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THE PHARMACOLOGICAL PROPERTIES OF CONESSINE, *ISO*CONESSINE AND *NEO*CONESSINE

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Conessine is an alkaloid obtained from the bark and seeds of *Holarrhena antidysenterica*, a tree growing in India. The alkaloid has been studied by many workers (Keidel, 1878; Burn, 1914; Chopra, Gupta, David and Ghosh, 1927; Chopra, 1933; Bakhsh, 1936) who have described various properties bearing little or no relation to one another. Conessine is a typical example of the many alkaloids described in reference books of pharmacology the properties of which are difficult to remember because there is no sign of connexion between them and because they appear to be entirely fortuitous.

A report from Frère Just. Gillet, S. J., missionary in the Belgian Congo, that the chewing of the leaves of the plant *Holarrhena congolensis* produced anaesthesia of the mucous membrane of the mouth, led Burn (1914) to examine conessine experimentally for local anaesthetic action, which he found it to possess. Further work on this action was carried out by Trevan and Boock (1927). The use of conessine as a local anaesthetic is, however, limited by the fact that when injected it causes necrosis. Two isomers of conessine, namely *iso*conessine and *neo*conessine, have been prepared by Dr. S. Siddiqui by treating conessine with sulphuric acid. Samples of conessine dihydrochloride, *iso*conessine hydrochloride and *neo*conessine hydrochloride were left with Prof. Burn by Dr. Siddiqui with the request that they should be compared with one another for local anaesthetic potency and for local irritant action. There was a possibility that one of these substances would prove as potent as conessine and not cause an inflammatory reaction when injected.

The three substances have therefore been compared for their irritant action, and the method used for making this comparison will be described in a later paper. They have also been compared for local anaesthetic action. In addition these substances have been examined for other properties; several local anaesthetics are known to depress the action of acetylcholine on the rectus muscle of the frog, and de Elío (1948) has even observed a quantitative parallelism. Dawes's work (1946) showed that many local anaesthetics had a quinidine-like action on the heart. Conessine and its isomers have therefore been examined on skeletal and on cardiac muscle. Since the results indicated a similarity to quinidine, and since de Elío (1948) had shown that quinidine had a spasmolytic action, reducing the action of acetylcholine on the intestine, conessine and its isomers have also been tested in this way.

Experimental Observations

Local anaesthetic action.—No figures exist for the relation between the local anaesthetic potency of procaine and conessine when given by injection. Conessine and its derivatives were therefore compared with procaine by intracutaneous injection into guinea-pigs according to the method of Bülbring and Wajda (1945). The results for conessine when compared with *neo*conessine and *iso*conessine on the same animals are shown in Fig. 1, from which it appears that *neo*conessine is the weakest, having 48 per cent of the strength of conessine, and *iso*conessine is intermediate, having 77 per cent of the strength of conessine. A careful comparison of

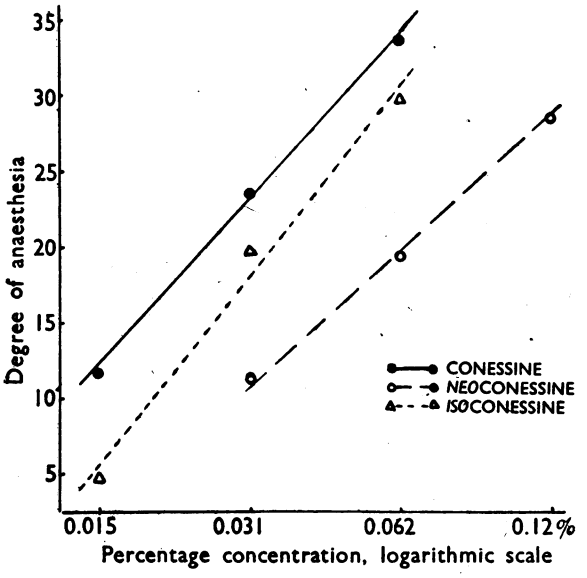

FIG. 1.—Graph showing the relative local anaesthetic potencies of conessine, *iso*conessine and *neo*conessine. Each point represents the mean degree of anaesthesia in 8 areas. If conessine = 100, then *iso*conessine = 77 and *neo*conessine = 48.

TABLE I

LOCAL ANAESTHETIC POTENCIES IN TERMS OF CONESSINE = 100
(Intracutaneous injection into guinea-pigs)

	Local anaesthetic action	Inhibition of ACh on frog rectus
Conessine ..	100	100
*Iso*conessine ..	77	73
*Neo*conessine ..	48	60
Cocaine.. ..	47	30
Quinidine ..	10	20
Procaine ..	7	10

conessine with procaine showed that the relative anaesthetic potencies can be expressed as in Table I, in which the figures for quinidine (de Elío, 1948) and for cocaine (Bülbring and Wajda, 1945) are also included for comparison.

Table I shows that conessine is appreciably stronger than its derivatives, but that the weakest, *neo*conessine, differs little in potency from cocaine.

Frog rectus.—The stimulant action of acetylcholine on the frog rectus muscle was found to be depressed by conessine and its isomers. An illustration of this action is given in Fig. 2 in which the effects of all three substances are shown. A direct comparison of *iso*conessine and quinidine showed that quinidine had 28 per cent of the potency of *iso*conessine in depressing acetylcholine on the frog rectus. When de Elío (1948) compared four local anaesthetics, procaine, cocaine, amethocaine and nupercaine, he found that they likewise depressed the action of acetylcholine on the frog rectus and moreover that their relative potency in doing so was similar to their relative potency as local anaesthetics. This was also true for conessine and its isomers as shown in Table I, in which the two sets of figures are placed side by side.

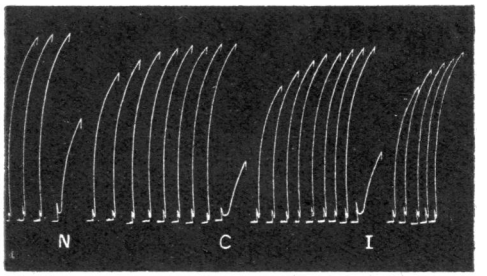

FIG. 2.—Record of contractions of the frog rectus abdominis produced by a concentration of 3.75×10^{-7} acetylcholine acting for 90 sec. The contractions were obtained at 6 min. intervals and those marked N, C and I were preceded by exposure of the muscle for 4½ min. to concentrations of 4×10^{-5} *neo*conessine, conessine and *iso*conessine respectively. If conessine = 100, then *iso*conessine = 73 and *neo*conessine = 60.

Denervated gastrocnemius of cat.—Similar experiments were also carried out on the denervated gastrocnemius muscle of the cat. The left sciatic nerves of a series of cats were divided under ether with aseptic precautions (these operations were kindly performed by Prof. Burn) and the animals were left for 5–26 days. The cats were then anaesthetized with ether and chloralose and the gastrocnemius muscle was detached from the os calcis and fastened to a tension lever. Injections were made through a cannula in the right iliac artery pointing towards the bifurcation of the aorta. Contractions were then obtained by the injection of acetylcholine, and the effect of a preceding injection of conessine on these contractions was determined. An illustration of two experiments is given in Fig. 3. The usual result was that injections from 1–10 mg. depressed the response. In the upper part of Fig. 3 this depression is shown produced by 10 mg. *iso*conessine and by 10 mg. conessine. In the lower part of Fig. 3 a less usual effect is shown, namely, that 1 mg. of these same alkaloids produced an augmentation of the acetylcholine response.

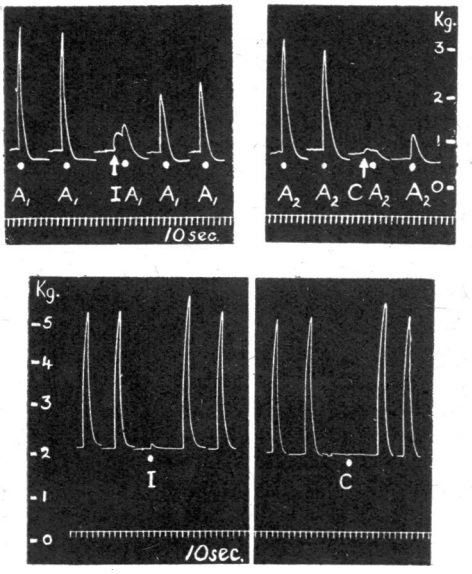

FIG. 3.—Cat. Chloralose. Record of contraction of denervated gastrocnemius in response to intra-arterial injections of acetylcholine.

Upper tracing: Muscle denervated 27 days before. Contractions at A_1 due to 10 μg. ACh. Intra-arterial injection of 10 mg. *iso*conessine produced a small contraction, and the following contraction in response to ACh was greatly reduced. Contraction at A_2 produced by 20 μg. ACh. 10 mg. conessine abolished the effect of the next injection of ACh.

Lower tracing: Muscle denervated 26 days before. Contractions produced by 10 μg. ACh. The intra-arterial injection of 1 mg. *iso*conessine at I, and of 1 mg. conessine at C, slightly increased the effect of the next injections of ACh.

Rat diaphragm preparation.—Since the work of Harvey (1939) it has been known that quinine has various actions on skeletal muscle among which are (1) the ability to increase the tension response to a single maximal stimulus, and (2) to act like curare in lowering the excitability at the motor end plate. Experiments were therefore carried out to see if these effects could be demonstrated when conessine and its isomers were used. It was found that the curare-like action was regularly demonstrable, but only once was the augmentor action seen. The isolated nerve-muscle preparation of the rat as described by Bülbring (1946) was used. The effect is shown in Fig. 4 in which the phrenic nerve was stimulated by maximal single shocks ; in the upper part of the figure the addition of 0.5 mg. *iso*conessine to the bath caused an increase in the response very similar to that seen in the lower part of the figure when 2 mg. quinidine was added. The usual response to the addition of *iso*conessine was

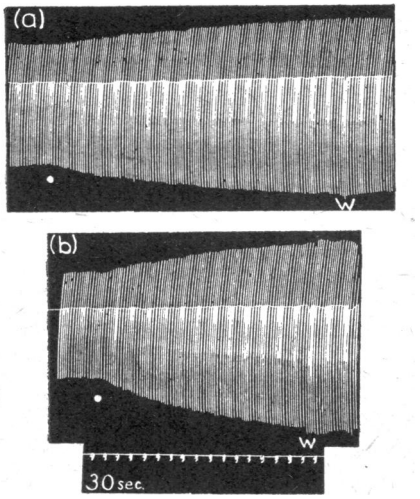

FIG. 4.—Rat diaphragm phrenic nerve preparation. Tyrode 37° C., bath 50 ml. Record of the contractions produced by stimulating the nerve with single maximal shocks 8–10 times per min. At (*a*) is shown the increase in amplitude produced by 0.5 mg. *iso*conessine. This is an unusual effect. At (*b*) is shown the action of 2 mg. quinidine.

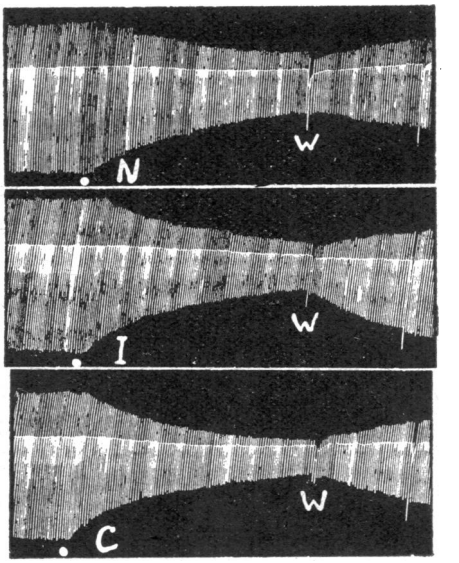

FIG. 5.—Rat diaphragm phrenic nerve preparation. Record of maximal contractions. Depression of contraction produced by 1 mg. *neo*conessine at N, 1 mg. *iso*conessine at I and 1 mg. conessine at C. All three substances were in the bath for 10 min. before washing out. The effects were reversible.

that shown in Fig. 5 in which the muscle twitches were steadily reduced as they are when *d*-tubocurarine is added; the same result was obtained with conessine and *neo*conessine. The doses used were 5–10 times as great as the dose of *d*-tubocurarine which would have been required to produce a similar effect.

Quinidine-like action on cardiac tissue.—The alkaloids were tested by Dawes's method (1946) in which the isolated rabbit auricles are suspended in a bath at 29° C. and driven electrically. The maximum rate at which the auricles will follow the electrical stimulus is determined before, and then after exposure for 10 min. to a given concentration of the substance. When quinidine is tested in this way it reduces the maximum rate, and this reduction persists for some time after the quinidine has been washed out. However, provided an interval is allowed, the preparation returns to its former state and further observations can then be made. When conessine or its isomers were used, a similar reduction in the maximum rate was observed (see Fig. 6), but the effect of a given concentration on different occasions was not the same, and often an increase in concentration produced little increase in effect. From the best experiments it appeared that conessine in a concentration of 2×10^{-5} was approximately equal to quinidine 10^{-5}. Thus the potency of conessine was 50 per cent of that of quinidine. *Iso*conessine was weaker than this.

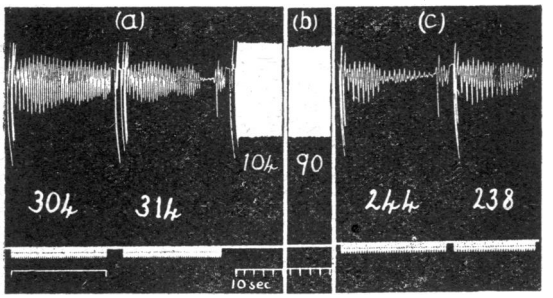

FIG. 6.—Isolated rabbit auricle driven electrically. Ringer-Locke at 29° C., bath 50 ml. (*a*) Auricle follows 304 but does not follow 314 stimuli per min. Spontaneous rate 104 beats per min. (*b*) Spontaneous rate after 5 min. exposure to 4×10^{-5} *iso*conessine. (*c*) After 10 min. exposure to *iso*conessine the auricle fails to follow 244 but follows 238 stimuli per min.

Rabbit auricles.—Acetylcholine causes diminution of the spontaneous contractions of the isolated rabbit auricles, as shown in Fig 7, in which the effect of 50 μg. acetylcholine is shown twice at the beginning of the record. After washing out, when the contractions had recovered their original size, 2 mg. *iso*conessine was added to the bath, and 1 min. later 50 μg. acetylcholine was added again.

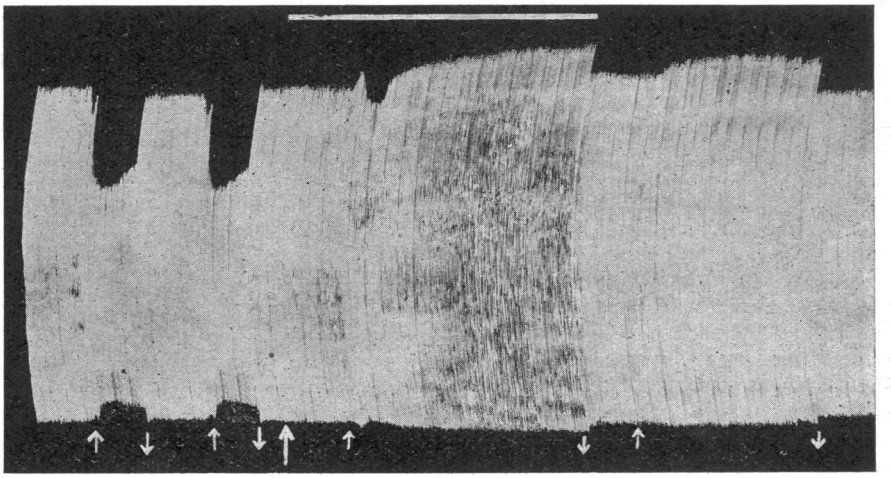

FIG. 7.—Isolated rabbit auricle beating freely. Ringer-Locke at 29° C., bath 50 ml. At the small arrows ↑, 50 μg. ACh was added to the bath and was washed out when the drum was stopped at the reversed arrows ↓. 2 mg. *iso*conessine was added to the bath at the large arrow (1 min. before the next dose of ACh) and remained in the bath during the period shown by the horizontal line. The inhibitory action of ACh was abolished and had not returned 10 min. after washing the *iso*conessine from the bath. The apparent augmentation by ACh in the presence of *iso*conessine was believed to be an artefact.

No diminution was seen, either then, or at a further addition of acetylcholine after the *iso*-conessine was washed out. After further washing out, the inhibitory effect of acetylcholine slowly returned, though not fully. Both conessine and *neo*conessine had the same effect.

Action on the heart.—In the rabbit heart perfused by Langendorff's method, the alkaloids were observed to have a dilator action on the coronary vessels which was most marked with *neo*conessine,

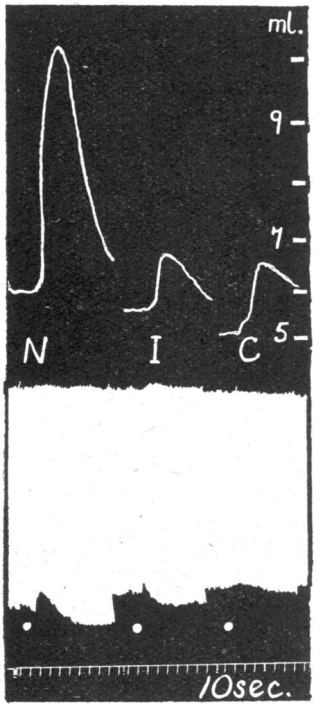

FIG. 8.—Isolated rabbit heart (Langendorff preparation). Perfused with Ringer-Locke at 37° C. Record from above downward: Coronary flow ml. per min., amplitude of heart beats and time marker. At N, I and C 200 μg. of *neo*conessine, *iso*conessine and conessine respectively were injected into the perfusion fluid. Note large dilator effect of *neo*conessine. (For method of recording, see Stephenson, 1948.)

and sometimes absent with conessine. A comparison of the dilator effect of the three substances is shown in Fig. 8, where they are also seen to cause a slight increase in amplitude of the beat. Bakhsh (1936) noted the dilator action of *iso*conessine on the coronary vessels.

Action on blood vessels.—To test the effect on the vessels, both the rat's hindlegs and the rabbit's ear have been used, perfused with Ringer's solution at room temperature. The rat's hindlegs were perfused through the abdominal aorta and the rabbit ear by the method of Gaddum and Kwiatkowski (1938). Conessine, when injected into the fluid perfusing the rabbit's ear, caused vasodilatation. It was curious to find that whereas the constrictor effect of an injection of adrenaline in the rabbit's ear was greatly reduced by an injection of *iso*-conessine, the constrictor effect of adrenaline in the rat's hindlegs was not affected. The difference was perhaps more apparent than real, since when an adrenaline tone was maintained in the rat's hindlegs, the injection of conessine (0.1 mg.) caused dilatation (Fig. 9a). In the rat's hindlegs, a single injection of acetylcholine during the maintenance of an adrenaline tone caused dilatation. When not only adrenaline but also conessine was present

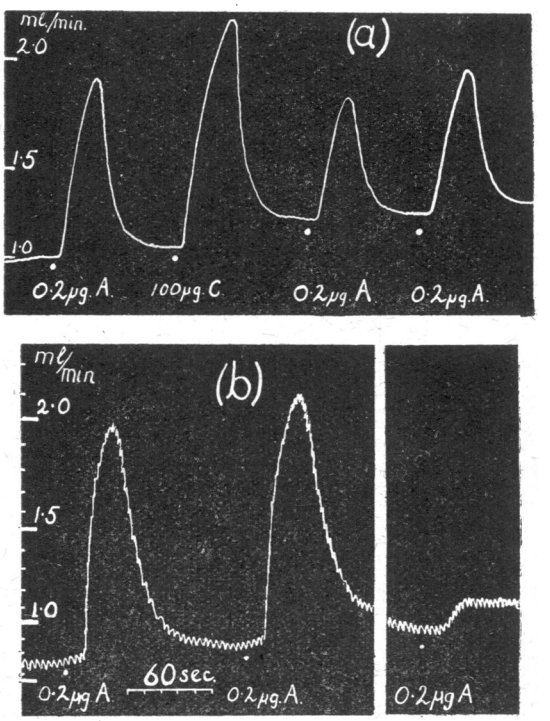

FIG. 9.—(*a*) Record of the flow from a perfusion of the hindlegs of a rat with Ringer-Locke at room temperature. The perfusion contained 10^{-7} adrenaline and dilatations were produced by 0.2 μg. ACh and by 100 μg. conessine. The effect of ACh is reduced after conessine. The record is continuous and the injections were made at 10 min. intervals. (*b*) Dilatations were produced by 0.2 μg. ACh. In the second part the perfusion fluid contained 10^{-5} conessine in addition to adrenaline. Note that, in the presence of conessine, the dilator effect of ACh was greatly reduced.

in the perfusing fluid, the dilator action of acetylcholine was almost abolished (Fig. 9*b*).

Action on blood pressure and respiration.—The actions of *neo*conessine and *iso*conessine were found to be indistinguishable from that of conessine on the blood pressure.

It was shown by Burn (1914) that a large dose of conessine produces a fall of blood pressure in

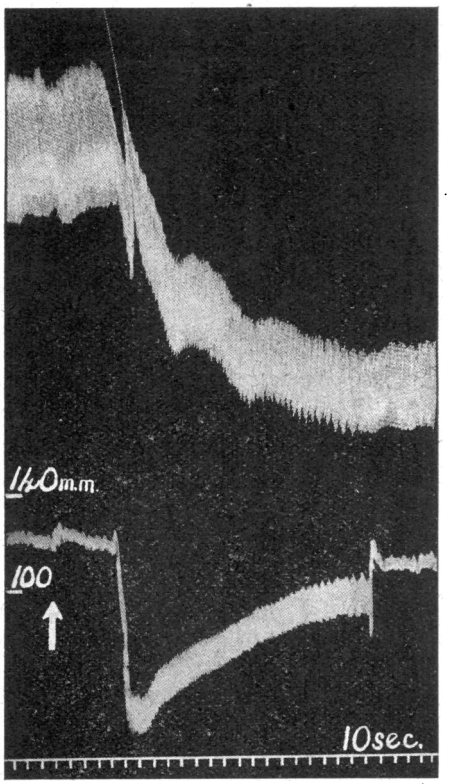

FIG. 10.—Rabbit, urethane. Record of respiration (above) and blood pressure. At the arrow 8 mg. conessine was injected. After about 1 min. this produced a sudden slowing of the heart rate accompanied by a fall of pressure. The pressure slowly recovered and the heart block suddenly disappeared. The respiration was depressed and recovered very slowly and incompletely.

which the heart action at first is unchanged. The fall is presumably due to the vasodilatation which has been described above. After some delay the heart is affected, the ventricular rate becoming very slow; the heart then appears to be in block. When smaller doses are used, either or both of these effects may be seen. In Fig. 10 when 8 mg. conessine was injected there was no initial fall, for vasodilatation did not occur; after a delay of 1 min. the heart became very slow and the pressure fell. Gradually the pressure rose again until suddenly the block disappeared and the blood pressure was fully restored.

Bakhsh (1936) suggested that the cardiac slowing was central in origin, but since conessine depressed the action of acetylcholine in so many directions it seemed unlikely that this would be so. The effect of conessine on vagal stimulation was therefore tested. In the experiment illustrated in Fig. 11 the injection of 8 mg. conessine abolished the fall of blood pressure produced by vagal stimulation, and diminished the depressor effect of 20 μg. acetylcholine by cutting out the slowing of the heart which this dose produced. When the respiration was recorded by Gaddum's method (1941), 8 mg. conessine was observed to diminish both rate and depth (see Fig. 10).

It is worth while to record the great tolerance of rabbits to the intravenous infusion of *iso*conessine at a uniform rate. No less than 106 mg. was infused into one rabbit, the heart continuing to beat strongly; only when the rate of infusion exceeded 2.5 mg. per min. was the heart seriously affected. Solmann (1942) states that quinidine leaves the blood stream very rapidly, and it may be that *iso*conessine does so also.

Spasmolytic action.—The spasmolytic action of four local anaesthetics and of quinidine was compared with that of atropine by de Elío (1948). He found that quinidine was six times more powerful than procaine, and procaine was equal to cocaine. Conessine has been stated both to relax and to stimulate smooth muscle but no one has hitherto shown that it depresses the action of acetylcholine. Fig. 12 is a record of the contractions of an isolated piece of rabbit intestine, in which it will be seen that in the presence of concentrations of conessine from 10^{-5} to 2×10^{-6}, the stimulant action of acetylcholine is proportionally reduced. Both *neo*conessine and *iso*conessine were found to be slightly stronger than conessine, and about equal to one another. When a comparison was made between conessine and quinidine on a loop of guinea-pig ileum, it was found that conessine was slightly less potent than quinidine.

Antimalarial action.—In view of the close similarity of conessine and its isomers to quinine and quinidine, a comparison was made with quinine in chickens infected with *Plasmodium gallinaceum*. This test was done under the supervision of Mr. L. G. Goodwin in the Wellcome Research Institute. Chicks, 6 days old, were inoculated intravenously with 0.2 ml. blood

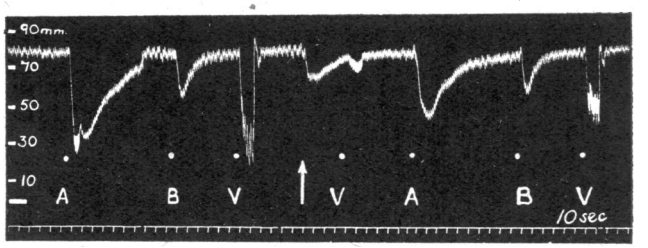

FIG. 11.—Rabbit, urethane. Record of blood pressure. At A 20 μg. ACh were injected intravenously, at B 1 μg. ACh was injected and at V the peripheral end of the right vagus was stimulated for 15 sec. At the arrow, 8 mg. conessine was injected and this abolished the effect of the vagus on the heart. The heart was no longer affected by 20 μg. ACh, though the injection still produced a fall of pressure. After 5 min. the heart was again sensitive to vagal stimulation but the effect was not as great as previously.

diluted to contain 10^8 parasitized red cells. The alkaloids were given by mouth, once on the day of inoculation, and twice on each of the three following days; 17 hours after the last dose, a blood smear was prepared from each bird in which 200 red cells were examined to determine the percentage which contained parasites. The results, recorded in Table II, indicate that, in the doses used, no antimalarial effect was exerted by conessine or its isomers.

TABLE II

ANTIMALARIAL ACTION IN CHICKS INFECTED WITH *P. Gallinaceum*

Substance	Dose mg./kg.	No. of birds	Mean percentage of cells with parasites
(Controls)	—	7	42
Conessine	40	4	32
,, ..	20	4	51
*Iso*conessine	80	4	46
,,	40	8	37
,,	20	8	42
*Neo*conessine	40	3	38
,,	20	3	21
Quinine ..	40	8	0.5
,, ..	20	8	11.4

Toxicity.—Conessine and its isomers were compared with one another by intravenous injection into mice in order to determine the mean lethal dose. Care was taken to inject at a uniform rate. For conessine 39 mice were used, for *iso*conessine 30 mice, and for *neo*conessine 50 mice. The lethal dose killing 50 per cent of mice was for conessine 28.7 mg. per kg., for *iso*conessine 33.2 mg. per kg., and for *neo*conessine 13.1 mg. per kg. Thus whereas conessine and *iso*conessine are very similar in toxicity, *neo*conessine is more than twice as toxic. It may be emphasized that while the actual figure for the toxicity of any one compound may vary greatly in different laboratories, the relative toxicities of the three compounds should remain constant.

DISCUSSION

Recent papers from this laboratory (Dawes, 1946; Dews and Graham, 1946; de Elío, 1948; Dutta, 1948) have brought out the similarity between various substances not previously considered to be related. Dawes's work showed that substances with a quinidine-like action on the heart included various local anaesthetics, spasmolytics and analgesics, and that quinidine and procaine reduced the action of acetylcholine in cardiac, skeletal and unstriped muscle. De Elío added to the evidence by testing four local anaesthetics, as well as atropine, pethidine and quinidine, on the frog rectus, the rabbit auricles and the rabbit intestine. In general all seven substances reduced the action of acetylcholine in these tissues and a close parallelism between local anaesthetic action and effect on the frog rectus was found to exist. Dutta tested pethidine, quinidine, atropine and benadryl to see whether they possessed the property, which procaine has long been known to possess, of lowering body temperature. He found that all these substances resembled procaine in this respect.

When conessine and its isomers were to be examined, their known local anaesthetic activity suggested that they also should be examined from the point of view described, and that since quinidine was also a plant alkaloid with local anaesthetic properties, the relation of their action to

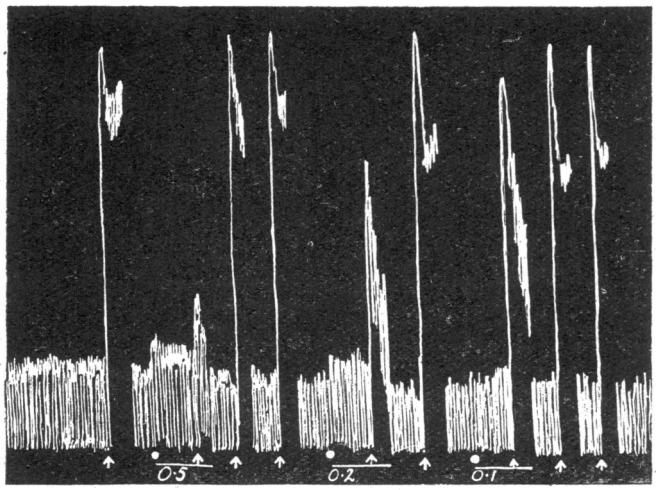

FIG. 12.—Isolated rabbit intestine, Ringer-Locke 36° C. The contractions at the small arrows were produced by the addition of 8 μg. ACh to the bath. Contractions produced in the presence of 0.5 mg., 0.2 mg. and 0.1 mg. of conessine (added at the white spot) were reduced in proportion to the amount of conessine. The fluid in the bath was changed at the end of the horizontal lines.

that of quinidine should be considered with special care. The outcome has been to demonstrate that in many respects conessine and its isomers are very similar to quinine and quinidine. So close indeed did the resemblance seem at one point that tests for anti-malarial activity were carried out. However, in doses in which quinine was active, conessine and its isomers were inactive. They have not yet been tested in higher doses.

Conessine resembles quinidine in diminishing the action of acetylcholine on the isolated intestine, the rabbit auricle, the frog rectus and on denervated mammalian muscle. The contractions of the rat diaphragm produced by nerve stimulation are occasionally augmented by *iso*conessine as they usually are by quinidine; the usual effect of conessine is depression and this is sometimes seen with quinidine. Conessine, like quinidine, lengthens the refractory period of cardiac tissue, and the effect of vagal stimulation on the heart in the anaesthetized rabbit is temporarily abolished by conessine as by quinidine (Starr, 1936) and by quinine in the dog (Babkin and Ritchie, 1945).

Of considerable interest is the parallelism between local anaesthetic action and inhibition of the action of acetylcholine on the frog rectus. This was first observed by de Elío, and the observations on conessine and its isomers make the parallelism still more complete. This is a further addition to the evidence that the action of acetylcholine is concerned with the sensation of pain. Harvey, Lilienthal and Talbot (1941) found that when acetylcholine was injected intra-arterially in men it caused very severe pain. Gray (1947) has detached a portion of the skin of an anaesthetized cat so as to leave only the artery, vein and nerve in connexion with the body. When he injected acetylcholine into the artery, he found that an action potential was set up in the nerve, and that this action potential was similar to that produced by mechanical pressure on the detached portion of skin. Thus a local anaesthetic may be a substance which reduces the action of acetylcholine at sensory nerve endings, and this would explain why local anaesthetic potency is related to the reduction of the action of acetylcholine on the frog rectus.

Hitherto conessine has been thought to be an alkaloid with its own peculiarities unlike those of any other plant alkaloid. It is now evident that this is not so, and that the action of conessine, which comes from the bark of Holarrhena, is very similar to that of the alkaloids quinidine and quinine which come from the bark of Cinchona. The actions of conessine also resemble those of papaverine, cocaine and atropine, three other plant alkaloids, all of which are local anaesthetics, spasmolytics and have a quinidine-like action on the heart. It is an obvious task to look at still more plant alkaloids in order to see how many others can be brought into this class, and to examine them for points of chemical and physical similarity.

SUMMARY

1. Conessine and its isomers, *iso*conessine and *neo*conessine, have been shown to possess properties very similar to those possessed by quinine and quinidine. Conessine, however, has no antimalarial action in chickens when tested in the same dose as quinine.

2. The local anaesthetic potency of conessine and its isomers is great; when tested by intracutaneous injection into guinea-pigs, conessine is about twice as active as cocaine, *iso*conessine is 50 per cent stronger than cocaine, and *neo*conessine is about equal to cocaine. The relative local anaesthetic potencies of conessine, its isomers, cocaine, quinidine and procaine are very similar to their relative activities in depressing the action of acetylcholine on the frog rectus muscle.

3. Conessine and its isomers have a quinidine-like action on the heart, and depress the action of acetylcholine on skeletal, cardiac and smooth muscle including that of the blood vessels, acting on all tissues very much like quinidine.

This work has been done while in receipt of a grant from the Therapeutic Research Corporation, to whom I wish to express my thanks. I am also indebted to Dr. S. Siddiqui for a supply of conessine, *iso*conessine, and *neo*conessine. The work has been carried out under the direction of Prof. J. H. Burn, and this paper has been written with his help.

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