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ACTION OF CALABASH CURARE AND RELATED CURARIFORM SUBSTANCES ON THE CENTRAL NERVOUS SYSTEM OF THE CAT

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In a previous paper (Salama and Wright, 1950) we described the effects of *d*-tubocurarine chloride on the central nervous system; we report in this paper the results of a study of the central actions of a number of other naturally occurring alkaloids with "curariform" action. The substances examined were: (i) calabash curare; (ii) *l*-bebeerine (curine) and *d*-bebeerine; (iii) curine dimethyl ether dimethiodide; (iv) β-erythroidine and dihydro-β-erythroidine. The chemistry of these substances is fully reviewed by Craig (1948). Some relevant chemical and physiological properties are briefly summarized below.

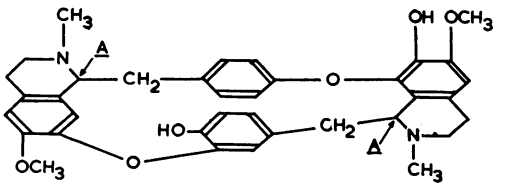
(i) *Calabash curare.*—The alkaloids isolated from calabash curare are called calabash curarines and toxiferines. The formulae of toxiferine I and calabash curarine II (which have powerful "curariform" actions) are $C_{20}H_{21}N_2^+,Cl^-$ and $C_{20}H_{23}N_2^+,Cl^-$ respectively. The non-quaternary nitrogen atom is secondary and non-basic and there is evidence for the quaternary nitrogen being in a reduced *iso*quinoline ring system and common to two rings (Karrer, 1946).

The sample used in this work was kindly provided by Dr. Harold King and was a mixture of calabash curare alkaloids. The literature on the central action of calabash curare is scanty, as earlier workers did not differentiate between the different curare alkaloids. Blume (1934) reported convulsions and other signs of central nervous stimulation in rats, mice, and guinea-pigs and to a less extent in cats after subcutaneous or intravenous administration of calabash curare.

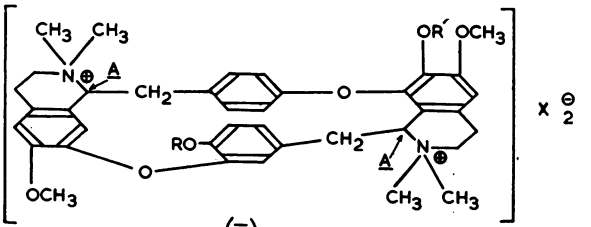
(ii) l-*Bebeerine (curine) and* d-*bebeerine.*—The samples were kindly provided by Dr. Harold King. These tertiary bases are stereoisomeric and differ only in having opposite configurations at each of the two asymmetric centres *A* shown in the structural formula (I). The closely related bis-quaternary salt *d*-tubocurarine chloride (II, R=R′=H, X=Cl) differs in addition in probably having one dextrorotatory and one laevorotatory asymmetric centre, whereas *d*-bebeerine has both dextrorotatory (King, 1948). According to West (1937) bebeerine has a central depressant action.

(iii) *Curine dimethyl ether dimethiodide* (II, R=R′=CH_3, X=I).—Although the term curine is the general name given by Boehm to the tertiary alkaloids which accompany the quaternary (e.g., tubocurarine) in crude curare preparations, it is now usually used as an alternative to *l*-bebeerine. The curine dimethyl ether dimethiodide is a bis-quaternary derivative of curine.

*The main results were reported to the Physiological Society (April, 1948) by S. Salama and incorporated in a Ph.D. thesis accepted by the University of London.



(I) l-Bebeerine (Curine)
A: asymmetric centres



(II)

d-Tubocurarine Chloride, R = R' = H, X = Cl.
Curine dimethyl ether dimethiodide, R = R' = CH_3, X = I.

(iv) *β-Erythroidine and dihydro-β-erythroidine.*—The active principles of *Erythrina americana* include two isomeric dextrorotatory alkaloids called α- and β-erythroidine. The latter is more readily purified and has the empirical formula $C_{16}H_{19}NO_3$; the single N atom is tertiary and common to two rings; a methoxyl group, two olefinic double bonds, a lactone ring, and an indole nucleus are also present. By catalytic hydrogenation, β-erythroidine is converted into dihydro-β-erythroidine and two tetrahydro isomers.

The erythroidines are peculiar in being tertiary nitrogen compounds with a marked peripheral paralysing action (Unna, Kniszul, and Greslin, 1944). Dihydro-β-erythroidine has a greater paralysing activity than β-erythroidine on the sciatic-gastrocnemius preparation of the cat. The peripheral paralysing activity of β-erythroidine is reduced to about a twentieth when it is converted to the quaternary methiodide. In all other bases studied the conversion of a tertiary into a quaternary compound greatly enhances the curariform action.

The samples of the erythroidine alkaloids used were kindly provided by Dr. J. W. Trevan.

METHODS

The methods employed to elicit and record the reflexes and the response of the peripheral nerve-muscle preparation were those described in the previous paper (Salama and Wright, 1950). Cats were used, usually anaesthetized with chloralose (0.06–0.08 g. per kg. body-weight). In our study of *d*-tubocurarine we found that the response of the animal to direct application of the drug to the central nervous system was the same whether the animal was anaesthetized with chloralose or decerebrated. The results to be described for all the alkaloids studied were also independent of the anaesthetic used.

Some of the alkaloids examined depressed the hind-limb reflexes. To determine whether these effects were due to an action on the central nervous system or to a peripheral action on the muscles, the ischaemic hind limb technique, described by Schweitzer and Wright (1937) was employed.

RESULTS

Action of calabash curare

Peripheral action.—The peripheral paralysing activity of the calabash curare preparation employed was 75 per cent that of *d*-tubocurarine when the drugs were injected intravenously in the cat, and the responses of the sciatic nerve-gastrocnemius muscles were studied. The relative activity was 50 per cent that of *d*-tubocurarine on the isolated rat nerve-diaphragm preparation.

Central actions

A. Intraventricular injection.—The effects of intraventricular injection of calabash curare in doses of 0.2–0.4 mg. (0.05–0.15 mg./kg.) into cats under chloralose anaesthesia resembled those produced by similar doses of *d*-tubocurarine injected by this route. Calabash curare produced hyper-reflexia, which was soon followed by seemingly " spontaneous " tonic and clonic movements. It also stimulated the respiration markedly, but the action on the blood pressure was generally not conspicuous.

Skeletal muscle reflexes.—Fig. 1 illustrates a representative experiment in which 0.2 mg. (0.055 mg./kg.) of calabash curare was injected intraventricularly. The knee jerk was immediately enhanced and a little later a shortening reaction was appended to it. After half a minute the height of the knee jerk had increased

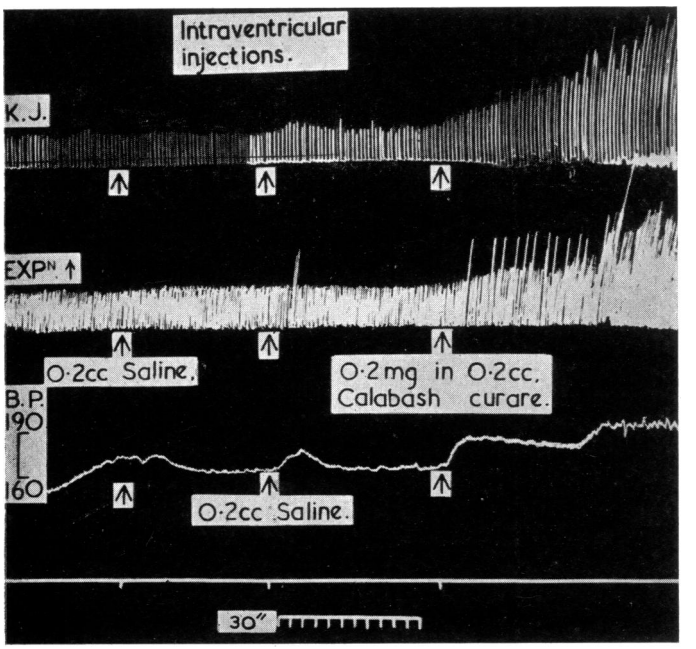


FIG. 1.—Cat, 3.6 kg., chloralose. Records from above downwards are: knee jerk; respiration (expiration upwards); carotid blood pressure; signal line; time in 30 sec. At first and second arrow, 0.2 c.c. saline injected intraventricularly. At third arrow 0.2 mg. of calabash curare in 0.2 c.c. saline injected intraventricularly.

fivefold. Within six minutes "spontaneous" movements appeared. As with *d*-tubocurarine, within one minute of the injection there was twitching of the ears and the facial muscles, followed by gross convulsive movements in the head, neck, and forelimbs. These convulsions spread quickly (in about one minute) to the hind limbs and gained in strength and frequency. In general the results were qualitatively indistinguishable from those which followed an injection of *d*-tubocurarine.

Respiration.—The effects of intraventricular injection of calabash curare were similar to those produced by *d*-tubocurarine. There was always a marked stimulation of respiration (Fig. 1). Sometimes the increase in rate preceded the increase in depth; at other times, the reverse occurred. This enhancement of respiration set in before there was any significant increase in general muscular activity and still occurred after both carotid sinus regions had been denervated and both vagi cut in order to eliminate the action of the chemoreceptors.

With the onset of generalized convulsions the respiratory movements became convulsive too. The spasm of the respiratory muscles, which was only occasionally observed after injection of *d*-tubocurarine, occurred more regularly with calabash curare.

Blood pressure.—The vasomotor centre was less intensely stimulated by calabash curare than by *d*-tubocurarine. Thus the rise of blood pressure was small in the experiment illustrated by Fig. 1; this slight rise followed immediately on the injection, and was sustained. When convulsions appeared, there was a further rise of blood pressure, which may be attributed to asphyxia, as it disappeared on applying artificial respiration. Additional factors which may contribute to the onset of asphyxia are tracheobronchial obstruction and pulmonary oedema (see below).

Glandular secretions.—The stimulation of salivary, lacrimal, and bronchial secretions was much more marked with calabash curare than with *d*-tubocurarine. In fact, in some of the fatal cases the trachea was found after death to be blocked with thick mucous secretion and the lungs were engorged with fluid; pressure on the abdomen in the intact animal led to the discharge of a large volume of watery fluid from the mouth.

Eye changes.—All the eye changes that occurred after an injection of *d*-tubocurarine were also produced by calabash curare. Thus there was a gradual increase in the size of the pupils; there was alternating contraction and dilatation of the pupils; the lids became retracted; finally the eyes became widely opened and there was full mydriasis.

It can be readily shown that the *immediate* effects on the spinal reflexes produced by intraventricular injection of calabash curare are due to an action on supraspinal centres. Thus similar changes in the hind-limb reflexes, both in magnitude and in time relationship, were obtained if the spinal theca was blocked in the midthoracic region to prevent the drug from reaching the lumbar cord via the cerebrospinal fluid (Salama and Wright, 1950). The findings in this respect are similar to those with *d*-tubocurarine. The absorption of calabash curare from the ventricles into the blood is slow as judged by the absence of any depressant effect on the response of the nerve-muscle preparation by the doses employed.

B. Effects produced by intrathecal injection.—In order to study the *direct* effect of calabash curare on the lower spinal centres a very tight ligature was tied round the meninges and spinal cord at the level of T6 so as to abolish transmission of all nervous impulses up and down the spinal cord as well as to block the upward flow of cerebrospinal fluid to the supraspinal levels (Salama and Wright, 1950); the drug was then injected intrathecally below the block. With this technique it was found that calabash curare had a *direct* excitant effect on the spinal centres. Spinal reflexes were, however, enhanced more rapidly and to a greater extent when calabash curare was applied directly to the supraspinal than when it was applied to the spinal centres.

The minimal dose of calabash curare needed to produce significant spinal excitation by a direct action on the cord was generally twice to four times as large as that needed when the drug was applied supraspinally. The same ratio held for the dose needed to produce maximal excitation of the cord. On supraspinal application the cord effects came on almost immediately; with spinal application there was a latent period of several minutes. The direct excitant action of calabash curare on the spinal cord was, however, considerably greater than that of *d*-tubocurarine.

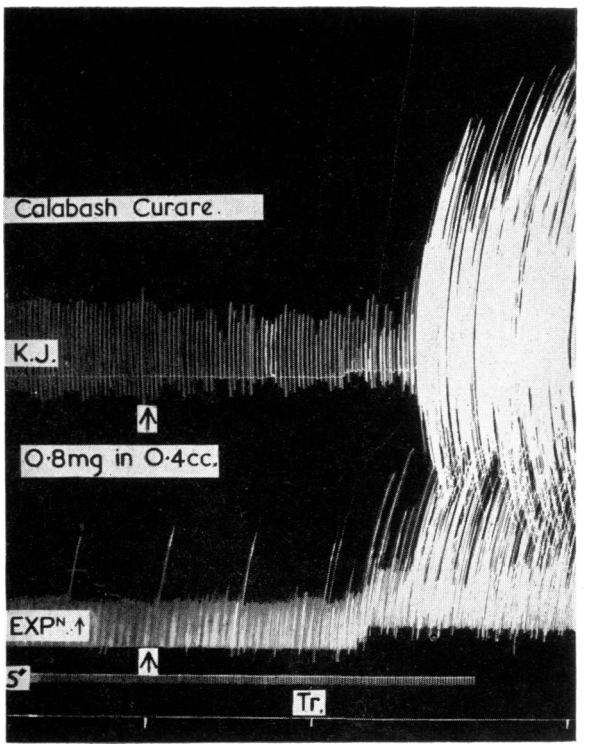


FIG. 2.—Cat, 2.5 kg., chloralose. Spinal cord transected and subarachnoid space blocked by tight ligature tied round meninges and spinal cord at level of T6. Records from above downwards are: knee jerk; respiration (expiration upwards); time in 5 sec.; signal line. At first arrow 0.8 mg. calabash curare in 0.4 c.c. saline injected intrathecally below the block. At Tr. a few drops of cerebrospinal fluid were allowed to escape in order to restore its pressure to normal.

The results of a representative experiment in which calabash curare was injected intrathecally below a complete spinal block at T6 were as follows: The dose (0.23 mg./kg.) injected was about twice as large as the average dose required to produce maximal reflex effects when given by the intraventricular route. Within three minutes of the injection, there was an increase in the knee jerk ; " spontaneous " movements appeared within 10 minutes. A response of similar magnitude only appeared as a rule after a delay of about one hour when a dose of *d*-tubocurarine three times the maximal intraventricular dose was injected intrathecally below a spinal block. After 25 minutes the convulsions were violent, but there was no change under these conditions in the blood pressure ; the eye changes and the secretory effects were also absent. Fig. 2 illustrates an experiment in which the direct excitant spinal action of calabash curare was exceptionally striking. Though the respiratory record in Fig. 2 seems to indicate that stimulation of respiration was taking place, careful inspection showed that the rhythmic respiratory movements were not changed either in rate or depth, but that the record was disturbed by the convulsive movements (owing to heightened spinal cord excitability) below the level of the block.

Action of l-*bebeerine and* d-*bebeerine*

The bebeerines had no central excitant or depressant actions when injected repeatedly intraventricularly or intrathecally in doses up to 10 mg./kg. body-weight. The peripheral curarizing action of *l*-bebeerine when tested on the isolated diaphragm preparation was less than 8 per cent that of *d*-tubocurarine.

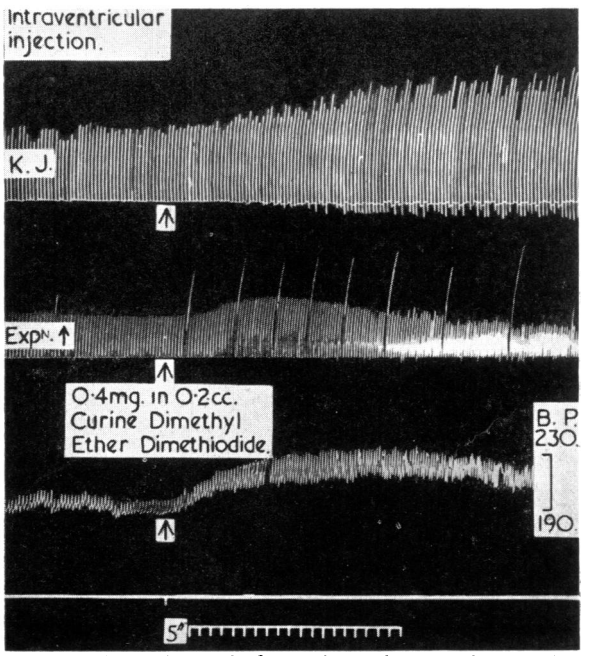


FIG. 3.—Cat, 5.5. kg., chloralose. Records from above downwards are: knee jerk; respiration (expiration upwards); blood pressure; signal line; time in 5 sec. At arrow 0.4 mg. curine dimethyl ether dimethiodide in 0.2 c.c. saline injected intraventricularly.

Action of curine dimethyl ether dimethiodide

When this curine compound was injected intraventricularly or intracisternally it had a central excitant action which was, however, weaker than that of *d*-tubocurarine or calabash curare. When injected intrathecally below a complete spinal block no central effects were produced. Its central excitant action is thus exerted solely on the supraspinal levels.

A. Intraventricular injection.—Fig. 3 illustrates the effects produced by intraventricular injection of doses of 0.4 mg. (0.07 mg./kg.) of this curine compound. Immediately after the injection, the knee jerk showed an increase in amplitude, but no " spontaneous " movements occurred during the first three minutes. A second dose of 0.4 mg. (Fig. 4A) produced a further increase in the knee jerk as well as " spontaneous " movements which were of small amplitude, but of high frequency.

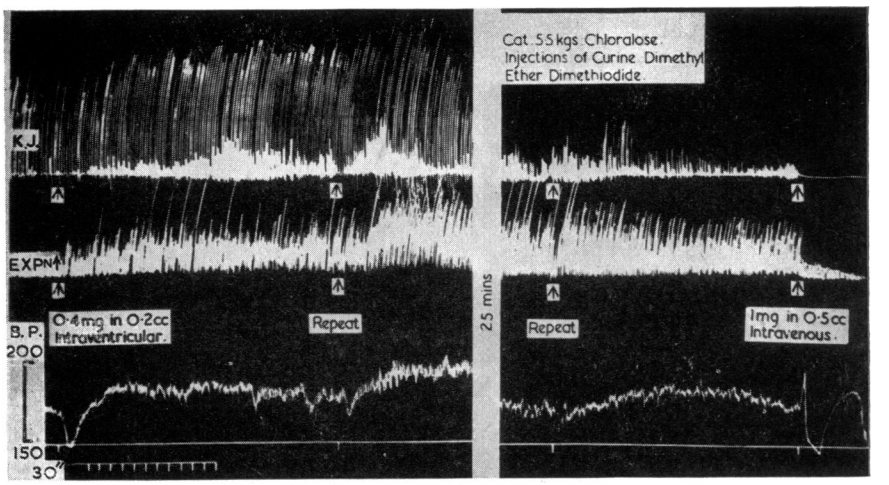


FIG. 4.—Cat, chloralose. Continuation of experiment in Fig. 3. (A) 3 min. after end of Fig. 3. At first and second arrows 0.4 mg. curine dimethyl ether dimethiodide injected intraventricularly. Pause of 25 min. between A and B. (B) At third arrow injection of 0.4 mg. curine dimethyl ether dimethiodide intraventricularly repeated. At fourth arrow 1 mg. of the same alkaloid injected intravenously.

After a few minutes, however, these " spontaneous " movements tended to die down, though the increase in the knee jerk persisted. A third injection of 0.4 mg. (Fig. 4A) produced another bout of " spontaneous " movements, which in their turn died down gradually over a period of 25 minutes. The knee jerk diminished as well. A fourth intraventricular injection (Fig. 4B) produced no response. An *intravenous* injection, however, of 1.0 mg. rapidly produced complete muscular paralysis (Fig. 4B).

Respiration.—After an initial considerable increase in depth (2.3 times), respiration soon decreased in amplitude but increased in rate. With further injections, however, the respiratory movements became involved in the general convulsions (Fig. 4A, B).

Blood pressure.—This showed a very slight and gradual increase, which was not sustained for more than one minute and then tended to decline gradually to its previous level.

B. Intrathecal injection.—2.0 Mg. doses of curine dimethyl dimethiodide were repeatedly injected intrathecally below a complete spinal block at the level of T6. There was no change in the reflexes, in the responses of the nerve-gastrocnemius preparation, in respiration or in blood pressure.

Action of the erythroidine alkaloids

The erythroidines depress spinal reflexes when large doses are employed.

Action of dihydro-β-erythroidine

Peripheral action.—The peripheral paralysing action of dihydro-β-erythroidine on intravenous injection in the cat (nerve-gastrocnemius preparation) or on the isolated rat nerve-diaphragm was approximately 30 per cent that of *d*-tubocurarine and about the same as that of curine dimethyl ether dimethiodide.

Central actions.—Injected intrathecally, intracisternally, or intraventricularly in doses of less than 20 mg. (about 7 mg./kg.) dihydro-β-erythroidine produced no

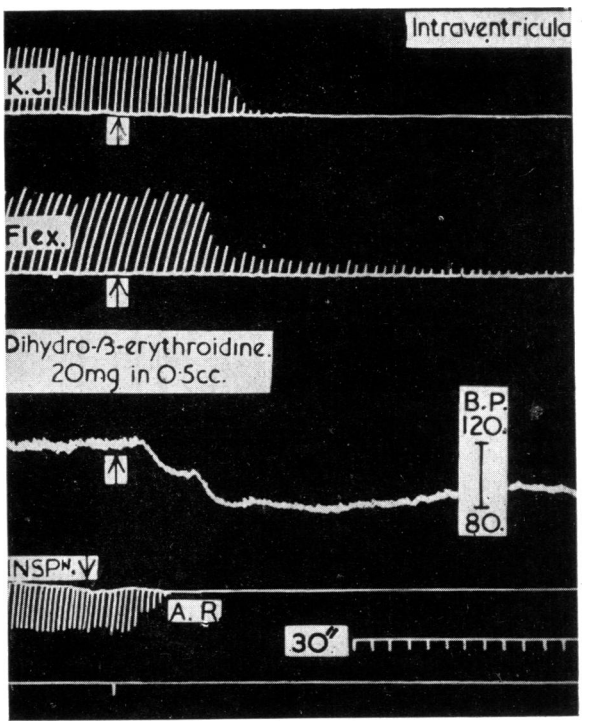


FIG. 5.—Cat, chloralose. Records from above downwards are: knee jerk (right side); flexor reflex (left side); carotid blood pressure; respiration; time in 30 sec.; signal line. At arrow 20 mg. dihydro-β-erythroidine in 0.5 c.c. saline injected intraventricularly. At A.R.: artificial respiration started.

effects. Two injections of 20 mg. intrathecally at intervals of a few minutes were likewise ineffective. Intracisternal or intraventricular injection of 20 mg., however, produced striking changes (Figs. 5 and 6). There was an immediate, moderate, but sustained fall of blood pressure, e.g., from 120 to 80 mm. Hg ; this fall is probably due to depression of the vasomotor centre. Breathing soon decreased in depth

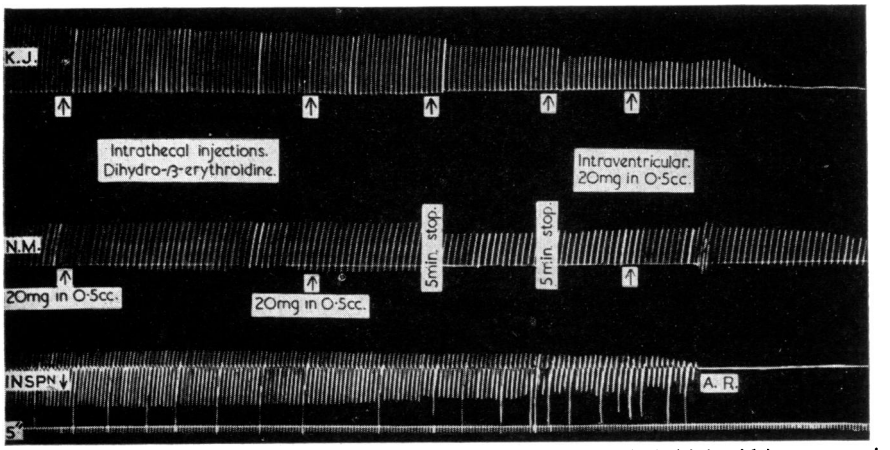


FIG. 6.—Cat, chloralose. Records from above downwards are: knee jerk (right side); contractions of gastrocnemius (left side) stimulated through its motor nerve; respiration; time in 5 sec. At the first and second arrows, 20 mg. dihydro-β-erythroidine injected intrathecally. At third arrow, 20 mg. dihydro-β-erythroidine injected intraventricularly. Drum stopped twice for periods of 5 min. At A.R.: start artificial respiration.

and finally stopped probably owing to depression of the respiratory centre. About two minutes after the injection, the spinal reflexes (knee jerk, flexor reflex) decreased rapidly in amplitude and disappeared after 3–5 minutes.

The depression of the spinal reflexes has been examined further. It is not due to asphyxia, as it occurs when the animal is maintained under artificial respiration. It is not due to ischaemia of the spinal cord, as in our experience a blood pressure level of 80 mm. Hg is adequate to maintain the normal excitability of the cord. To some extent it is due to dihydro-β-erythroidine being absorbed into the circulation and exerting a peripheral paralysing action on the muscles. The spinal reflexes were, however, depressed when the ischaemic hind-limb technique was employed in order to prevent the drug from reaching the muscles. The depression of the spinal reflexes was therefore mainly due to a central action. But, as already explained, though such depression was produced with supraspinal injection it did not occur with intrathecal injection. Dihydro-β-erythroidine must therefore produce its effects by an action on the supraspinal levels. This action may consist of depression of facilitatory neurones or stimulation of suppressor neurones. We have no evidence to enable us to decide between these two alternatives.

Action of β-erythroidine

Peripheral action.—The peripheral paralysing action of β-erythroidine is much smaller than that of its dihydro-derivative and is less than 10 per cent that of *d*-tubocurarine. Owing to shortage of material the number of experiments per-

formed was inadequate. It was found, however, that in 20 mg. doses β-erythroidine had a weak central depressant action, which was in part exerted on the spinal cord itself and partly on the supraspinal levels.

Discussion

The main results are summarized in the Table.

1. *Calabash curare.*—Calabash curare has a marked excitatory action on the central nervous system similar to that produced by *d*-tubocurarine except in the following respects:

(i) Intraventricular administration has a smaller excitatory action on the vasomotor centre.

(ii) The enhancement of salivary, lacrimal, and bronchial secretions is more pronounced.

(iii) The direct excitatory action on the spinal cord is produced by smaller doses and after a somewhat shorter latent period than with *d*-tubocurarine.

These quantitative differences, however, do not alter the fundamental fact that calabash curare, like *d*-tubocurarine, has a strong central excitatory action and that with both alkaloids the main central action is on the supraspinal levels. The central action of calabash curare, like that of *d*-tubocurarine, is in the opposite direction to the peripheral activity.

2. *The bebeerines* (*l*- and *d*-) show no significant peripheral or central actions. The relationship between bebeerine and *d*-tubocurarine has been mentioned earlier (page 459), and while the *lack* of central action of the former would seem to be related

Substance	Central action	Analysis of central actions			Relative potencies of peripheral action	
		Intra-thecal	Intra-cisternal	Intra-ventricular	Curarizing the diaphragm	Curarizing intravenous cat
d-Tubocurarine ..	Strong central excitant	+	+++	+++	100	100
Calabash curare ..	Strong central excitant	++	+++	+++	50	75
Curine dimethyl ether dimethiodide	Weak central excitant	0	+	+	30	30
l-Bebeerine Cl ..	No central action	0	0	0	<8	0
Dihydro-β-erythroidine	Very weak inhibition of reflexes	0	—	—	30	30
β-erythroidine ..	Very weak inhibition of reflexes	—	—	—	4–10	3–6

+ = Central excitant action. — = Central depressant action.

in some way to its tertiary nature and the *marked* activity of the latter to the presence of quaternary groupings, it is unwise to generalize in this connexion, in view of the many factors, known and as yet unknown, which should be taken into consideration. For example, direct comparison of the activity of bebeerine with *d*-tubocurarine will serve little to relate their physiological action to chemical structure, since stereochemical factors, now realized to be so important in drug-receptor relationships, differ in these alkaloids in a way not yet absolutely determined. It is also salutary to note that the *tertiary* erythroidines possess a peripheral action similar to that of *d*-tubocurarine (see below). Presumably, even the possession of a tertiary nitrogen atom in the *correct molecular environment* can generate a peripheral activity of this type, but to generalize on the basis of the few parameters known would be dangerous.

3. *Curine dimethyl ether dimethiodide.*—This quaternary compound is stereoisomeric with *d*-tubocurarine dimethyl ether, the former being laevorotatory. This curine compound has a central excitatory and a peripheral paralysing action like that of *d*-tubocurarine but weaker. As mentioned above the tertiary base curine (*l*-bebeerine) has no central or peripheral action. The central actions of *d*-tubocurarine dimethyl ether or of *l*-tubocurarine and its dimethyl ether have not yet been examined.

4. *The erythroidines.*—These compounds contain one tertiary nitrogen atom which is common to two rings but no *iso*quinoline grouping. They seem to constitute a distinct pharmacological group. They are obtained from a specific botanical source and are not found in crude curare preparations. Though they are tertiary bases, they display a peripheral paralysing action. Unlike the natural curare alkaloids, their quaternary derivatives are less active peripherally than the tertiary compounds. Therefore their mode of action peripherally may turn out to be different in some respects from that of curare. By their central action they depress the spinal reflexes. As these effects on the nervous system are only produced when the drugs are applied in very large doses they may be non-specific in character.

SUMMARY

1. The actions on the central nervous system of calabash curare, *l*- and *d*-bebeerine, curine dimethyl ether dimethiodide, β-erythroidine, and dihydro-β-erythroidine were studied in cats.

2. The quaternary curare alkaloids, *d*-tubocurarine and calabash curare, and the quaternary compound curarine dimethyl ether dimethiodide have a central excitant action and a peripheral paralysing action. The central and peripheral actions of curine dimethyl ether dimethiodide are weaker than those of *d*-tubocurarine and calabash curare. The central activity of calabash curare is approximately equal to that of *d*-tubocurarine ; it has, however, a greater direct stimulating action on the spinal cord centres.

3. The bebeerines, which are tertiary curare alkaloids, are inactive centrally and have a very feeble peripheral paralysing action.

4. The erythroidines, which are tertiary compounds, have considerable peripheral paralysing activity, no central excitant action, and a weak (possibly non-specific) central depressant action.

We are greatly indebted to Dr. S. J. Holt, of the Courtauld Institute of Biochemistry, for very useful discussions of the chemical questions considered in this paper.

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