THE INFLUENCE OF EMULSIONS OF OLIVE OIL UPON THE BIOLOGICAL ACTIONS OF ALKALOIDS

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(With 1 Figure in the Text)

Introduction

In 1932 and 1934, Myers showed that lethal doses of certain bacterial toxins when mixed with emulsions of olive oil, in a fine state of division, and injected subcutaneously into animals failed to produce their lethal effects. Similar results were obtained by Walsh & Frazer (1934). Cataphoresis experiments have shown that oil globules in emulsions of oil in water type carry a negative charge. On this basis it was assumed that an adsorption phenomenon might be the explanation of the failure of the bacterial toxins to produce their lethal effects when mixed with emulsions of oil in water type and injected into animals.

If these small oil globules can adsorb toxins at their surfaces then there appears to be no reason why other substances should not be adsorbed on these surfaces and so have their biological effects modified. Certain varieties of charcoal, kaolin, etc., are able to adsorb alkaloids quite readily and are used extensively in the treatment of alkaloidal poisoning. In view of this fact it was thought that emulsions of olive oil in water might be able to adsorb alkaloids and so modify their biological effects in the same way as they modify the action of toxins.

The present investigation was conducted with the object of determining whether such emulsions are able to modify the biological effects of alkaloids, and, if the result were positive, whether emulsions could be utilized in the treatment of cases of alkaloidal poisoning in a similar way to that in which charcoal and kaolin are used.

Methods

It was thought advisable to choose such alkaloids as could be easily obtained and which produce very definite effects, readily determined and measured by the ordinary laboratory methods. It was decided to use salts of arecoline, pilocarpine, and atropine, and to measure their effects upon the blood pressure in the common carotid artery. Morphine hydrochloride was also selected because its depressant effects upon the respiration can be conveniently recorded in the cat, and strychnine because of its spinal convulsant effect. In most of the experiments, cats under the influence of chloralose or decerebrate cats were used. Frogs were used in the experiments with strychnine.

Preparation of the olive-oil emulsion, 50% (*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 passed several times through a mechanical emulsifier until the average size of the oil particles is 0·5–1μ. This emulsion is very stable, mixes well with water and retains its characters when mixed with an equal amount of water. The emulsion was mixed with aqueous solutions of the alkaloids and allowed to stand at 37·5° C. for varying periods, ranging from 1 to 24 hr., before being injected into the animals. The maximum time of 24 hr. was thought to be long enough for adsorption to take place if it was going to take place at all.

The emulsions were kindly made for me by Mr E. Saville Peck, of Cambridge.

Experiments

(*a*) *Arecoline hydrobromide and atropine sulphate*

5 mg. of arecoline hydrobromide were dissolved in 0·5 c.c. of distilled water and added to 19·5 c.c. of the emulsion. The whole was well mixed and allowed to stand for 6 hr. at a temperature of 37·5° C. The final concentration of arecoline hydrobromide in this mixture was 0·25 mg./c.c. In the same way a mixture of atropine sulphate and emulsion was made having a concentration of 1 mg. atropine sulphate per c.c. and set aside under the same conditions for 6 hr. Seven experiments were conducted on separate cats. Four of the animals were given chloralose as a general anaesthetic, and three were decerebrate preparations. Blood pressure was recorded from a cannula ligatured into the common carotid artery and respiration was natural. All injections were made into either the left external jugular vein or a branch of the left femoral vein.

In five of the experiments, three decerebrate and two chloralose cats, agreement was perfect. The injection of 1 c.c. of the emulsion without the admixture of any alkaloids, had little or no effect upon the general blood pressure. Any change recorded was a negligible decrease in the general level of the blood pressure in three animals. The injection of 0·25 mg. of arecoline hydrobromide in 1 c.c. of emulsion produced a typical fall in blood pressure caused by slowing of the heart rate. The effect was always rapidly abolished by the injection of the atropine-emulsion mixture after which the injection of a further 0·25 mg. of the arecoline hydrobromide in emulsion produced no further effects upon the blood pressure.

From these results it was apparent that the effects of arecoline and atropine were not modified in any way when the drugs were mixed with the emulsion for as long as 6 hr.

The remaining two experiments, which yielded almost identical results, differed, however, from the other five only in so far as the effects of the emulsion alone and the arecoline-emulsion mixture were concerned. In both the animals were under the influence of chloralose. Table I is a protocol of

Table I. *The influence of arecoline hydrobromide and atropine sulphate mixed with olive-oil emulsion, upon the blood pressure*

Time	Drug	B.P. mm. Hg
1.30 p.m.		196
1.33	1 c.c. 50% olive-oil emulsion	
1.35		190 falling
1.38		136 steady
1.48		136 steady
1.49	0·25 mg. arecoline HBr in ice emulsion	
1.50		198 max. point of rise
1.55		114
2.00		116 steady
2.02	Do.	
2.03		198 max. point of rise
2.07		76
2.10		76 steady
2.15		76 steady
2.16	Do.	
2.17		184 max. point of rise
2.22		90
2.40		150
2.43		150 steady
2.45	0·25 mg. arecoline in 1 c.c. Ringer	
2.46		52 max. point of fall
2.47	1 mg. atropine sulphate in 1 c.c. emulsion	
2.48		148
2.49		164
2.58		164 steady
2.59	0·25 arecoline HBr in 1 c.c. Ringer	164
3.01		164

Emulsion and alkaloid mixed 5 hr. at 37·5° C. before use.
Cat 2·2 kg. Chloralose anaesthesia.
B.P. Right common carotid artery.
Injections via left external jugular vein.

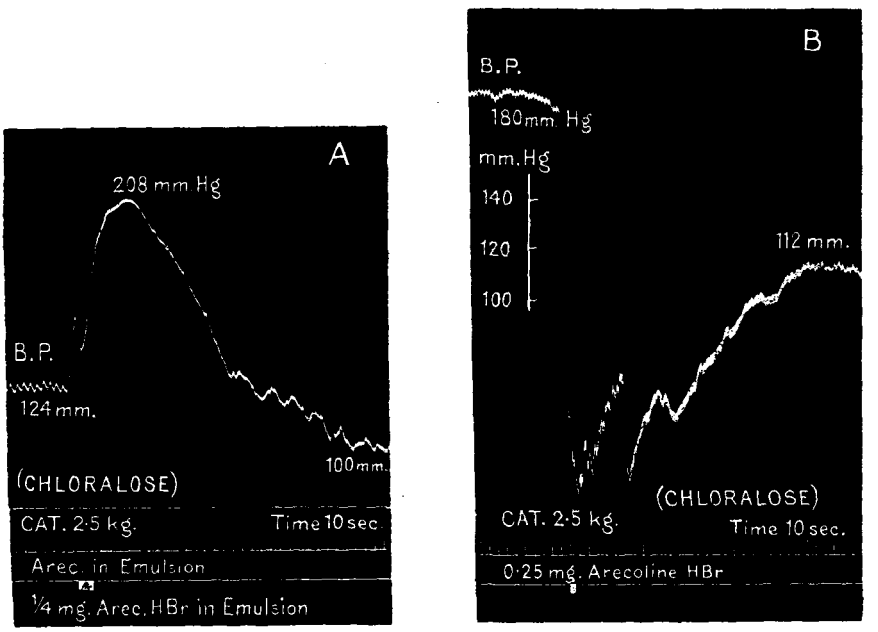

Fig. 1. A shows the unusual effect of arecoline hydrobromide, when mixed with emulsion, upon the blood pressure of a 2·5 kg. cat under the influence of chloralose as a general anaesthetic. This effect was constant in two out of seven cats and shows the nicotine and adrenalin-like effects of the drug. B shows the effect of the same amount of arecoline hydrobromide in aqueous solution. All injections were intravenous.

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one of these experiments. It shows that the injection of 1 c.c. of the emulsion alone caused a marked fall in the blood pressure from 196 to 136 mm. Hg in 5 min. The injection a few minutes later of 0·25 mg. arecoline in 1 c.c. emulsion, caused a rapid rise in blood pressure from 136 to 198 mm. Hg in 1 min. after which it fell to 116 mm. Hg, where it was stationary for some time. Second and third injections of the same quantity of arecoline-emulsion mixture given at suitable intervals produced the same effect. These rises in blood pressure are difficult to explain in view of the observation that the emulsion alone caused a fall in the blood pressure of this animal, and that 0·25 mg. arecoline in Ringer's solution (given at 2.45 p.m.) caused a typical fall in blood pressure from 156 to 52 mm. Hg. The animal was perfectly still and quiet throughout the experiment. The remainder of the experiment agrees with the other experiments of this series.

(*b*) *Pilocarpine nitrate and atropine sulphate*

Two decerebrate and two chloralosed cats were used in this series. The pilocarpine-emulsion mixture was made up to the same strength and in the same way as the arecoline-emulsion mixture of the previous experiments. The atropine-emulsion mixture was exactly the same as before and was allowed to stand for 6 hr. at 37·5° C. In all four experiments there was a close agreement in results. In every case the pilocarpine-emulsion mixture caused a depression of the blood pressure while the atropine-emulsion mixture antagonized this effect. In all the animals the injection of 1 c.c. of the emulsion alone caused very little or no alteration in the general blood pressure level. From these results it is evident that the emulsion does not modify the effects of the pilocarpine or atropine and so are in agreement with the majority of the experiments outlined in the previous section.

(*c*) *Morphine hydrochloride*

In these experiments blood pressure was recorded in addition to respiration and different concentrations of morphine hydrochloride ranging from 0·5 to 3 mg. per c.c. in the olive-oil emulsion were used. The protocols of four separate experiments are given in Tables II, III, IV and V.

Table II shows that the intravenous injection of 0·25 mg. morphine in 0·5 c.c. emulsion caused a marked depression in the rate and depth of respiration, as well as in the blood pressure. During an interval of 63 min. the respiratory rate fell from 14 to 7 per min., while the depth of respiration decreased from 17/17 (normal) to 14/17 of the normal amplitude. During the same interval of time the blood pressure fell from 168 to about 148 mm. Hg. Although there was a marked depression of the central nervous system respiratory failure did not occur; in fact the subsequent injection of three further doses of 3, 1 and 5 mg. of morphine in emulsion at intervals of 20, 25 and 10 min. did not cause failure of the respiratory centre, although further depression of the central nervous system was produced as is shown by the state of the

respiration and blood pressure at the termination of the experiment. One might be inclined to believe that the emulsion had exerted some protection in view of the large amounts of morphine injected without producing a fatal termination, but it is a common observation that cats will tolerate enormous amounts of morphine or its derivatives when injected in aqueous solution, without the addition of any emulsion, provided that a small initial dose of the drug is given (Myers, 1933). The evidence suggests that the olive-oil emulsion does not influence the depressant action of morphine hydrochloride upon the central nervous system.

In the next experiment the concentration of morphine hydrochloride in the emulsion was altered to 1 mg. in 1 c.c. emulsion and the mixture set aside for 4 hr. at 37·5° C. before use. Table III shows that the intravenous injection of 0·25 c.c. of the emulsion alone caused an immediate cessation of respiration in the expiratory phase and a fall in blood pressure of 26 mm. Hg. This period of apnoea lasted 2 min. after which the respiratory rate quickly increased to twice its normal rate while the amplitude remained almost normal. The blood pressure began to return slowly to normal after the asphyxial rise produced during the period of apnoea. Both respiration and blood pressure had returned to normal limits 25 min. after the injection had been made. These were still constant 7 min. later when 0·25 mg. of morphine hydrochloride mixed with 0·25 c.c. of the emulsion was injected intravenously. The result of this injection was a progressive depression of the central nervous system as shown by the decreased respiratory rate and fall in the systemic blood pressure. It is interesting to note that the amplitude of the respiratory movements was never decreased by the morphine, and remained more or less constant until 4·5 hr. after the morphine injection had been made when the experiment was terminated. The effects of the morphine-emulsion mixture were found to be practically the same as those produced in the two control cats of 2·5 and 2·8 kg. respectively, under the influence of chloralose. In these two control experiments 0·25 mg. of morphine hydrochloride in 0·25 c.c. of Ringer's solution was substituted for the 0·25 c.c. of morphine-emulsion mixture, otherwise the experiments were conducted under identical conditions. In neither case did the experiment have a fatal termination and the degree of depression and the time required to produce it were approximately the same. Referring to Table III it will be seen that the state of depression became more or less maximal after 20 min. The corresponding times for the control animals were 25 and 30 min. respectively. The results of one of the control experiments are given in Table IV. The close approximation of these results shows that the effects of morphine upon the respiration and blood pressure are not modified in any way by the olive-oil emulsion.

It might be argued that the time allowed for the possible adsorption of the alkaloid to the oil particles was not sufficiently great, so the next experiment was designed to elucidate this point.

The morphine-emulsion mixture was made up in exactly the same con-

Table II. *The influence of morphine hydrochloride, mixed with olive-oil emulsion, upon respiration and blood pressure*

Time	Drug	Respiration rate/min.	Depth of respiration in relation to normal	B.P. mm. Hg
10.15 a.m.		14	17/17	166
10.20		14	17/17	168
10.25		14	17/17	166
10.30		14	17/17	168
10.32	0·25 mg. morphine HCl in 0·5 c.c. emulsion			
10.40		10	16/17	168
10.50		12	16/17	166
11.00		12	15/17	164
11.35		7	14/17	Clot (? 148)
11.50		7	14/17	130
11.52	3 mg. morphine HCl in 2 c.c. emulsion			
11.55		4	14/17	96
12.15 p.m.		4	14/17	66

Further doses of 1 mg. and 5 mg. morphine HCl in emulsion failed to produce respiratory failure.
Emulsion and alkaloid mixed 3 hr. at 37·5° C. before use.
Cat 3 kg. Chloralose anaesthesia.
Injections via left external jugular vein.

Table III. *The influence of morphine hydrochloride, mixed with emulsion, upon respiration and blood pressure*

Time	Drug	Respiration rate/min.	Depth of respiration in relation to normal	B.P. mm. Hg
9.30 a.m.		13	19/19	96
9.35		13	19/19	94
9.40		13	19/19	96
9.42	0·25 c.c. emulsion alone	Apnoea in expiratory phase	—	70
9.44		Respiration beginning again	—	114
9.47		28	18/19	60
9.52		14	23/19	76 rising
9.57		14	20/19	106
10.03		11	19/19	116
10.08		12	19/19	112
10.13		12	19/19	102
10.15	0·25 mg. morphine HCl in 0·25 c.c. emulsion			
10.20		10	18/19	90
10.25		9	19/19	78
10.30		8	19/19	74
10.35		7	18/19	76
11.30		7	19/19	62
2.30 p.m.		7	18/19	50

Emulsion and alkaloid mixed 4 hr. at 37·5° C. before use.
Cat 2·7 kg. Chloralose anaesthesia.
Injections via left femoral vein.

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Table IV. *Control experiment. The influence of morphine hydrochloride in Ringer's solution upon respiration and blood pressure*

Time	Drug	Respiration rate/min.	Depth of respiration in relation to normal	B.P. mm. Hg
2.20 p.m.		18	22/22	106
2.30		18	22/22	110
2.40		18	22/22	108
2.42	0·25 mg. morphine HCl in 0·25 c.c. Ringer's solution			
2.47		14	23/22	99
2.52		12	23/22	87
2.57		10	21/22	85
3.02		10	21/22	83
3.07		9	20/22	78
3.12		9	20/22	79
3.22		9	20/22	78
4.22		9	21/22	74

Cat 2·5 kg. chloralose anaesthesia. Injections via left femoral vein.

Table V. *The influence of morphine hydrochloride, mixed with emulsion, upon respiration and blood pressure*

Time	Drug	Respiration rate/min.	Depth of respiration in relation to normal	B.P. mm. Hg
9.5 a.m.		12	12/12	94
9.10		12	12/12	95
9.15		12	12/12	94
9.17	0·25 mg. morphine HCl in 0·25 c.c. emulsion			
9.22		12	11/12	80
9.27		12	10/12	75
9.32		12	9/12	74
9.37		12	8/12	62
9.40	Respiration failure	—	—	Asphyxial rise
9.41	Artificial respiration			
9.49	Stopped artificial respiration			
9.50	Respiration failure	—	—	Asphyxial rise

Emulsion and alkaloid mixed and left for 17 hr. at 37·5° C. before use.
Cat 2·6 kg. Chloralose anaesthesia. Injections via left femoral vein.

Table VI. *The influence of morphine hydrochloride, mixed with emulsion, upon respiration and blood pressure*

Time	Drug	Respiration rate/min.	Depth of respiration in relation to normal	B.P. mm. Hg
3.05 p.m.		15	25/25	156
3.10		15	25/25	150
3.15		15	25/25	154
3.17	6 mg. morphine HCl in 2 c.c. emulsion			
3.20		15	22/25	138
3.22		14	19/25	130
3.23		10	22/25	130
3.27		10	22/25	120
3.32		12	19/25	90
3.38		12	19/25	86
3.45	Respiration failure			

Emulsion and alkaloid mixed for 4 hr. at 37·5° C. before use.
Cat 3 kg. Chloralose anaesthesia. Injections via intraperitoneal route.

centration as before (1 mg./1 c.c.) but was left at 37·5° C. for 17 hr. The two animals chosen for the tests were in good condition and about the same weight as the others used in the previous experiments. The results clearly showed that the morphine produced its depressant effects in the same way as in the previous experiments. The results of one of these experiments are tabulated (Table V) because of its fatal termination when only 0·25 mg. of morphine hydrochloride was used. Table VI shows the results of another experiment using a larger dose of morphine hydrochloride in a larger volume of the emulsion. The injection consisted of 6 mg. of morphine hydrochloride in 2 c.c. of emulsion and was made by the intraperitoneal route. This experiment terminated fatally at the end of 28 min. with a serious depression of the respiratory centre caused by the morphine hydrochloride.

Diacetylmorphine hydrochloride

A further experiment was made using diacetylmorphine hydrochloride (heroin) instead of morphine. The depressant action of heroin upon the central nervous system is approximately 50% greater than that of morphine and in this experiment a fatal dose of heroin (8 mg.) was mixed with 1 c.c. of the emulsion. The mixture was allowed to stand for 4 hr. at 37·5° C. before use. The injection was given intravenously into the left femoral vein and the results are set out in Table VII where it will be seen that the animal died from respiratory failure 23 min. after the injection of heroin mixture.

Table VII. *The influence of diacetylmorphine hydrochloride, mixed with emulsion, upon the respiration and blood pressure*

Time	Drug	Respiration rate/min.	Depth of respiration in relation to normal	B.P. mm. Hg
10.30 a.m.		28	22/22	166
10.35		26	22/22	164
10.40		26	22/22	166
10.45	8 mg. heroin in 1 c.c. emulsion			
10.50		24	19/22	140
11.00		16	25/22	90
11.10		14	15/22	60
11.15		14 irreg.	Irreg.	40
11.17				
11.18				
11.20	Artificial respiration started	12	—	—
11.30		12	—	60
11.32	Artificial respiration stopped	10	—	70
11.34				
11.40	Respiration failure	0	0	Asphyxial rise
11.55	0·25 c.c. coramine (5%)	5	50/22	100 falling
12.05 p.m.	Death. Respiration failure			

Emulsion and alkaloid mixed for 4 hr. at 37·5° C. before use.
Cat 2·8 kg. Chloralose anaesthesia.
Injections via left femoral vein.

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Strychnine hydrochloride

Spinal frogs were used in this group of experiments, except where the emulsion alone was tested, when intact frogs were used. Eight frogs were each given an injection of 0·1 c.c. of 0·25% solution of strychnine hydrochloride. All the injections were made into the dorsal lymph sac and the time noted between the moment of injection and the onset of spinal convulsions. The time was found to be from 4 to 15 min. and all the frogs convulsed. A second group of frogs were each injected into the dorsal lymph sac with 1 c.c. of freshly prepared emulsion and their behaviour observed for 2 hr. Nothing unusual was seen and they all appeared to be as active at the end of this period as they were before the emulsion was injected. These frogs were still quite normal 7 days later. It is therefore obvious that the emulsion caused no alteration in their general behaviour. It was then decided to mix this dose of strychnine in 1 c.c. of the emulsion and inject this amount of the mixture into the dorsal lymph sac of each of a fresh group of frogs. 60 c.c. of the strychnine-emulsion mixture was made and divided into six portions of 10 c.c. each. Each portion was then allowed to stand for a different interval of time at 37·5° C. The times selected were $\frac{1}{2}$, 1, 4, 8 16 and 24 hr. As these periods expired 1 c.c. of the portion was injected into the dorsal lymph sac of each of a group of four frogs, and the times recorded of the injection—convulsion interval. Every frog in each group convulsed within 14 min. except one in the 4 hr. group which did not convulse or show any signs of spinal hyperexcitability. These results show that the emulsion did not influence the action of strychnine hydrochloride on the spinal cord of these frogs.

DISCUSSION

The results of this investigation have shown quite clearly that when the salts of certain alkaloids are mixed with emulsions of olive oil in a fine state of division the biological actions of the alkaloids are not changed or modified in any way. Only a very limited number of alkaloidal salts were tested but they represented groups acting upon the cardiovascular system, the central nervous system, and the spinal cord. The only unusual feature observed in the whole investigation was the rise in blood pressure produced by the injection of arecoline-emulsion mixtures in two cats under the influence of chloralose. There was no doubt about the constancy of this effect which was produced several times in each animal and the effect was a most marked one. The same dose of arecoline in Ringer's solution always produced a typical depressor effect in the same animals. This unusual response was produced in two out of seven cats. In one of these animals the injection of a small amount of the emulsion alone produced no effect while in the other a small depression in the level of the blood pressure was observed. It appears therefore, as if the emulsion alone does not account for this action. The type of curve in tracings from these animals was not unlike that produced by adrenaline and it may be that

they are adrenalin effects. The animals were quite quiet throughout the experiments and there were no tremors or struggles which might have accounted for a liberation of adrenalin from the suprarenal glands. The pressor effect was only produced immediately after the arecoline-emulsion mixture was injected into the vein of the animal. Apart from this I have no explanation to offer for this effect although one might speculate upon it being due to some direct irritant action upon the capsule of the suprarenal gland caused by the emulsion-alkaloid mixture. It is generally recognized that in cats under the influence of atropine, large doses of choline or acetylcholine given intravenously result in a rise in blood pressure thought to be due to the irritant effect of these drugs on the suprarenal capsule. While the animals described in this communication had not been given atropine at the stage when this unusual blood pressure effect was recorded it is possible that the arecoline may have acted upon the suprarenal capsule with a consequent liberation of adrenalin.

Even small doses of morphine hydrochloride were not modified by the presence of the emulsion, and toxic doses always produced their lethal effects in a normal space of time.

There is therefore no evidence to show that these alkaloidal salts are adsorbed by emulsions of olive oil of the type used in these experiments. Though this type of emulsion is able to modify the effects of bacterial toxins it seems that it is unable to modify the biological actions of these alkaloidal salts. In the experiments using bacterial toxins it was found that the toxin must be mixed with the emulsion for a minimum period of 15–30 min. before injection into the animal if the toxin was not to have a lethal action. Shorter periods were not sufficient to produce this effect. In the experiments outlined in the present communication the alkaloids were mixed with emulsions for varying periods ranging from $\frac{1}{2}$ to 24 hr., without the production of any alteration in their biological effects and therefore it appeared useless to extend this period. An explanation of the different results obtained in the emulsion-toxin and emulsion-alkaloid investigations is difficult to suggest, but they may depend upon the molecular structure of bacterial toxins of which little is known. It is possible that the emulsions act by adsorbing toxins on their oil surfaces. On the other hand there may be something in the nature of the structure of the molecules of these alkaloidal salts which prevents their adsorption by the oil particles in emulsions. Further work will be required to solve this problem.

Summary

1. When certain alkaloidal salts of arecoline, pilocarpine, atropine, morphine, diacetylmorphine, or strychnine are mixed with an olive-oil emulsion in a fine state of division, allowed to stand at 37·5° C. for varying periods up to 24 hr., and then injected into animals, their biological actions are apparently not modified in any way.

2. This suggests that the droplets of the emulsion have not the power of adsorbing alkaloidal salts at their surfaces.

3. Arecoline hydrobromide when mixed with an olive-oil emulsion and injected intravenously sometimes showed a pressor action in chloralosed cats, whereas an aqueous solution of arecoline hydrobromide always produced a normal depressor effect in the same animals. This effect was seen in two animals under the influence of chloralose.

4. The intravenous injection of small amounts of the emulsion usually had no effect upon the blood pressure level, although, in a few instances it produced a slight depressor effect.

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