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MECHANISM OF NEUROTOXICITY OF CARDIOTONIC GLYCOSIDES

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1 In cats intracerebroventricular administration of 5, 10, 20 μg of peruvoside, a cardiac glycoside obtained from the plant, *Thevetia neriifolia*, and 10 and 20 μg of ouabain, produced marked neurotoxicity. This was dose-related.

2 Prior administration of reserpine (2 mg/kg i.m., 500 μg i.c.v.) or tetrabenazine (25 mg/kg i.v., 50 mg/kg i.v. and 2 mg/kg i.c.v.) suppressed the neurotoxicity, but lithium carbonate (100 mg/kg i.p., 2 mg i.c.v.) and haloperidol (200 μg i.c.v.) were ineffective.

3 Prior administration of 2-bromolysergic acid diethylamide (BOL-148, 200 μg i.c.v.) or *p*-chlorophenylalanine (PCPA) (400 mg/kg i.p.) suppressed the neurotoxicity induced by peruvoside and ouabain.

4 Perfusion of the lateral ventricles of cats with 10, 20 and 30 μg of peruvoside or ouabain produced a massive release of 5-hydroxytryptamine (5-HT). This was dose-related. Prior administration PCPA suppressed the release of 5-HT.

5 The results of the findings indicate the involvement of 5-HT in the genesis of neurotoxicity induced by peruvoside or ouabain.

Introduction

Cardiac glycosides have been reported to produce effects referable to the central nervous system. Gaitondé, McCarthy & Borison (1965), while studying the central emetic mechanism of digitalis glycosides in cats, reported a digitalis toxicity syndrome characterized by emesis, nystagmus, salivation, panting, defaecation, convulsions, hyperpnoea and death. Similar toxic manifestations in cats after administration of peruvoside, a cardiac glycoside obtained from the plant, *Thevetia neriifolia*, have been reported by Gaitondé & Joglekar (1972). Buterbaugh & Spratt (1970) suggested that monoamines might have a role in acute digitoxigenin toxicity. Intracerebroventricular (i.c.v.) injection of digitalis glycosides in minute doses produces marked neurotoxicity (Melville & Shister, 1957; Gaitondé *et al.*, 1965). In this paper we have investigated the mechanism of neurotoxicity induced by cardiac glycosides in cats and elucidated the role of putative neurotransmitters in its genesis.

Methods

Cats of either sex weighing 3 to 4 kg were used. A cannula was implanted in the lateral cerebral ventricle as described by Feldberg & Sherwood (1954) and experiments were carried out after the cats made a complete recovery (6 to 8 days).

A drug or vehicle was injected i.c.v. in a volume not exceeding 0.25 ml, the effective volume being 0.2 ml. Implantation of the cannula in the cisterna magna of the cat was performed as described by McCarthy & Borison (1966). At the end of each experiment the position of the cannula was verified as described by Feldberg (1963). Those experiments in which the cannula was not in the desired position, were discarded. In all, 110 cats were used in experiments involving i.c.v., i.m. and i.p. injections and 10 cats in experiments involving intracisternal (i.c.) administration.

The severity of the toxic symptoms following administration of cardiotonic glycosides by i.c.v. or i.c. injection in conscious cats was given a score as follows: (a) Each symptom of severe toxicity, as listed in Table 1, received a score of '2'. (b) Mild toxic effects each received a score of '1'. Scoring was done by a 'blind' observer. The total toxicity score for each cat was found by addition. Five cats were used for each dose and the grouped data compared.

The cerebral ventricles of 14 anaesthetized cats were perfused as described by Bhattacharya & Feldberg (1958). The inflow was 0.1 ml per minute. The fluid used for perfusion was artificial

Table 1 Toxic symptoms following intracerebroventricular administration of various doses of peruvoside or ouabain in conscious cats

Severe	*Mild*
Nystagmus	Piloerection
Tachypnoea	Defaecation
Panting	Micturition
Restlessness	Licking
Ataxia	Pupillary changes
Tremor	Ear flutter
Vocalization	Scratching
Psychosis	Laboured respiration
Rage	Drowsiness
Convulsions	
Death	

cerebrospinal fluid prepared as described by Merlis (1940). The effluent was collected in a graduated test tube kept in chilled ice and the volume was recorded every 15 min; the samples were kept at −30°C until assayed for the presence of biogenic substances. Peruvoside or ouabain or 0.9% w/v NaCl solution (saline), was injected into the lateral ventricles via the inflow. The perfusion was stopped for 15 min and the outflow of the polythene tube was closed with a metal cap. This was done in order to allow the full action of the injected drug. At the end of this period, perfusion was restarted and the perfusate collected as described above. Rectal temperature was monitored continuously by a rectal probe (APLAB).

The perfusate was assayed on guinea-pig ileum for acetylcholine and histamine and on rat fundal strip for noradrenaline and 5-hydroxytryptamine (5-HT) (Vane, 1957). The sensitivity of the assays was: 5-HT 2.5 pg/ml, acetylcholine 5 ng/ml, noradrenaline 1 ng/ml, histamine 10 ng/ml.

The following drugs were used: peruvoside (supplied by the Director-General, Indian Council of Medical Research, New Delhi, India), ouabain and 2-bromolysergic acid diethylamide (BOL-148, Sandoz India Ltd., Bombay, India), reserpine (Ciba Research Centre, Bombay, India), tetrabenazine (Roche Pharmaceuticals, Bombay, India) and haloperidol (a gift from Janssen Pharmaceuticals, Belgium). Other drugs were obtained from local commercial firms. A treatment schedule of the cats challenged with peruvoside or ouabain is given in Table 2.

Results

Peruvoside or ouabain-induced neurotoxicity

Intracerebroventricular administration of 5, 10 and 20 μg of peruvoside or 10 and 20 μg of ouabain produced a sequence of similar toxic effects such as ataxia, nystagmus, panting, piloerection, defaecation, vocalization and death (Table 3). Intracisternally, peruvoside or ouabain produced toxic effects of much less severity. The average toxicity score after

Table 2 Schedules of drug treatment of cats challenged with peruvoside or ouabain

Treatment	*Dose*	*Route*	*Time of challenge with cardiac glycosides*
Peruvoside	5, 10, 20, 30 μg	i.c.v.	—
Peruvoside	12.5 μg/kg	i.c.	—
Ouabain	10, 20, 20 μg	i.c.v.	—
Ouabain	6.0 μ/kg	i.c.	—
Reserpine	2 mg/kg	i.m.	24 hours
Reserpine	500 μg in divided doses	i.c.v.	4 hours
Tetrabenazine	25 mg/kg	i.v.	4 hours
Tetrabenazine	50 mg/kg	i.v.	4 hours
Tetrabenazine	2 mg	i.c.v.	30 minutes
BOL-148	200 μg	i.c.v.	30 minutes
Haloperidol	200 μg	i.c.v.	30 minutes
Lithium carbonate	100 mg/kg	i.p.	9th day
Lithium carbonate	2 mg	i.c.v.	30 minutes
PCPA	400 mg/kg	i.p.	24 hours

BOL-148 = 2-bromolysergic acid diethylamide; PCPA = *p*-chlorophenylalanine; i.c.v. = intracerebroventricular; i.m. = intramuscular; i.p. = intraperitoneal; i.c. = intracisternal.

administration of 5 μg of peruvoside i.c.v. was 6.2 ± 0.48 (s.e.); the toxicity score increased three times when the dose of peruvoside was doubled. Further increase in the dose of the drug produced almost maximum toxicity. A dose of 10 μg of ouabain produced a toxicity score of 21.2 ± 1.84 (s.e.) and this was comparable with that produced by 37.5 μg of peruvoside. There was 100% mortality in the group receiving 10 μg of peruvoside or ouabain; the average time required for death to occur was 6 h (range 5–8 hours).

Effects of catecholamine-depleting drugs

Cats pretreated with reserpine or tetrabenazine were challenged with peruvoside. Prior reserpine treatment by the intramuscular or i.c.v. route resulted in a substantial reduction in the toxicity score of almost 50% (Figure 1). A similar effect was noted after treatment with tetrabenazine (Figure 2). Tetrabenazine in an intravenous dose of 25 mg/kg reduced the toxicity score by almost 40% and a higher dose i.e. 50 mg/kg (i.v.) produced a further reduction. Intracerebroventricular tetrabenazine was effective in about one-tenth the intravenous dose.

Effect of 2-bromolysergic acid diethylamide (BOL-148) treatment

Prior i.c.v. administration of BOL-148 in a dose of 200 μg suppressed the toxicity of peruvoside and ouabain (Table 4). The toxicity score was reduced and the incidence of death was substantially lowered.

Effects of p-chlorophenylalanine (PCPA)

PCPA in a dose of 400 mg/kg intraperitoneally 24 h before challenge with 20 μg of peruvoside or 10 μg ouabain suppressed the severity of the toxic reaction (Figure 3). There was no mortality in the group of cats receiving PCPA before ouabain or peruvoside challenge, compared with 100% mortality in the untreated group.

Table 3 Toxicity score in cats after administration of different doses of peruvoside or ouabain

Drug	*Dose (μg i.c.v.)*	*Average score (± s.e.)*	*No. of cats died/tested*
Peruvoside	5	6.2 ± 0.48	0/5
	10	18.0 ± 1.14	5/5
	20	19.8 ± 0.25	5/5
	37.5	21.6 ± 0.12	5/5
Ouabain	10	21.2 ± 1.84	5/5
	20	27.3 ± 0.66	5/5

Table 4 Effect of 2-bromolysergic acid diethylamide (BOL-148) on peruvoside or ouabain-induced toxicity in cats

Drug	*Dose (μg i.c.v.)*	*Average toxicity score (± s.e.)*	
		Normal	*BOL-148*
Peruvoside	20	19.8 ± 0.25 (5/5)	6.2 ± 1.14 (0/5)
Ouabain	20	27.3 ± 0.66 (5/5)	11.0 ± 0.57 (1/5)
	10	21.2 ± 1.84 (5/5)	5.6 ± 0.34 (0/5)

BOL-148 = 200 μg i.c.v. 30 min before challenge. Figures in parentheses indicate no. of cats died/no. of cats tested.

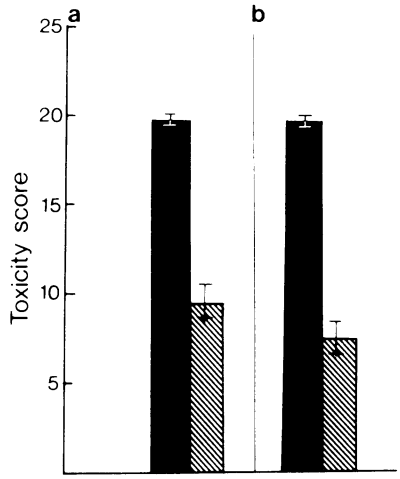

Figure 1 The effect of reserpine treatment on peruvoside toxicity score in cats. Solid columns—untreated; cross-hatched columns—treated animals (a) Reserpine (2 mg/kg i.m.); (b) reserpine (500 μg i.c.v.).

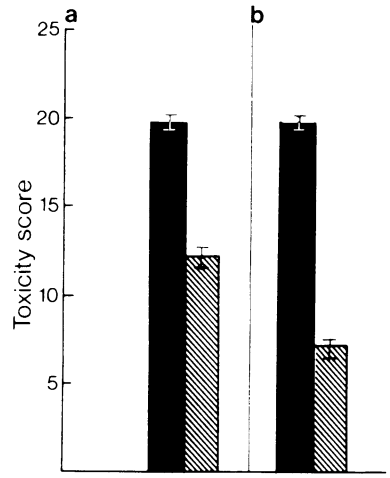

Figure 2 The effect of tetrabenazine treatment on peruvoside toxicity score in cats. Solid columns—untreated; cross-hatched columns—treated animals (a) Tetrabenazine (25 mg/kg i.v.); (b) tetrabenazine (50 mg/kg i.v.).

Effects of haloperidol and lithium carbonate

Prior i.c.v. administration of 200 μg of haloperidol or 2 mg of lithium carbonate failed to suppress the neurotoxicity induced by peruvoside or ouabain (Table 5). Chronic lithium carbonate treatment in a dose of 100 mg/kg (i.p.) for eight days also failed to influence the toxicity. Lithium carbonate (2 mg) in combination with haloperidol (200 μg, i.c.v.) also failed to influence the toxicity of the cardiac glycosides.

Release of biogenic substances into perfusates of the lateral ventricles

Injection of peruvoside or ouabain resulted in a detectable release of 5-HT into the perfusate (Figure 4). The greatest amounts of 5-HT appeared in the first 15 min collection period after injection, when the release was dose-dependent. The response to a second injection of peruvoside or ouabain showed tachyphylaxis. Since traces of blood in the perfusate could cause a high 5-HT content we established that no

Table 5 Effects of haloperidol or lithium carbonate alone or in combination, on peruvoside or ouabain toxicity in cats

		Average toxicity score (± s.e.) after treatment with				
Challenge drug	*Dose (μg i.c.v.)*	*No drug*	*Haloperidol (i.c.v.)*	*Lithium (i.p.)*	*Lithium (i.c.v.)*	*Haloperidol + lithium (i.c.v.)*
Peruvoside	20	19.8 ± 0.25 (5/5)	19.0 ± 1.0 (5/5)	19.0 ± 1.0 (5/5)	19.0 ± 2.0 (5/5)	19.0 ± 1.0 (5/5)
Ouabain	10	21.2 ± 1.8 (5/5)	20.0 ± 1.0 (5/5)	20.0 ± 2.1 (5/5)	19.5 ± 1.1 (5/5)	20.0 ± 1.1 (5/5)

Haloperidol = 200 μg i.c.v.; lithium i.p. = 100 mg/kg i.p. for 9 days; lithium i.c.v. = 2 mg i.c.v. Figures in parentheses indicate no. cats died/no. cats tested.

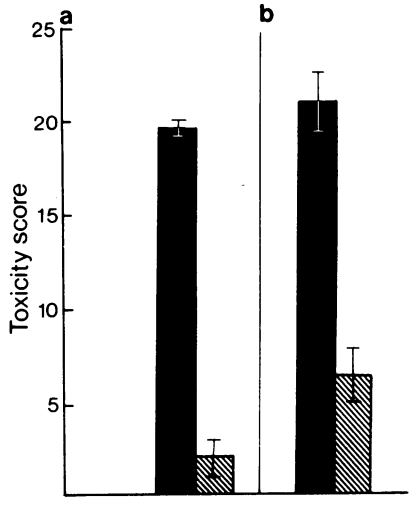

Figure 3 The effect of *p*-chlorophenylalanine (PCPA) (400 mg/kg i.p.) treatment on peruvoside and ouabain toxicity score in cats. Solid columns—untreated; cross-hatched columns—treated animals. (a) Peruvoside (20 μg i.c.v.) toxicity score; (b) ouabain (10 μg i.c.v.) toxicity score.

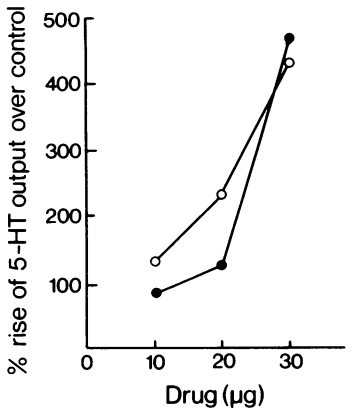

Figure 4 The rise in 5-hydroxytryptamine (5-HT) output after intracerebroventricular administration of 10, 20 and 30 μg of peruvoside (O) and ouabain (●). The 5-HT content (pg/ml) of the first 15 min collection of perfusate after glycoside injection was expressed as a percentage of that in the 15 min collection immediately before injection. Each point is the mean of 3 experiments.

blood was present by centrifugation and microscopic examination.

Prior administration of PCPA suppressed the 5-HT release induced by peruvoside or ouabain (Figures 5 and 6).

Any histamine, acetylcholine or noradrenaline present in the perfusate was too small in amount to be detected by the assay procedures used.

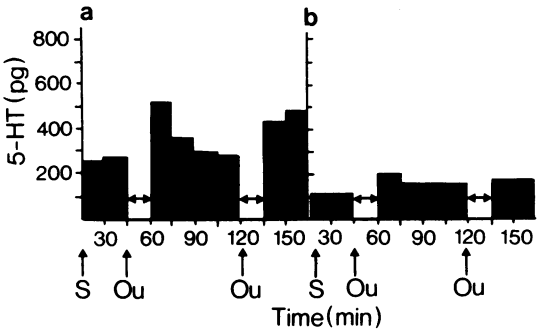

Figure 5 The release of 5-hydroxytryptamine (5-HT) in the perfusate from lateral ventricles of (a) untreated or (b) *p*-chlorophenylalanine (PCPA)-treated cats. Perfusate was collected every 15 min, except during the 15 min immediately after injection of ouabain (20 μg i.c.v.) when the perfusion was stopped to allow the drug to act (indicated by two-headed arrow). S—saline; Ou—ouabain.

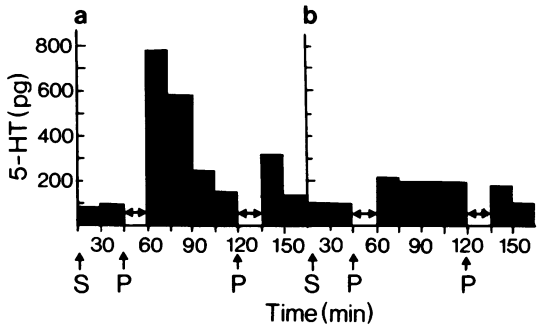

Figure 6 The release of 5-hydroxytryptamine (5-HT) in the perfusate from lateral ventricles of (a) untreated or (b) *p*-chlorophenylalanine (PCPA)-treated cats. Perfusate was collected every 15 min, except during the 15 min immediately after injection of peruvoside (20 μg i.c.v.) when the perfusion was stopped to allow the drug to act (indicated by two-headed arrow). S—saline; P—peruvoside.

Discussion

The distribution of a drug introduced into a lateral ventricle of the brain is governed by a number of factors such as volume of injection, concentration of the drug, site of injection, the extent of uptake by periventricular brain tissue or by choroid plexus and the rate of CSF formation. Feldberg (1963) has demonstrated that drugs administered i.c.v. in cats in a volume of 0.25 ml will not only fill the lateral ventricles, but will also fill the 3rd and 4th ventricles and tend to escape into the subarachnoid space more anteriorly and ventrally. Our results indicate that the cardiac glycosides produced a typical 'digitalis toxicity' syndrome. Intracerebroventricular

administration of various doses of peruvoside or ouabain produced strikingly severe toxic effects which were dose-related. However, intracisternal administration produced less toxic effects, twitching of the ear and muscles of the neck suggesting a local irritation of the upper cervical segment of the spinal cord.

The neurotoxicity produced by digitalis-like drugs is in all probability due to penetration of these substances from the ependyma into deeper areas of the brain. It is also possible that the ependymal lining may contain chemosensitive receptors which are activated by such drugs and thus reflexly bring about neurotoxicity.

Biologically active substances like catecholamines, 5-HT, histamine and prostaglandins have been postulated to serve as neurotransmitters in specific brain areas by a number of investigators (Vogt, 1954; 1973; Koelle, 1959; Carlsson, 1966; Horton, 1969). Vogt (1954) has shown that administration of various drugs intramuscularly or i.c.v. results in changes in the levels of brain catecholamines. We have found that prior administration of reserpine intramuscularly or i.c.v. suppressed neurotoxicity produced by peruvoside or ouabain. Thus, mortality which is invariably seen after i.c.v. digitalis was almost totally prevented in reserpine-treated animals. Reserpine has been reported to prevent accumulation of catecholamines and 5-HT in the presynaptic storage site (Pletcher, Shore & Brodie, 1955; Shore, Silver & Brodie, 1955; Holzbauer & Vogt, 1956; Muscholl & Vogt, 1958).

Catecholamines or 5-HT, or both, may be involved in the 'digitalis toxicity' syndrome. Our results on cats treated with tetrabenazine also tend to support this hypothesis. The relative role of these two types of transmitters in the genesis of the digitalis toxicity syndrome was further revealed by our studies in animals treated with lithium carbonate. Lithium carbonate has been used therapeutically to suppress 'psychoses' (Cade, 1949; Schoue, 1959; Gershon & Yuwiler, 1960). Since some aspects of digitalis toxicity syndrome resembled 'psychosis', it was decided to investigate the effect of lithium carbonate. The results of the study indicate that digitalis toxicity is unaffected by prior lithium carbonate administration. Stern, Fieve, Neff & Costa (1967) have reported that lithium carbonate stimulates catecholamine turnover in the brain by producing a change in ionic equilibrium at the neuronal membrane. If this is so, our results then indicate catecholamines may not be involved to any large extent in the genesis of digitalis toxicity.

In our previous study we reported that prior administration of haloperidol and pimozide to cats suppressed vomiting induced by peruvoside or ouabain (Gaitondé & Joglekar, 1975). However, administration of haloperidol failed to suppress other neurotoxic effects. Haloperidol has been reported to be a dopamine antagonist (Fuxe & Sjoqvist, 1972). Failure of haloperidol to protect against digitalis toxicity syndrome may be taken to indicate that dopamine may not be involved in its genesis.

BOL-148 (i.c.v.) suppressed considerably the toxicity of digitalis. This drug has been reported to be an antagonist to 5-HT (Garattini & Valzelli, 1965). Buterbaugh & Spratt (1970), while studying the role of brain monoamines in acute digitoxigenin toxicity, suggested that intact brain amine levels have a permissive role in the development of digitoxigenin toxicity, and lethality. In the present study, since BOL-148 suppressed toxicity, we investigated further the possibility of affording protection by pretreating the cats with a drug that reduces the 5-HT concentration in the brain. Our results with PCPA pretreatment clearly demonstrated that reduced brain 5-HT levels suppressed digitalis toxicity. Our findings with BOL-148 and PCPA treatment lend strong support to the hypothesis that the neurotoxicity of digitalis glycosides may be due to the massive release of 5-HT over widespread areas in the brain.

Perfusion experiments in anaesthetized cats in fact do indicate a release of 5-HT into the ventricular perfusate after i.c.v. administration of peruvoside or ouabain. Although the cats were anaesthetized, this release of 5-HT was associated with a number of signs of neurotoxicity such as dilatation of pupils, twitching of facial muscles, ear flutter, movement of the fore legs, defaecation, marked increase in respiration, etc. Rectal temperature showed a drop of almost 1°C. Prior treatment of the cats with PCPA suppressed 5-HT release and also all the associated phenomena of neurotoxicity.

Neurotoxicity becomes one of the problems in therapy with digitalis glycosides. Administration of BOL-148 or a related antagonist to 5-HT may be one of the rational ways of treating digitalis toxicity.

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References

BHATTACHARYA, B.K. & FELDBERG, W. (1958). Perfusion of cerebral ventricles: effects of drugs on outflow from cisterna and aqueduct. *Br. J. Pharmac. Chemother.*, **13**, 156–162.

BUTERBAUGH, G.G. & SPRATT, J.L. (1970). The possible role of brain monoamines in the acute toxicity of digitoxigenin. *J. Pharmac. exp. Ther.*, **175**, 121–130.

CADE, J.F.J. (1949). Lithium salts in the treatment of psychotic excitement. *Med. J. Aust.*, **36**, 349–352.

CARLSSON, A. (1966). In *Mechanisms of Release of*

Biogenic Amines ed. Von Euler, U.S., Rosell, S. & Yvnas, B., pp. 331–346, Oxford: Pergamon Press.

FELDBERG, W. (1963). *A Pharmacological Approach to the Brain from its Inner and Outer Surface.* Evarts Graham Memorial Lectures, 1961, London: Edward Arnold (Publishers) Ltd.

FELDBERG, W. & SHERWOOD, S.L. (1954). Injections of drugs into the lateral ventricle of cat. *J. Physiol., Lond.*, **123**, 148–167.

FUXE, K. & SJOQVIST, F. (1972). Hypothermic effect of apomorphine in the mouse. *J. Pharm. Pharmac.*, **24**, 702–705.

GAITONDÉ, B.B. & JOGLEKAR, S.N. (1972). Mechanism of peruvoside induced emesis. *Neuropharmacology*, **11**, 427–433.

GAITONDÉ, B.B. & JOGLEKAR, S.N. (1975). Role of catecholamines in the central mechanism of emetic response induced by peruvoside and ouabain. *Br. J. Pharmac.*, **54**, 157–162.

GAITONDÉ, B.B., McCARTHY, L.E. & BORISON, H.L. (1965). Central emetic action and toxic effects of digitalis in cats. *J. Pharmac. exp. Ther.*, **147**, 409–415.

GARATTINI, S. & VALZELLI, L. (1965). *Serotonin.* New York: American Elsevier Publishing Co.

GERSHON, S. & YUWILER, J. (1960). Lithium in a specific psychopharmacological approach to the treatment of mania. *J. Neuropsychiatry*, **1**, 229–241.

HOLZBAUER, M. & VOGT, M. (1956). Depression by reserpine of noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.*, **1**, 8–11.

HORTON, E.W. (1969). *Prostaglandins.* London: International Encyclopaedia of Pharmacology, Pergamon Press.

KOELLE, G.B. (1959). *Neurohumoral Agents as a Mechanism of Nervous Integration.* Reprinted from *Evolution of Nervous Control.* Copyright 1959 by the American Association for the Advancement of Science, Washington, D.C.

McCARTHY, L.E. & BORISON, H.L. (1966). Volumetric compartmentalization of the cranial cerebrospinal fluid system determined radiographically in the cat. *Anat. Rec.*, **155**, 303–310.

MELVILLE, K.L. & SHISTER, H.E. (1957). General systemic effects and electrocardiographic changes following injections of digitalis glycosides into lateral ventricles of the brain. *Am. Heart. J.*, **53**, 425–438.

MERLIS, J.K. (1940). The effects of changes in the calcium content of cerebrospinal fluid on spinal reflex activity in the dog. *Amm. J. Physiol.*, **131**, 67–72.

MUSCHOLL, E. & VOGT, M. (1958). The action of reserpine on the peripheral sympathetic system. *J. Physiol., Lond.* **141**, 132–155.

PLETSCHER, A., SHORE, P.A. & BRODIE, B.B. (1955). Serotinin release as a possible mechanism of reserpine action. *Science*, **122**, 374–375.

SCHOUE, M. (1959). Lithium in psychiatric therapy: Stocktaking after ten years. *Psychopharmacologia*, **1**, 65–78.

SHORE, P.A., SILVER, S.L. & BRODIE, B.B. (1955). Interaction of serotinin and lysergic acid diethylamide (LSD) in the central nervous system. *Experientia*, **11**, 272–273.

STERN, D.N., FIEVE, R., NEFF, N. & COSTA, E. (1967). The effect of lithium on turnover of brain and heart catecholamines. *Pharmacologist*, **9**, 210.

VANE, J.R. (1957). A sensitive method for assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol. Lond.*, **123**, 451–481.

VOGT, M. (1973). Functional aspects of catecholamines in central nervous system. *Br. med. Bull.*, **29**, 168–171.

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