PHARMACOLOGICAL STUDIES IN THE APOCYNACEOUS GENUS ASPIDOSPERMA MART. & ZUCC. *ASPIDOSPERMA OBLONGUM* A.DC.

BY J. N. BANERJEE*† and J. J. LEWIS*

From the Pharmacology Laboratories, Pharmacy Department, University of Nottingham

Received December 17, 1953

THE Aspidosperma species are South and Central American trees varying in height from 2 to 60 metres and growing in various habitats. They have received a certain amount of attention from taxonomists (Markgraf[1–4], Pichon[5], Woodson[6], Milanez[7], Standley[8], Lundell[9] and Kuhlmann[10]). The timbers are dealt with by Record and Hess[11], Record and Mell[12] and Danks[13]. In general, the chemistry and pharmacology of the constituents of the plant organs have been neglected. Aspidospermine and aspidosamine were isolated from *A. quirandy* Hassler by Floriani[14] and their pharmacological actions investigated. Becker[15] found extracts of the bark of *A. nitidum* Benth. inactive against *Plasmodium lophuræ* infection in the white Pekin duck. Floriani[16–18] and Wasicky *et al.*[19] studied the chemistry and the pharmacological and chemotherapeutic actions of the alkaloids of the bark of *A. polyneuron* Muell. Arg. Rothlin[20] and Planchon *et al.*[21] have investigated *A. peroba* F. Allem. Orazi[22] has isolated aspidospermine from *A. australe* Muell. Arg. *A. quebracho-blanco* Schlecht, is the best known and most studied species and yields aspidospermine and yohimbine (quebrachine)[23–32]. Galenical preparations from the bark of this species have been used as antimalarials, febrifuges and as respiratory stimulants. For the early literature on preparations containing the quebracho alkaloids, Cow[31] should be consulted. Froude, as quoted by Waugh and Abbott[32], used aspidospermine in typhoid fever as an antipyretic and respiratory stimulant. Schiffler (quoted by Waugh and Abbott[32]) found quebracho bark extract caused muscular weakness and paralysis in a rabbit together with hyporeflexia and narcosis. Death was preceded by convulsions. Since potent pharmacologically active substances have been isolated from various Apocynaceæ, we felt it worth while to report the continuation of a systematic study[33] of the alkaloids of *A. oblongum* A.DC.

Materials. The total alkaloids were extracted from the powdered bark by classical methods; the mixed hydrochlorides of these were obtained by further purification as a white well-crystalline solid. These were used in the experiments as a neutral 1 per cent. solution in the appropriate saline solution.

Methods and Results. There was a progressive decline in the response to stimulation of the sciatic nerve of the frog gastrocnemius-sciatic nerve preparation, when the solution was applied to the muscle or nerve.

* Present address: Department of Materia Medica and Therapeutics, University of Glasgow.

† Nottingham University Research Scholar.

Depression was more marked when the solution was applied to the former. Inhibition in both cases progressed to completion; the effect upon the nerve was irreversible, although the muscle still responded to direct stimulation. Addition of 2 ml. of 1 in 10^4 eserine salicylate to a depressed preparation resulted in a temporary recovery.

In the frog rectus abdominis preparation the addition of 0·18 to 0·2 mg. of the alkaloids to a 10-ml. bath containing 0·1 ml. of 1 in 10^4 of acetylcholine chloride caused inhibition (Fig. 1).

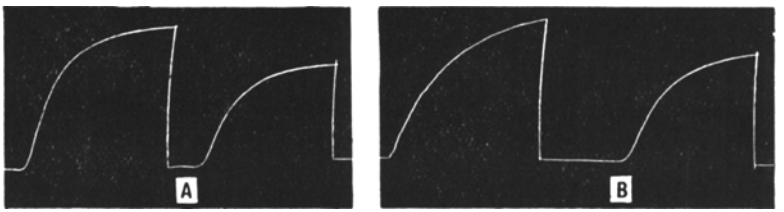

FIG. 1. Antagonism by the oblongum alkaloid hydrochlorides of the action of 10 μg. of acetylcholine on the frog rectus abdominis muscle preparation in a 10-ml. bath. Dose of acetylcholine preceded 3 minutes earlier at: A. by 0·2 mg.; B. by 0·18 mg. of alkaloids.

In preparations of the small intestine of the rat, guinea-pig and rabbit, the response to a standard submaximal dose of acetylcholine chloride was inhibited (Fig. 2). Similarly, there was inhibition of the stimulant action of 5 mg. barium chloride. The stimulant action of submaximal doses of histamine upon the guinea-pig small intestine was also inhibited. Normal peristaltic movements and tone were reduced in all three cases, rabbit intestine showing marked relaxation. In all instances the degree of inhibition was proportional to the dose of drug administered.

The virgin guinea-pig uterus was stimulated by 1 mg. of the drug. In rat and rabbit uterus the amplitude of the normal rhythmic contractions was reduced by 2 mg. of total alkaloid hydrochloride, and the stimulant actions of 10 μg. of acetylcholine chloride and 5 mg. of barium chloride on the rat uterus were inhibited. No modification of the relaxant action of 10 μg. of adrenaline could be demonstrated in the rat uterus preparation by a bath concentration of 1 in 25,000 of the alkaloids, but in the rabbit uterus its spasmogenic action was inhibited by 3 mg. of the drug.

The rate, amplitude and tone of the frog heart *in situ* were gradually decreased by application to the surface of the exposed heart of 0·06 to 0·6 mg. of the drug in solution; this effect was not modified by prior atropinisation (10 μg. atropine) and after application of the alkaloids to the heart, stimulation of the vagus did not produce its characteristic effects. Similar effects were noted with the isolated frog's heart perfused through the aorta or through the sinus venosus with normal Ringer's solution containing 1 in 200,000 of the drug, or with "half-calcium" Ringer's solution containing 1 in 200,000 of the drug.

The rate and amplitude of Langendorff preparations of the rat, guinea-pig, rabbit and kitten heart were reduced by perfusion with a 1 in 200,000

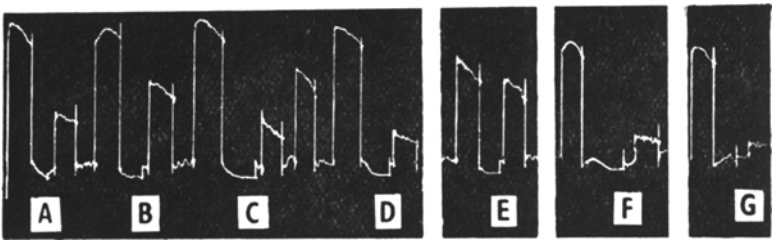

FIG. 2A. Antagonism by the oblongum alkaloids of the action of 2 μg. of acetylcholine on the isolated rat intestine in a 50-ml. bath. Dose of acetyl choline preceded 3 minutes earlier at: A. by 0·5 mg.; B. by 0·2 mg.; C. by 0·8 mg.; D. by 1·1 mg.; E. by 0·1 mg.; F. by 1·6 mg.; G. by 2·2 mg. of alkaloids.

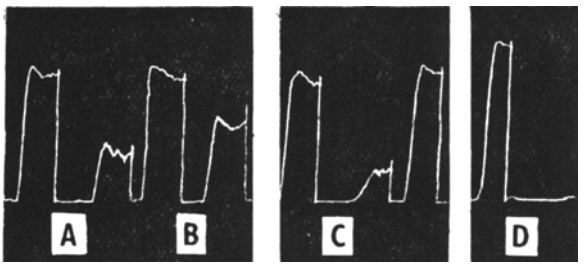

FIG. 2B. Antagonism by the oblongum alkaloids of the action of 5 μg. of acetylcholine on the guinea-pig ileum in a 50-ml. bath. Dose of acetylcholine preceded 3 minutes earlier at: A. by 0·5 mg.; B. by 0·3 mg.; C. by 0·7 mg; D. by 0·9 mg. of alkaloids.

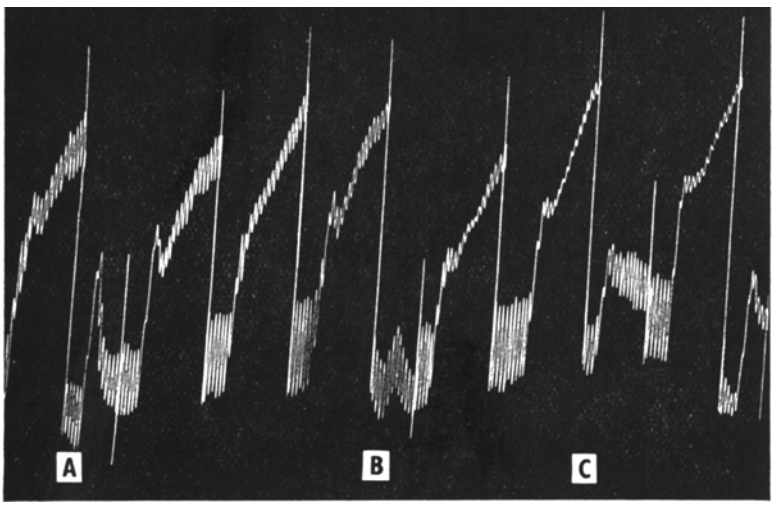

FIG. 2C. Antagonism by the oblongum alkaloids of the action of 1 μg. of acetylcholine on the rabbit small intestine in a 5-ml. bath. Dose of acetylcholine preceded 90 seconds earlier at: A. by 0·5 mg.; B. by 1·5 mg.; C. by 1·0 mg. of alkaloids.

solution of drug in Ringer–Locke fluid. After continued perfusion, the ventricles ceased to follow the auricles and partial auricular-ventricular block developed. The block became more complete as perfusion continued, but was reversible. Similar, but more dramatic, results followed injection of stronger solutions of the drug directly into the cannula. Thus a quantity of solution containing 3 mg. of alkaloid stopped the heart within 30 seconds and a typical auricular-ventricular blocking effect was seen during the period of recovery. Outflow from the heart decreased after administration of the drug.

In the electrocardiogram of the rabbit and rat, doses which caused marked slowing of the heart were seen to reduce the amplitude of the P wave, to invert the QRS wave and to prolong and increase the amplitude of the T wave. The PT interval was almost doubled.

In the isolated rabbit-auricle preparation suspended in oxygenated Ringer–Locke solution at 29° C., the depressant effect of acetylcholine chloride was reversibly inhibited. 2 μg. of acetylcholine chloride were added to a 50-ml. bath at 10-minute intervals, until a constant level of inhibition was obtained. The duration of contact with the auricular tissues was 2 minutes. 2 mg. of the alkaloids were then added to the bath followed in 1 minute by 2 μg. of acetylcholine chloride. Further additions of acetylcholine chloride were made until recovery.

The broncho-constrictor actions of acetylcholine and histamine in the *in vivo* guinea-pig lung preparation[34] were antagonised by intravenous administration of the drug. Guinea-pigs were anæsthetised by intraperitoneal injection of a 25 per cent. solution of urethane at a dose level of 1·8 g./kg. The trachea was cannulated and the cannula connected to a Starling respiration pump. The ventilation was 10 to 12 ml. per minute. Drug solutions were administered via the jugular vein which was cannulated. The bronchoconstrictions produced by two successive doses of 20 μg. of acetylcholine chloride and by two successive doses of 2 μg. of histamine acid phosphate were recorded, 8 minutes elapsing between each administration. The alkaloids were then injected into the jugular vein and the bronchospasm produced by subsequent doses of 20 μg. of acetylcholine chloride and 2 μg. of histamine acid phosphate observed. 25 mg./kg. of alkaloid antagonised to an equal extent both acetylcholine chloride and histamine acid phosphate at the above dose levels. The antagonism was not apparently specific since both drugs were antagonised to an equal extent.

When infused into the abdominal aorta of the perfused rat hind legs, preparation at room temperature 0·6 mg. of the drug reversed the constrictor actions of both adrenaline (5 to 10 μg.) and acetylcholine chloride (5 μg.). The drug solution itself caused marked vasoconstriction.

In the cat, anæsthetised with ether and chloralose, administration of small doses of drug (1 to 10 mg.) into the jugular vein caused no noticeable effects upon depth or frequency of respiration; larger doses (20 mg.) caused respiratory paralysis. Intravenous administration of the drug caused a sharp fall in blood pressure, the fall being proportional to the dose given. In some animals larger doses caused a relatively prolonged

period of low blood pressure. In others the fall, though well marked, was transient. Antagonism to histamine and acetylcholine was not demonstrated. On the other hand there was well marked antagonism to adrenaline and reversal of the pressor action of this drug. It was noted that there was no recovery to the original level of response to adrenaline (Fig. 3).

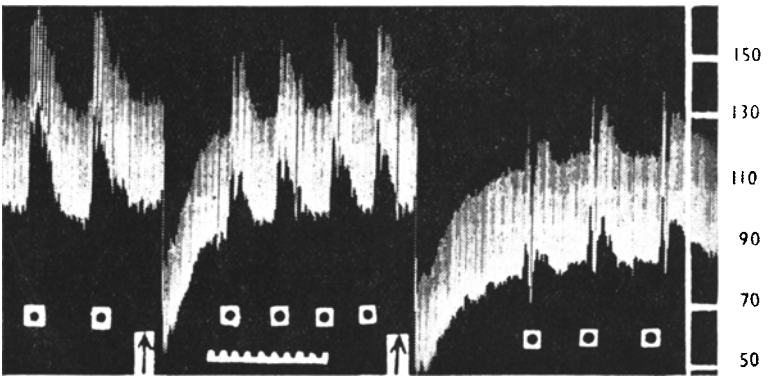

FIG. 3. Reversal of the pressor response to adrenaline on the blood pressure of the chloralose cat.
10 μg. of adrenaline was given at the points marked by dots and 5 mg. of oblongum alkaloids at the points marked by arrows. Time base, 10 seconds.

Injection of 3 mg. of the drug into the ventral lymph sac of frogs caused miosis, muscular weakness, loss of reflexes and then paralysis; death occurred in from 40 to 70 minutes. 6 mg. produced similar but exaggerated effects and death took place within 50 minutes. Convulsions were not seen. In mice, doses of 18 to 30 mg. by mouth or by subcutaneous injection caused the animals to become quiet and sleepy. 40 mg. caused clonic convulsions followed by death. Similar effects were seen in rats.

As would be expected from the findings of Dutta[35], intraperitoneal injection into mice, weighing 23 to 24 g., of 3 mg. of these alkaloidal hydrochlorides caused a fall in body temperature (as compared with controls) amounting to 5° F.—the body temperature not returning to normal for 2½ hours.

When tested by the method of Sollmann[36] as modified by Bülbring and Wajda[37], a graded local anæsthetic activity was found. To rule out the possibility of a general toxic effect upon nervous tissues or of a neuromuscular block, immediately after sensory paralysis to the highest concentration of acid, the sciatic nerve was exposed and stimulated, when the gastrocnemius muscle was found to respond normally.

12 mg. of the drug showed no antimalarial activity when tested by the method of Rollo[38] against *Plasmodium berghei* in mice weighing 20 g. When tested *in vitro*, against *Entamœba histolytica*, a 1 in 20,000 solution of the drug was approximately as effective as a 1 in 800,000 solution of emetine hydrochloride.

The drug completely protected mice against the effects of lethal doses of adrenaline[39]—3 mg. of alkaloid protecting against 0·28 mg. adrenaline hydrochloride.

Discussion

The Apocynaceæ have yielded a number of pharmacologically interesting substances, including the glycosides of *Strophanthus* spp., aspidospermine from *Vallesia glabra*, etc., conessine from *Holarrehna antidysenterica*. Lately much interest has centred upon *Rauwolfia serpentina*, which yields alkaloids with hypotensive actions. Yohimbine and aspidospermine are found in the genus *Aspidosperma*. Extracts of the barks and other plant organs of *Aspidosperma* spp. are still used in folk medicine in South and Central America against fevers, rheumatism, liver disorders, dysentery and toothache, whilst during the 19th century extracts of the bark of *Aspidosperma quebracho-blanco* were used in orthodox European medicine in the treatment of asthma and dyspnœa. More recently, Dominguez[40] has noted the treatment of malaria with aspidospermicine-containing extracts of *Aspidosperma quebracho-blanco f. pendulæ*.

The alkaloids of *Aspidosperma oblongum* produce hypotension and markedly reverse the pressor effects of adrenaline in the cat, this action being quite prolonged. The protection of mice against lethal doses of adrenaline is also worthy of note as is the reversal of the vasoconstrictor effects of adrenaline in the rat hind legs preparation. Antagonism to the spasmogenic action of adrenaline in the rabbit uterus was also seen.

Acetylcholine antagonism was shown on the frog rectus abdominis, the gut and uterus, the isolated auricles and the *in vivo* guinea-pig bronchi. As pointed out by de Elio[41] acetylcholine antagonists may show local anæsthetic activity and we were able to demonstrate this with our preparation. The point made by Stephenson[42] that other vegetable alkaloids might show properties similar to those of conessine is of interest here. Specific antagonism to any one of the spasmogenics used was not shown.

Summary

1. The bark of *Aspidosperma oblongum* A.DC. contains alkaloids which antagonise the actions of acetylcholine, histamine, barium and adrenaline, and reverse the actions of the latter.

2. The alkaloids markedly depress the isolated heart's action and change the electrocardiogram pattern.

We thank Mr. D. B. Fanshawe, Conservator of Forests, British Guiana for the bark of *Aspidosperma oblongum*; Dr. L. G. Goodwin for supplying us with a strain of *Plasmodium berghei* and for advice; Major J. Buchanan of Messrs. Cossor Ltd., for advice on the use of the Cossor 1314 E.C.G., and Dr. F. Hawking, Miss G. M. Harmer and Mr. E. Wilmhurst for help and advice. We are especially indebted to Mr. G. E. Trease, Director of Pharmaceutical Studies, University of Nottingham, who aroused our interest in the work.

REFERENCES

1. Markgraf, *Notizbl. bot. Gart. Berl.*, 1935, **12**, 553.
2. Markgraf, *ibid.*, 1935, **12**, 295.
3. Markgraf, *ibid.*, 1937, **13**, 464.
4. Markgraf, *ibid.*, 1938, **14**, 128.
5. Pichon, *Bull. Mus. Nat. d'Hist. Nat. ser.* 2, 1947, **19**, 362.
6. Woodson, *Annal. Missouri Bot. Gard.*, 1951, **38**, 119.
7. Milanez, *Physis., Paris*, 1939, **15**, 429.
8. Standley, *Tropical Woods*, 1933, **36**, 13.
9. Lundell, *Phytologia*, 1939, **1**, 337.
10. Kuhlmann, *Ann. Reun. sul-amer. Bot.*, 1938, **3**, 75.
11. Record and Hess, *Timbers of the New World*, Yale University Press, 1943, p. 107.
12. Record and Mell, *Timbers of Tropical America*, Yale University Press, 1924, p. 507.
13. Danks, *Notes on the British Guiana Timbers*, Forest Dept., British Guiana, 1945, p. 22 to 23.
14. Floriani, *Rev. Cent. Estud. farm. bioquim.*, 1935, **25**, 375, 723.
15. Becker, *Iowa St. Coll. J. Sci.*, 1949, **23**, 189.
16. Floriani, *Rev. farm., B. Aires*, 1938, **80**, 47, 135.
17. Floriani, *Ann. Farm. Bioquim.*, 1930, **1**, 135.
18. Floriani, *Rev. farm., B. Aires*, 1932, **72**, 70.
19. Wasicky, Unti and Barbieri, *Ann. Fac. Farm. Odontol., Univ. Sao Paulo*, 1942–43, **3**, 137.
20. Rothlin, *Bull. Sci. pharm.*, 1921, **28**, 54.
21. Planchon, Bretin and Manceau, *Precis de Matiere Medical*, 5th Ed., Vol. 2, Librairie Maloine, Paris, 1946, pp. 1644 to 1645.
22. Orazi, *An. Asoc. quim. argent.*, 1946, **34**, 158.
23. Fraude, *Ber. dtsch. chem. Ges.*, 1878, **11**, 2189.
24. Hesse, *Leibigs Annalen*, 1882, **211**, 249.
25. Fourneau and Page, *Bull. Sci. pharm.*, 1914, **21**, 7.
26. Labriola, *An. Asoc. quim. argent.*, 1938, **27**, 150.
27. Fraude, *Ber. dtsch. chem. Ges.*, 1879, **12**, 1560.
28. Ewins, *J. chem. Soc.*, 1914, **105**, 2738.
29. Bizet and Hamet, *C.R. Acad. Sci., Paris*, 1937, **204**, 1754.
30. Penzoldt, *Klin. Wschr.*, 1879, **16**, 279.
31. Cow, *J. Pharmacol.*, 1913–14, **5**, 341.
32. Waugh and Abbott, *A Textbook of Alkaloidal Therapeutics*, Clinic Publishing House, Chicago, 1904.
33. Banerjee and Lewis, *Nature, Lond.*, 1953, **171**, 802.
34. Konzett and Rössler, *Arch. exp. Path. Pharmak.*, 1940, **195**, 71.
35. Dutta, *Brit. J. Pharmacol.*, 1948, **3**, 246.
36. Sollmann, *J. Pharmacol.*, 1918, **11**, 1.
37. Bülbring and Wajda, *ibid.*, 1945, **85**, 78.
38. Rollo, *Trans. R. Soc. trop. Med. Hyg.*, 1952, **5**, 567.
39. Loew and Micetich, *J. Pharmacol.*, 1948, **93**, 434.
40. Dominguez, *Rev. farm., B. Aires*, 1932, **73**, 82.
41. de Elio, *Brit. J. Pharmacol.*, 1948, **3**, 108.
42. Stephenson, *ibid.*, 1948, **3**, 237.