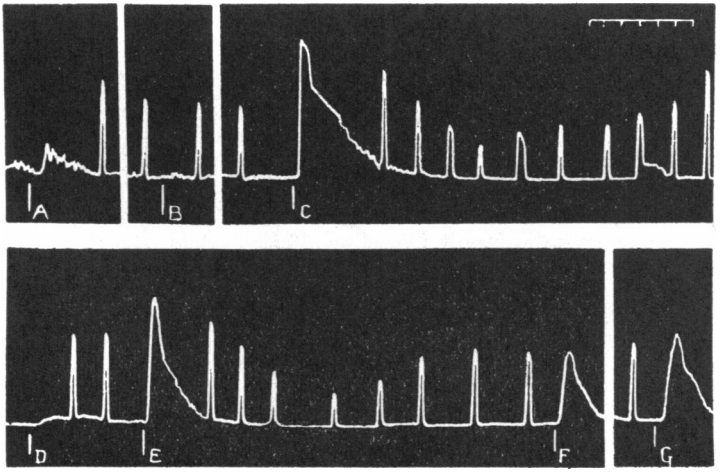

Fig. 7. Responses of the isolated guinea-pig's jejunum to perfusate collected from monkey's liver (at A, B, E, F, G) and to cobra venom (at C and D). Time in minutes. (Details in text.)

injection. The total output after the first injection was 0·45 g., that after the second 0·40 g.

Fig. 7 shows the assay of perfusate from this experiment on the guinea-pig's jejunum. The perfusate collected before the injection of lysocithin was without effect in a dose of 0·4 c.c. At A, 0·4 c.c. of a sample collected between 3 and 15 min. after the first injection of lysocithin was tested. There was a small contraction with a latency of 35 sec. The following samples of perfusate were devoid of activity. At B, 0·4 c.c. of the sample collected in the second quarter of an hour was shown to have no stimulant effect. The gut was now treated with two successive doses of 10 μg. of cobra venom (at C and D). The contraction at C started after a latent period of 8 sec. and was followed by typical changes in excitability. The gut was now desensitized and failed to react at D. At E 0·2 c.c. of the sample of perfusate, which had been tested at B, was retested after incubation with 4 μg. of cobra venom for half an hour. It gave a typical slow contraction of the muscle with a latency of 8 sec. and the characteristic after-changes in excitability. This sample was now active in a dose of 0·04 c.c. (containing 0·8 μg. of venom) at F. The contraction started after a latent period of 15 sec. The newly acquired activity of this sample of perfusate cannot have been due to the cobra venom present in it, but must have resulted from the action of substances formed in it by the venom. The decreased excitability is probably an effect of lysocithin, the delayed contraction and the after increase in excitability to the formation of a muscle stimulant substance. At G is seen the effect of 0·2 c.c. of a sample of perfusate which was collected 5–10 min. after a later injection into the liver of 5 mg. of cobra venom. The contraction was followed by changes in excitability similar to those seen after C and E. It will be observed that the effects at C, E and G are closely similar, and it is reasonable to assume that they are produced in a similar manner by the action of venom on the gut, in the perfusate and in the liver.

Circulatory effects in the cat. The intravenous injection of 20 mg. of our lysocithin preparation after a latent period of 7–10 sec. caused a steep evanescent fall in systemic blood pressure and a concomitant rise of pressure in the pulmonary artery. No desensitization occurred as after the injection of venom, and the blood-pressure changes could be obtained repeatedly. In the experiment of Fig. 8 the effects of three injections of 20 mg. given at intervals of about 5 min. are shown. With larger doses of lysocithin no recovery from the steep fall of systemic pressure occurred; there was a greater rise of pressure in the pulmonary artery and death occurred within a few minutes. Such an experiment is illustrated in

Fig. 9 A. When the systemic blood pressure had fallen, pulsatory oscillations could no longer be seen on the mercury manometer and the record became a thin line. On the other hand, vigorous pulsations were maintained in the pulmonary artery even after the pressure had fallen below

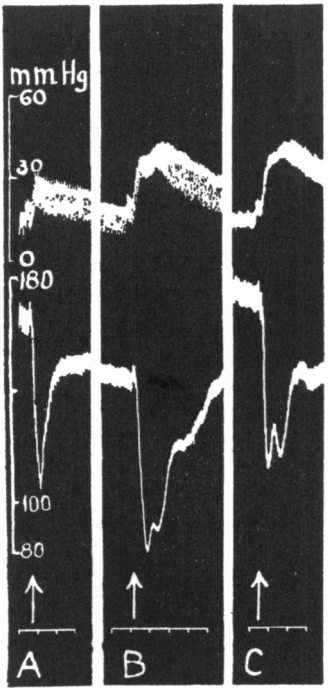

Fig. 8.

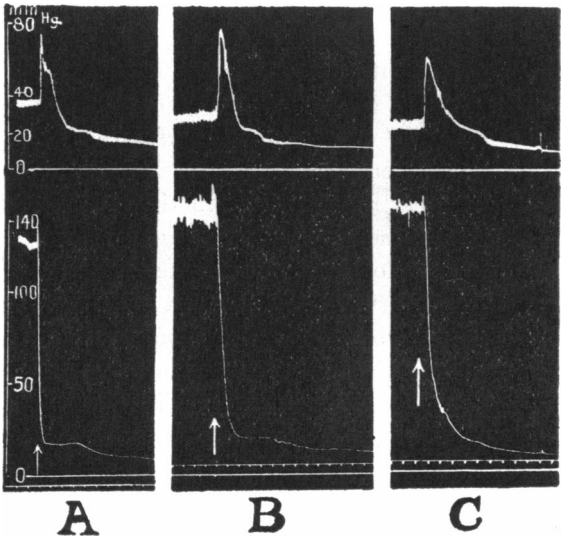

Fig. 9.

Fig. 8. Arterial blood pressure (lower tracing) and pressure in the pulmonary artery (upper tracing) from a cat under chloralose anaesthesia. At A, B and C 20 mg. of lysocithin intravenously. Time in half minutes.

Fig. 9. Arterial blood pressure (lower tracings) and pressure in the pulmonary artery (upper tracings) from three cats. At the arrows 100 mg. of lysocithin (at A), 0·5 mg./kg. cobra venom (at B) and 100 stings of bee venom (at C). Time in half minutes.

its original level. Similar observations were made after the intravenous injection of large doses of snake venom and bee venom. In Fig. 9 is shown for comparison the effects on the systemic pressure and on the pressure in the pulmonary artery of 0·5 mg./kg. of cobra venom (B) and of 100 stings of bee venom (C). The records are indistinguishable from those obtained after lysocithin. The tracings B and C are taken from our previous publications [1937].

The effects of lysocithin resembled those produced by snake venoms and bee venom in another respect. When large doses of these venoms were injected intravenously into cats haemorrhagic oedema of the lungs invariably resulted. Lysocithin produced similar changes. Post-mortem in the experiment of Fig. 9 the lung showed haemorrhagic patches and was tough in consistency. When the survival period was longer, as in the experiment of Fig. 8, there was extensive haemorrhagic oedema of the lung.

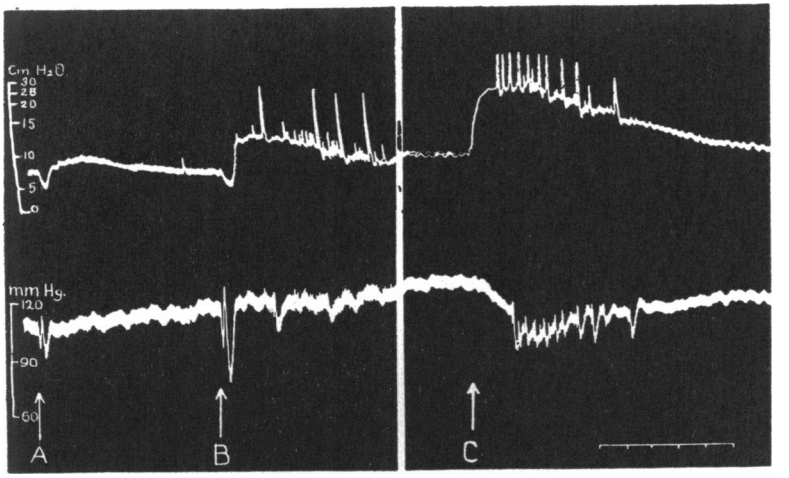

Fig. 10. Arterial blood pressure (lower tracing) and pressure in the portal vein (upper tracing) from a 8 kg. dog under chloralose. At A and B intravenous injections of 50 and 100 mg., at C intraportal injection of 75 mg. of lysocithin. Time in minutes.

Circulatory effects in the dog. The intravenous injection of 50–100 mg. of our lysocithin preparation causes an evanescent steep fall of systemic pressure. There is no desensitization and repeated doses give approximately similar responses. In some instances the fall is interrupted by a short upward fluctuation of pressure (Fig. 10 A and B). The fall in systemic pressure is accompanied by a preliminary slight fall of pressure in the portal vein, which is probably passive, followed by a rather prolonged rise. This results from increased resistance in the liver, since it is stronger when the same or a smaller dose of lysocithin is injected into the portal vein (at C). In this case the rise in pressure in the portal vein is followed by a gradual fall of systemic pressure, which recovers as the portal pressure returns to normal. The fall in systemic pressure in this instance appears to result from the hepatic effects of the lysocithin. The

experiment of Fig. 10 shows another effect of lysocithin, which is often observed when snake venoms are injected. The artificial ventilation is overcome by deep spasmodic inspirations, the effects of which are seen in the record of the arterial and particularly of the portal pressure (B and C).

In heparinized dogs we have collected the lymph from the thoracic duct and have injected 100 and 200 mg. of our preparation of lysocithin intraportally and intravenously. There was a transient increase in the lymph flow and a few minutes later the lymph was coloured with pigment from haemolysed blood and contained a few intact red blood cells.

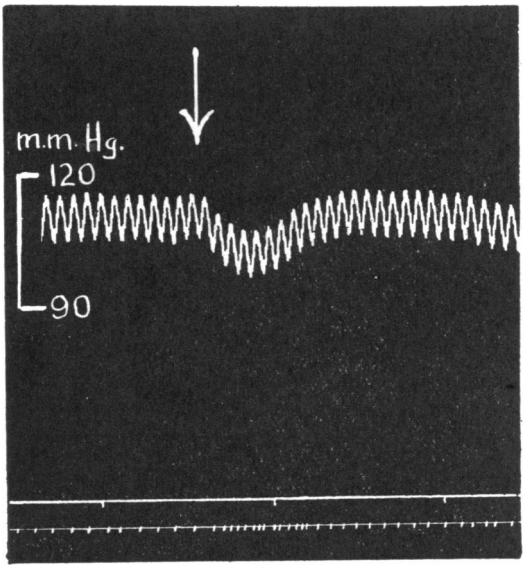

Fig. 11. Arterial blood pressure (upper tracing) time in half minutes (middle tracing) and outflow from the right femoral vein (lower tracing). At the arrow 5 mg. lysocithin injected into the femoral artery.

The evanescent steep fall in systemic pressure produced by the intravenous injection of lysocithin probably results from peripheral vasodilatation. When lysocithin is injected into the femoral artery transient vasodilatation occurs in the limb. After the injection of 1 mg. the dilatation lasted for 20 sec., after 5–10 mg. the outflow from the femoral vein was doubled for 10–20 sec. and had returned to normal within 30 sec. These effects could be obtained repeatedly. Fig. 11 shows a record of the venous outflow after the arterial injection of 5 mg. of lysocithin, each signal corresponding to 3·2 c.c. of outflow.

Discussion

The experiments reported in this and in the preceding paper show that when venom is allowed to act *in vitro* upon lymph, egg yolk or upon a sample of lecithin there is formed in addition to lysocithin a substance which causes slow contraction of the isolated jejunum of the guinea-pig; that these substances are also formed in the tissues by the action of venom on perfused organs and that they may account for many of the symptoms produced by venoms.

The mode of formation of the gut stimulant substance is unknown. Its formation by venom appears to be linked with that of lysocithin, but it is neither oleic acid nor a simple oleate. We do not even know the constituent from which it is derived, which may be another lipin. Its formation in the gut probably accounts for the stimulating action of venom and for the succeeding increase in the excitability of this muscle. Since a similar action on the gut is produced by cell injurious agents which do not form lysocithin it may prove to be an intermediary in these forms of cell injury. In such cases its formation could not be linked with that of lysocithin and it is even possible that it is liberated because a similar substance is present in extracts of normal organs.

The close similarity between the effects of lysocithin and those produced by venoms is easily explained if these actions of venom are caused by the formation of lysocithin, which in some instances acts not only directly but by the liberation of histamine. The formation of lysocithin may continue for some time or it may have a prolonged action in the tissues where it is formed. This would explain the difference in the duration of the effects observed after the injection of venom and of lysocithin into the circulation or into perfused organs. Our experiments with lysocithin have been confined to a few actions only. We have not determined whether it plays any part in the neurotoxic manifestations of venom poisoning. The varying actions of venom on different animals and of different venoms on the same animal may be related to differences in the actions of the phosphatidases of the venoms upon various tissues. Since no desensitization occurs against the action of lysocithin, desensitization against venom must be accounted for by failure to produce further lysocithin in response to a second dose of venom.

Conclusions

1. Lymph and egg yolk treated with venom acquire a lytic action on red blood cells and cause contraction of the isolated jejunum of the guinea-pig, which has been desensitized to venom. These activities are

the properties of at least two substances. The haemolytic activity must be attributed to lysocithin. The gut stimulant substance has not been identified.

2. Many symptoms produced by venom can be attributed to the effects of these two substances which are formed in the tissues by venom.

3. Lysocithin in addition to its haemolytic action causes decreased excitability of the isolated jejunum and contraction of the uterus of the guinea-pig, opacity of the cornea if injected into the anterior chamber of the rabbit's eye and haemorrhagic oedema of the lung when injected intravenously into the intact guinea-pig. It causes the liberation of protein, pigments and histamine from the perfused liver of the dog, and of protein and pigments from the perfused liver of the monkey. In the cat and dog it causes a steep fall of systemic blood pressure which in the former is associated with a rise of pressure in the pulmonary artery and haemorrhagic oedema of the lung and in the latter with a rise of pressure in the portal vein. Injected into the femoral artery of the dog it causes evanescent vasodilatation. These effects resemble those produced by venoms.

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