Some examples of the effect of asymmetric nitrogen atoms on physiological activity / by P.P. Laidlaw.

No. 71

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BY

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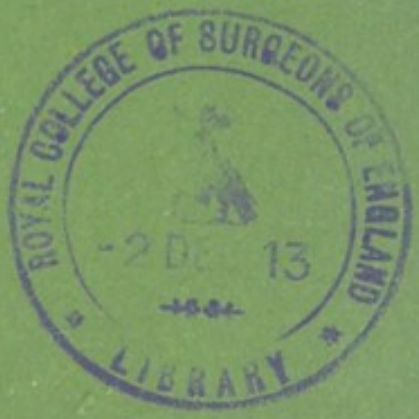

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SOME EXAMPLES OF THE EFFECT OF ASYMMETRIC NITROGEN ATOMS ON PHYSIOLOGICAL ACTIVITY

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The experiments of Cushny[1] and others have shown that optical isomers frequently have very different physiological activities. In most of the examples so far studied the isomerism has been due to the presence of an asymmetric carbon atom in the molecule. Hildebrandt,[2] however, records some examples of variations in activity due to asymmetric nitrogen atoms; working with quaternary coniine complexes he found that the β form was always more powerful than the α variety. The differences in activity observed by him were small (about 1 to 2), and from his examples it might be concluded that the asymmetric nitrogen atom did not have such a profound influence on activity as an asymmetric carbon atom. I have recently had the opportunity of investigating some further examples of asymmetric nitrogen compounds, and find that the difference in activity may be large.

Jowett and Pyman[3] recently isolated from the bark of Xanthoxylum brachyacanthum the chloride of an alkaloid, which they identified as a methochloride of *l*-canadine. They further found that, of the two *l*-canadine methochlorides which are possible, owing to the asymmetry of the nitrogen atom (Voss and Gadamer),[4] the salt from Xanthoxylum corresponded to the α

[1] Cushny, Jour. of Phys., xxx p. 176, 1904; Cushny and Peebles, Journ. of Phys. xxxli, p. 501, 1905; Cushny, Journ, of Phys., xxxviii, p 263, 1909.

[2] Hildebrandt, Arch. f. exper. Path. u. Pharm., liii, p. 76, 1905.

[3] Jowett and Pyman, Trans, Chem. Soc., ciii, p. 290, 1913.

[4] Voss and Gadamer, Arch. d. Pharm., ccxlviii, p. 43, 1910.

variety. This appears to be the first recorded case of the isolation from a natural source of a substance presenting this type of asymmetry: and, since pharmacological activity has been attributed to several species of Xanthoxylum, it seemed of interest to make a quantitative comparison between some characteristic action of this methochloride and that of the isomeric β variety, which can be obtained synthetically.

Owing to the presence of an asymmetric carbon atom in canadine a dextro-canadine also exists and, therefore, two more isomers are possible, viz, α and β dextro-canadine methochlorides. The ratio of activities of these was obtained indirectly through the α and β tetrahydroberberine methochlorides, which are α and β racemic canadine methochlorides. The possibilities are shown in the following scheme.

α *l*-canadine methochloride } α tetrahydroberberine methochloride
α *d*-canadine methochloride }

β *l*-canadine methochloride } β tetrahydroberberine methochloride
β *d*-canadine methochloride }

The four bodies actually compared were thus.

α *l*-canadine methochloride
β *l*-canadine methochloride
α *ld*-canadine methochloride
β *ld*-canadine methochloride

For the supply of these I am indebted to Dr. F. L. Pyman.

In common with other ammonium bases these alkaloids paralyse the motor endings in striped muscle, and it is this particular activity which has been studied; the other activities were not thoroughly investigated, and it is very probable that the ratios of activities obtained do not hold good for the other actions of these bases.

It was soon evident from a few preliminary experiments that the β salts were much more active than the α salts. Thus if a series of frogs is injected with diminishing doses of α and β canadine methochlorides, and the onset of "curare action" observed, a result like that in Table I is obtained, from which one

may say that β canadine methochloride is about ten times as active as the α salt.

TABLE I

Frogs' average weight 20 grams

β *l*-CANADINE METHOCHLORIDE		α *l*-CANADINE METHOCHLORIDE	
Dose	Effect	Dose	Effect
mgm.		*mgm.*	
4.0	Complete paralysis in 7 min.	4.0	Complete paralysis in 30 min.
2.5	Complete paralysis in 8 min.	2.5	Nearly complete paralysis in 45 min.
1.0	Complete paralysis in 10 min.	1.0	Partial paralysis in 45 min.
0.25	Nearly complete paralysis in 50 min.		
0.10	Partial paralysis in 50 min.		

It appeared unlikely that an accurate comparison could be made by this method. Variations in individual frogs, apart from weight, and the difficulty in gauging the degree of paralysis, make comparisons difficult. More accurate comparison is obtained by the following method. The hind legs of a frog were perfused with Ringer's solution through the aorta just above the bifurcation into the iliac arteries. One sciatic nerve was stimulated at intervals of ten seconds, with single maximal induction shocks, and the height of contraction of the gastrocnemius muscle was recorded. The opposite limb was then excluded from circulation by a small clip on the iliac artery and Ringer's solution containing a known dilution of alkaloid was perfused through the stimulated limb. The height of contractions slowly diminishes as perfusion is continued and a curve of the onset of paralysis for a given dilution is thus obtained. Plain Ringer's solution is then substituted to study the rate of recovery. The other leg is then treated in a precisely similar manner, except that a known dilution of the other isomer is perfused through it. In this way a fairly accurate comparison of activities is obtained (see fig. 1 and 2.) The perfusion pressure was kept high in order to exclude effects of differences in perfusion rate and was, of course, the same for right and left legs.

Some experiments were performed by perfusing Ringer's solution through a paralysed or partially paralysed limb, until recovery was about complete; but it was found that, when once a muscle had been poisoned by one isomer, it never recovered completely within the rather narrow time limits of a perfusion experiment; so that it remained more susceptible to the same, or opposite isomer, and paralysis could be easily obtained with weaker dilutions than that originally needed. This method was, therefore, abandoned and a comparison of one limb, one isomer, against opposite limb, opposite isomer, was adopted.

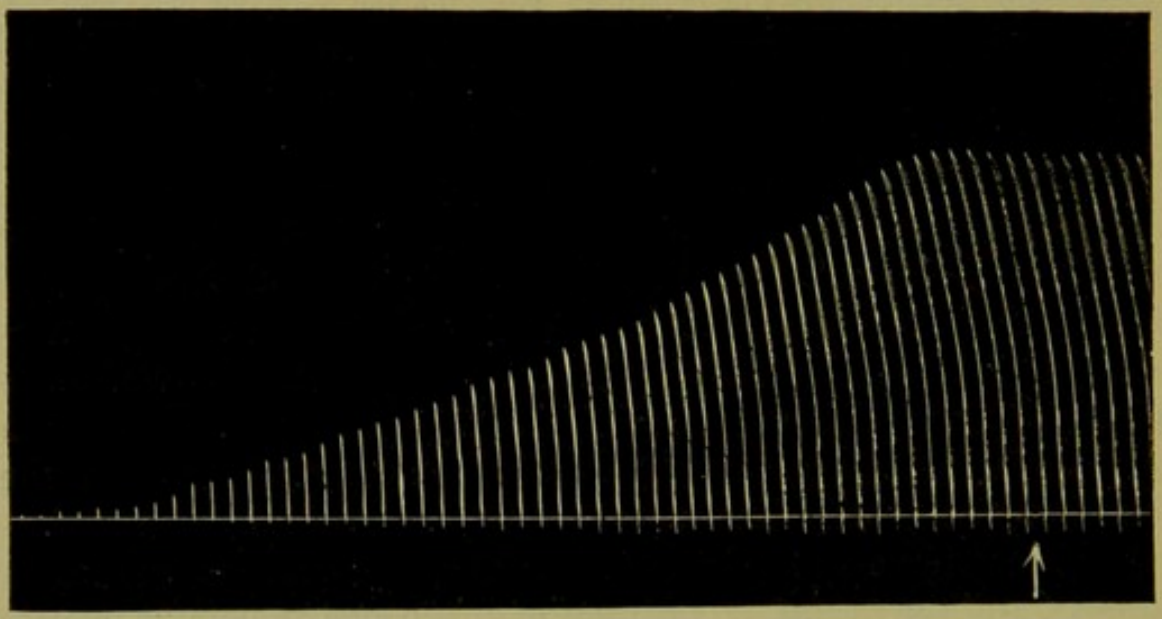

FIG. 1. EFFECT OF 1 IN 20,000 α TETRAHYDROBERBERINE METHOCHLORIDE

Figure 1 is a typical tracing of the effect of 1 in 20,000 α tetrahydroberberine methochloride; and figure 2 that of 1 in 20,000 β tetrahydroberberine methochloride. It will be observed that the α compound is much weaker than the β. By comparing weaker strengths of β tetrahydroberberine methochloride with 1 in 20,000 α on other frogs it was found that 1 in 80,000 β produced approximately an equal effect.

Figure 3 is a chart representation of two experiments. The ordinates represent the height of gastrocnemius contractions, the abscissa time in minutes of perfusion with the drug. The records with 1 in 50,000 α racemic canadine methochloride and 1 in

FIG. 2. EFFECT OF 1 IN 20,000 β TETRAHYDROBERBERINE METHOCHLORIDE

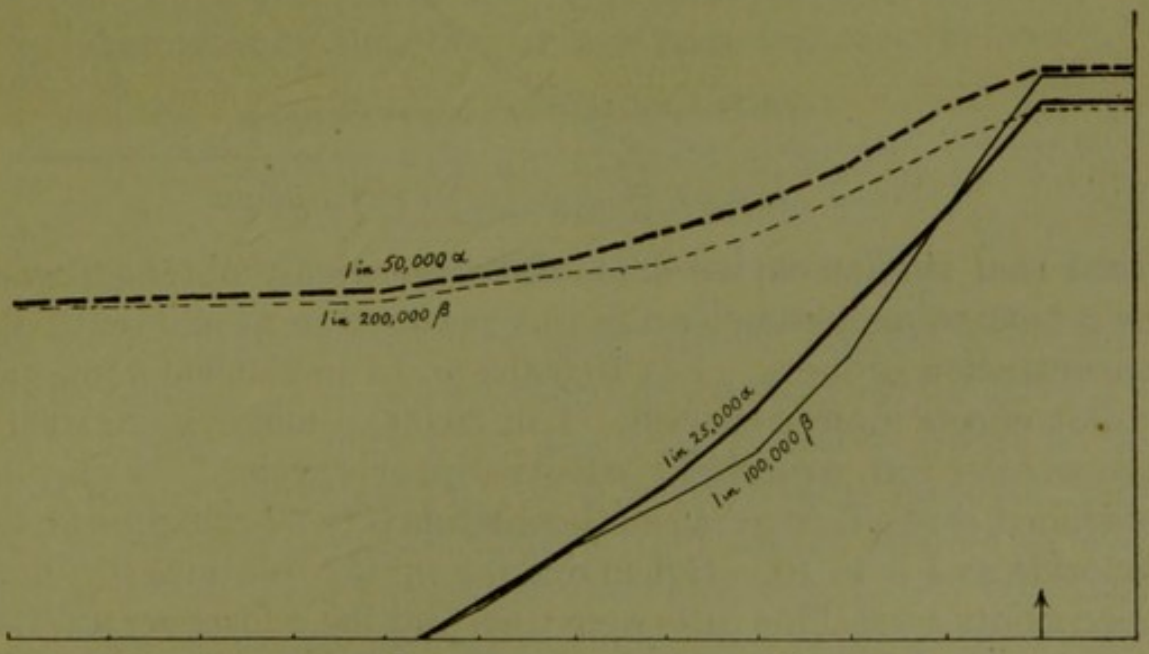

FIG. 3. α AND β TETRAHYDROBERBERINE METHOCHLORIDES

200,000 β racemic canadine methochloride (upper curves) show that, with these concentrations, the rate of onset of paralysis is very slow and not complete yet the two effects appear to be equal. The two lower curves are from another experiment where the concentrations of the alkaloids in the perfusion fluids were doubled, and it is evident that the curves are again closely similar. It may be concluded that α racemic canadine methochloride is only one-fourth as powerful as the β form.

Figure 4 is another chart representation of two experiments with α and β *l*-canadine methochlorides. In this case it was

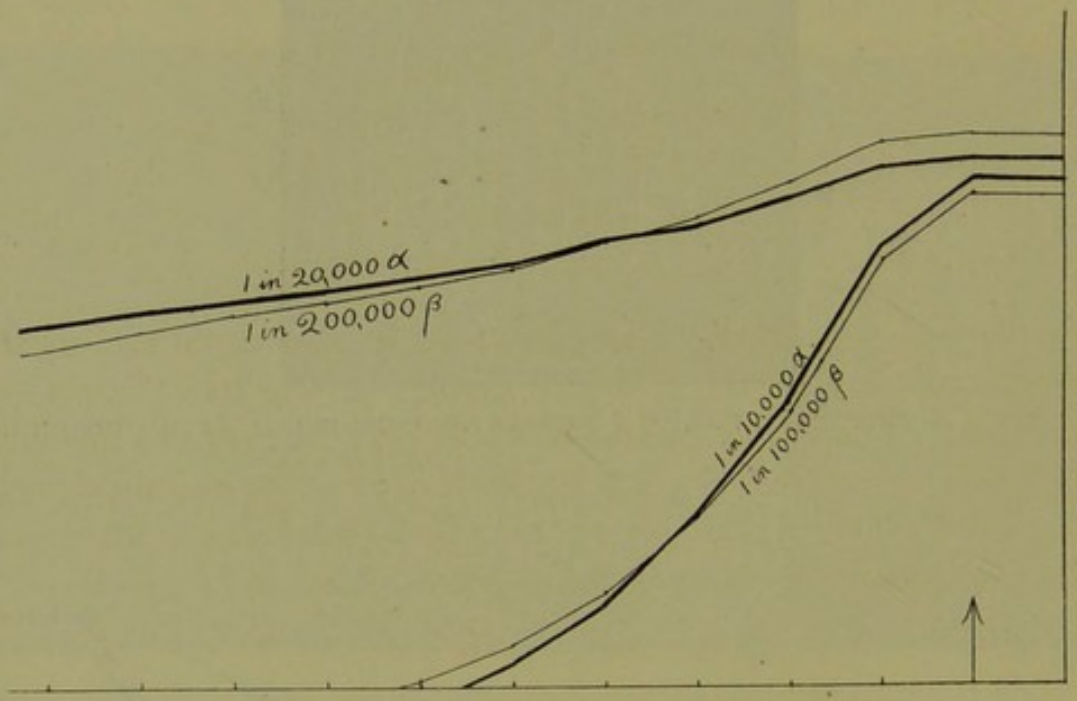

FIG. 4. α AND β *l*-CANADINE METHOCHLORIDES

found that similar curves of paralysis were only obtained when the β *l*-canadine methochloride was perfused in at one-tenth the concentration of the α. 1 in 10,000 α and 1 in 100,000 β produce similar effects (lower curves). 1 in 20,000 α and 1 in 200,000 β give smaller but equivalent effects (upper curves). It may be concluded that α *l*-canadine methochloride is to β *l*-canadine methochloride as 1 is to 10. But in making up the solutions for these experiments crystalline salts were used and the β form crystallises with six molecules of water of crystallisation and the α with only one. Correcting for this error, the true ratio is obtained of about 1 : 12 instead of 1 : 10.

In a precisely similar way the ratio of activity of α *l*-canadine methochloride to α *dl*-canadine methochloride was found to be 1 to 5.

The ratio of the activity of α dextro-canadine methochloride to that of β dextro-canadine methochloride can easily be deduced from these data.

The previous experiments show that

(1) $\alpha l : \beta l :: 1 : 12$, or $12\,\alpha l = \beta l$.
(2) $\alpha l : (\frac{1}{2}\,\alpha l + \frac{1}{2}\,\alpha d) :: 1 : 5$ or $9\,\alpha l = \alpha d$.
(3) $(\alpha l + \alpha d) : (\beta l + \beta d) :: 1 : 4$, or $4\,\alpha l + 4\,\alpha d = \beta l + \beta d$.

Substituting αl where possible in the last equation

$$\begin{aligned} 4\,\alpha l + 36\,\alpha l &= 12\,\alpha l + \beta d \\ 28\,\alpha l &= \beta d \\ \therefore \alpha d : \beta d &:: 9\alpha l : 28\,\alpha l \\ \text{or } 1 &: 3 \text{ approximately.} \end{aligned}$$

The asymmetry of the nitrogen atom in the canadine methochlorides produces very different results in the two closely related *d*- and *l*-canadine methochlorides. In laevo-canadine methochloride the ratio is 1 : 12, in dextro-canadine methochloride it is 1:3. Further if the activity of α laevo canadine methochloride be taken as unity the other isomers have values as follows.

α laevo canadine methochloride, 1
α dextro canadine methochloride, 9
β laevo canadine methochloride, 12
β dextro canadine methochloride, 28

At present no explanation of these various degrees of activity is forth-coming. The dextro canadine methochlorides have not been available in a pure state, and their properties must be included in any generalisation. It is worth noting, however, that α laevo canadine methochloride is very easily soluble in cold water and that β laevo canadine methochloride is only sparingly soluble; also it crystallises with six molecules of water of crystallisation, as compared with one in the α form. Some solubility differences might account for the different activities, but it must

be borne in mind that the solubility in water or saline is only one factor, and the solubility in the tissue of the myoneural junction is equally, if not more important. There does not seem to be any method available for testing the partition coefficient for these substances between circulating fluid and the junctional tissues. So that we may leave the speculation on one side until some method is devised for testing the theory.

ADDENDUM

A simple apparatus for excluding make shocks

In cases where stimulation at regular intervals is required, it is convenient to use some form of clock which makes and breaks at regular intervals the primary current of an induction coil. In this series of experiments the Palmer clock, which is designed to act as a time marker, was employed. The make shock was excluded from the stimulations by the following simple device.

A lever with unequal arms was mounted so that the short arm, carrying a piece of soft iron, could be drawn downwards by means of an electro magnet. The long arm was fitted with a light metal bar from which depended two platinum pins. These, in the position of rest, dip into two mercury pots insulated from each other. Each pot is connected with one terminal of the secondary coil used for stimulation, and the primary circuit includes battery, clock, electro magnet of apparatus and primary coil. At make of primary, the secondary coil is short circuited through mercury pots and platinum pins carried by the lever. It thus fails to stimulate. Almost immediately this short circuit is broken by the electro magnet raising the platinum pins out of the mercury cups, so that, on break of primary, the break induced shock passes through the stimulating electrodes before the platinum pins drop back into mercury cups, and so short circuit the secondary again (see diagram). The smooth working of the apparatus depends, apart from cleanness of all contacts, on the degree of dip of the platinum pins into the mercury. This must be sufficient to exclude all the make shock, and the pins must be

raised sufficiently high from the mercury to allow time for the break shock to pass before the secondary is short-circuited again.

It is evident that break shocks could be excluded, and make shocks employed to stimulate, by reversing the position of the mercury cups to the other end of the lever.

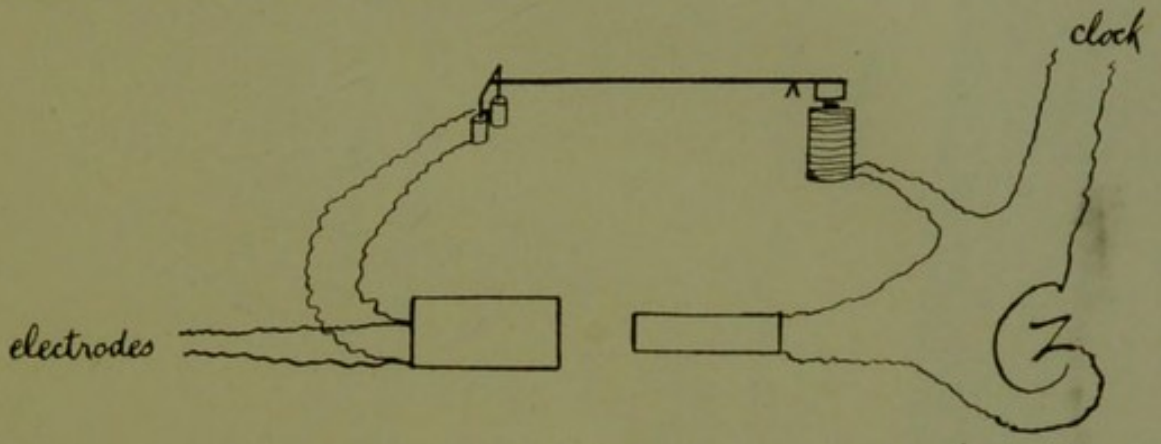

FIG. 5. DIAGRAM OF APPARATUS AND CONNECTIONS FOR OBTAINING SINGLE INDUCED BREAK SHOCKS BY MEANS OF CLOCK AND INDUCTION COIL

SUMMARY

1. An asymmetric nitrogen atom may exercise a profound influence on physiological activity.

2. In the case of the *l*-canadine methochlorides the β salt is 12 times as active as the α isomer.

3. In the case of the *d*-canadine methochlorides the β variety is only about 3 times as active as the α form.

4. In both cases the laevorotary base is weaker than the dextrorotary but not in equal degree.

5. No explanation of these variations in activity of the four isomers is satisfactory. It may possibly be connected with differences in solubility.

6. A simple apparatus is described for the exclusion of make shocks, when employing a clock and induction coil for single stimuli at regular time intervals.