Studies on the Cardiovascular Actions of Chlorpromazine.

by

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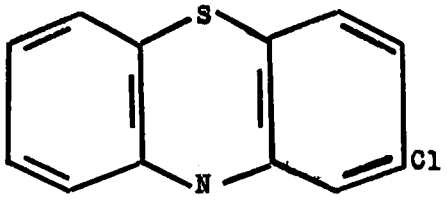
INTRODUCTION

During the past decade, several chemical derivatives of the phenothiazine nucleus have been synthesized in France, and their various pharmacological actions investigated. As a result of these studies, a number of new compounds of this type have already been introduced as therapeutic agents. The drugs in this series are of special pharmacological interest because of the wide scope of physiological functions which they have been reported to affect, and this is particularly so in respect to the chlorophenothiazine derivative, known as chlorpromazine. Indeed, as stated by Dundee (1954), "in the history of modern medicine, there have been few drugs to which so many properties have been ascribed or which have found so many clinical uses as chlorpromazine".

Chlorpromazine is chemically 3-chloro-10 (3-dimethylamino-n-propyl) phenothiazine, and used in medicine in the form of the hydrochloride, as represented by the following formula:

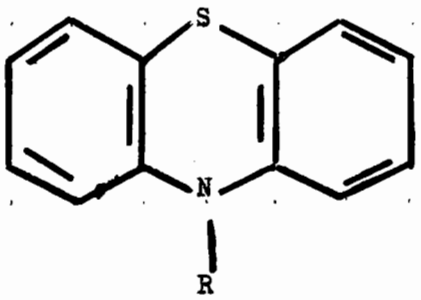


$CH_2 . CH_2 . CH_2 . N(CH_3)_2 . HCl.$

The drug was first synthesized by Charpentier (1950) working in the research laboratories of Rhône-Poulenc-Specia in France, and was originally known in that country as 4560 R.P. It has since been marketed under various proprietary or trade-names, including "Largactil", in France, Great Britain

and Canada; "Thorazine", in the United States, and "Megaphen" in Germany.

The discovery of chlorpromazine was a direct result of earlier investigations of the pharmacology of several related phenothiazine derivatives, notably among which are diethazine and promethazine. The close chemical similarity of these compounds and their relationship to chlorpromazine are evident from their chemical structures, as shown hereunder:



R = CH_2. CH_2 . N (C_2H_5). HCl

(Diethazine Hydrochloride, 2987 R.P., or "Diparcol")

R = CH_2. CH . (CH_3). N $(CH_3)_2$. HCl

(Promethazine Hydrochloride, 3277 R.P., or "Phenergan")

Generally speaking, all of these phenothiazine derivatives exert ~~rather~~ several similar types of pharmacological actions, although they possess considerable quantitative differences in respect to one or other specific type of action. Thus, Bovet, Fournel and Charpentier (1947) and Heymans, Estable and de Bonneveaux (1949a, 1949b) have emphasized the marked depression of ganglionic transmission induced by diethazine. It has also been observed that this compound depresses central nervous system function and diminishes or abolishes convulsions induced by both strychnine and metrazol. In addition, the drug can be shown to exert anticholinergic and anti-adrenergic actions, and some local anaesthetic activity, but little or

no antihistaminic action. In contrast, as shown by Halpern (1947), Viaud (1947), Winter (1947) and Bovet (1948), promethazine exerts marked antihistaminic and local anaesthetic actions, but again, has been demonstrated to induce depression of both ganglionic transmission and central nervous system functions.

Finally, as shown by Courvoisier, Fournel, Ducrot, Kolsky and Koetschet (1953), chlorpromazine produces the most marked central nervous system action, and is also a potent anti-adrenergic and local anaesthetic agent. However, the drug shows little or no antihistaminic action. More will be said concerning these and other actions of chlorpromazine later.

It is apparent from the above observations that pharmacological analysis of the cardiovascular actions of these agents presents a complex and difficult problem, and it is still uncertain to what extent many of the observed changes in circulatory dynamics are related to one or other of the multiple associated effects of these agents on other body functions.

In his recent review of the pharmacology of chlorpromazine, Dundee (1954) states that "the majority of the cardiovascular effects observed after administration of chlorpromazine are secondary to its effects on the autonomic nervous system". It is evident, however, from the earlier studies of Courvoisier and associates (1953) that the drug may also exert a direct dilating action on blood vessels, as judged by its action on the isolated perfused rabbit's ear. Moreover, it is conceivable that secondary changes in the cardiovascular system might result from the well-recognized actions of the drug on the central nervous system. In view of the increasing widespread clinical use of chlorpromazine, more exact knowledge on these questions is highly desirable, and it appeared worthwhile to undertake a systematic investigation of its cardiovascular actions.

In the first series of experiments, the general cardiovascular effects of the drug were studied in the animal as a whole with the circulation intact. In the second series of experiments, the heart-lung preparation was employed, in order to dissociate as far as possible cardiac and peripheral effects of the drug. And, in the third series of experiments, the effects of the drug on isolated perfused hearts were investigated. It was hoped that by correlating the results from these different approaches, a better understanding of the mechanism of the cardiovascular actions of chlorpromazine and perhaps also of its related agents, might be achieved.

HISTORICAL REVIEW

The earliest publication in the literature concerning the actions of chlorpromazine is that by Laborit and Huguenard (1951) in respect to "artificial hibernation" for anaesthesia. Courvoisier and her associates (1953) reported an extensive study of the general pharmacology and toxicology of the compound.

The multiplicity of actions of chlorpromazine as shown by these workers, and some of which have already been referred to in the Introduction, immediately intrigued both pharmacologists and clinicians in many countries. It is not surprising, therefore, that within the short period of four years, an extensive literature on the drug has already accumulated. In his recent review, Dundee (1954) lists no less than five hundred such publications.

With the increasing numbers of studies appearing in the literature in such rapid succession, it becomes rather difficult to bring together an entirely complete review of all these publications. As stated in the general introduction, we were primarily interested in the pharmacology of the cardiovascular actions of the drug, and consequently will consider mainly the various experimental observations concerning its effects on this system. Some miscellaneous observations concerning its actions on other systems of the body will also be appended.

I. Observations on the Cardiovascular System as a Whole.

ACTIONS ON THE BLOOD PRESSURE. There is now considerable experimental and clinical evidence to indicate that chlorpromazine in sufficient dosage exerts a fall in blood pressure. Courvoisier et al. (1953) first described this hypotensive action in chloralosed dogs, and concluded that following intravenous injection of dosages ranging from 1 to 5 mg./kg., only a negligible and transitory depressor effect was observed. Huidobro (1954),

using cats anaesthetized with sodium pentobarbital has reported that chlorpromazine in doses of 0.8 to 8.5 mg./kg., injected intravenously, produces a rapid and marked fall in blood pressure -- with higher doses this effect lasts longer than 26 hours. There was no evidence of any lessened blood pressure response on repeated injections.

In man, Moyer et al. (1954) have reported that chlorpromazine produces a fall in blood pressure. They conclude that the drug reduces the blood pressure by decreasing the peripheral resistance. Other clinicians (Foster, 1954; Dobkin et al., 1954) have also described similar effects. Morris et al. (1955) stated that following intravenous injection of 50 mg. of chlorpromazine in normotensive man, the blood pressure was reduced "to a significant degree". However, this was not seen as frequently after intramuscular administration. These observations were made in connection with a study of the effect of chlorpromazine on the cerebral circulation, using the Nitrous oxide method and it is difficult to assess the significance of the findings.

Stevenson et al. (1954) have reported that chlorpromazine (50 mg. intramuscularly) induced a definite decrease in the blood pressure in man during standing and in some instances led to definite "postural hypotension". However with the patient in the recumbent position, the blood pressure showed no significant changes.

The mechanism of the above-described depressor effects of chlorpromazine is still uncertain. Whether the observed decrease in the blood pressure is a purely peripheral action or whether it results partially or wholly from the central actions of the drug, has not yet been established. With regard to the latter point, Cathala et al. (1952) have injected chlorpromazine into the lateral cerebral ventricles of the unanaesthetized dog in doses (2 mg./kg.) sufficient to produce profound narcosis, abolition of corneal reflex and

complete muscular relaxation. It was observed that notwithstanding these central effects, the arterial pressure remained unchanged ranging between 110 to 120 mm. Hg. On the other hand, Dasgupta et al. (1954a) have reported that intracisternal injections of small amounts (0.25 to 0.5 mg.) of chlorpromazine in Rhesus monkeys under allobarbitone anaesthesia, can produce some fall in blood pressure (30 to 50 mm. Hg.) and conclude that under certain conditions, chlorpromazine can exert a central depressant effect on vasomotor tone.

ACTIONS ON THE HEART. According to Courvoisier et al. (1953) in the unanaesthetized rabbit, no changes in the electrocardiogram were found following intravenous injections of chlorpromazine in doses of 1 to 10 mg./kg. With repeated subtoxic doses at ten minute intervals, there was an increase in the atrio-ventricular and intraventricular conduction times, an increase in P-R interval and widening of the QRS complex. Finally, in toxic doses totally 70 mg./kg. (10 mg./kg. repeated 7 times), there developed bundle branch block and auricular fibrillation, followed by death.

In most clinical studies, the main electrocardiographic effects reported have been an increase in heart rate and sinus arrhythmia (Laborit et al., 1952; Dobkin et al., 1954; Foster, 1954; Foltz, 1955). However, Moyer et al. (1954) could observe no significant changes in the electrocardiogram in most cases in man following oral administration, but in a few patients there was an increase in heart rate, partial auriculo-ventricular block and minor T wave changes. The significance of these changes is rather doubtful, since in some of these instances, other factors were present which could have given rise to such cardiac irregularities, as stated by the authors themselves. These workers have also reported that chlorpromazine exerts a variable effect on cardiac output in man. Thus there was a slight initial increase following small doses of the drug, but as the dose was augmented

the cardiac output was reduced.

ACTIONS ON PERIPHERAL VESSELS. Pickering and Ahlquist (1955) using a rotameter have studied the blood flow in several different vascular areas in anaesthetized dogs. They observed that following intra-arterial injection of 1 mg./kg. of chlorpromazine, there is a marked increase in femoral blood flow. There was no significant change in renal blood flow. Moyer et al. (1954) also reported no changes in renal haemodynamics in man following the administration of chlorpromazine. Foster et al. (1954) measured the blood flow in the extremities of man using venous occlusion plethysmography. They recorded a marked increase (284 percent) in the hand and a less marked increase (72 percent) in the forearm and calves, following intravenous injection of 25 mg. of chlorpromazine. In order to elucidate further the mechanism of this vasodilatation, the drug was infused into the brachial artery. Under these conditions, the blood flow in the hand was increased only 50 percent. The authors concluded then that the observed vasodilator effect of chlorpromazine in the hand was due to both local and central effects. In addition, they also found that the drug reduces the response of the blood vessels in the hand to the "cold constrictor test". Finally, Morris et al. (1955) reported that following intravenous injection of chlorpromazine (50 mg. intravenously in man), there was a reduction in the cerebral blood flow. However, they attributed this decrease to the associated general fall in blood pressure, since after intramuscular administration neither fall in blood pressure nor diminution in cerebral blood flow occurred.

ANTI-FIBRILLATORY ACTIONS. Courvoisier et al. (1954) first described the "anti-fibrillatory" actions of chlorpromazine. They studied the effects on the cardiac irregularities produced by adrenaline, aconitine nitrate and adenosine phosphoric acid. In the rabbit, 2.5 to 5 mg./kg. of chlorpromazine injected intravenously diminishes the deleterious effects of aconitine nitrate

(0.1 mg./kg. intravenously). In the guinea pig, chlorpromazine (2.5 to 5 mg./kg.) counteracts the arrhythmias produced by adrenaline and can diminish or prevent the auriculo-ventricular block induced by adenosine phosphoric acid (1 mg./kg. intravenously). Melville (1954) has reported that chlorpromazine (0.5 to 1 mg./kg. injected intravenously) effectively prevents ventricular extrasystoles and fibrillation following epinephrine during chloroform inhalation in dogs. However, the ventricular irregularities associated with the actions of either ouabain or pressor pituitary extract were not prevented by repeated injections of the drug. Dilalme et al. (1955) state that chlorpromazine can prevent cardiac irregularities produced by petroleum ether and adrenaline. Following doses of 0.5 to 1 mg./kg. protection was complete and lasted for 1 to 3 hours. However, the drug did not raise the electrical threshold to atrial fibrillation, whereas quinidine and procaine amide were effective. In addition, chlorpromazine did not prevent the P wave disappearance from the electrocardiogram, as induced by petroleum ether. For the above reasons, the authors conclude that chlorpromazine exerts this protective action by virtue of an anti-epinephrine effect rather than by a direct quinidine-like depressant action on the myocardium.

II. Observations on Isolated Cardiovascular Preparations.

ACTIONS ON ISOLATED PERFUSED HEARTS. On the isolated frog heart, Courvoisier et al. (1953) have reported that chlorpromazine has no inotropic, chronotropic or tonotropic action. The concentrations employed were not stated. Further, the same workers using the isolated perfused rabbit's heart (Langendorff method) reported that the drug (0.1 to 1 g./litre) increased the coronary flow by 60 to 100 percent. However, associated changes in the heart contractions were not recorded in these experiments. Using the isolated rabbit heart, Melville (1954) has reported that chlorpromazine (0.5 to 1 mg./kg.) induces a marked increase in the coronary flow and a

concomitant depression of the heart contractions.

ACTIONS ON ISOLATED CARDIAC TISSUE PREPARATIONS. On the isolated rabbit's auricle, Courvoisier et al. (1953) again reported no significant activity of chlorpromazine. Finkelstein et al. (1954a) have observed, on the papillary muscle of the cat's heart, that chlorpromazine in concentrations of 0.1 to 0.5 mg./100 ml. produces a reversible negative inotropic action, resulting in a 40 to 60 percent reduction in contractile force. There was also a decrease in the irritability of the heart muscle, as indicated by an increase in the threshold voltage required to drive the muscle following chlorpromazine.

Further, Coraboeuf et al. (1954) have studied the effects of chlorpromazine on the electrical activity of the conductile fibres from the dog's heart (Purkinje fibres). Concentrations of 1 in 25,000 of the drug produced a slight increase in the electrical rhythm with a few irregularities. With repetition of this concentration there occurs a decrease in the duration of the action potential, with an increase in the threshold, and a correlated decrease in the amplitude. In addition, there was a slowing of the rhythm. If the same concentration was again repeated, there appeared a condition which the authors designated as "flutter" -- a gradual fall in membrane potential and a rapid rhythm where the upstroke was as long as the downstroke -- followed by cessation of all activity. These latter effects were irreversible in spite of frequent washings.

ACTIONS ON ISOLATED PERIPHERAL VESSELS. Courvoisier et al. (1953) perfusing isolated rabbit's ears, reported that chlorpromazine (0.1 to 1 g./litre) caused an increase of flow of 50 to 100 percent. Injection of the drug into the medial artery of the rabbit's ear (intact animal) was followed by a visible vasodilatation of the capillaries. This was accompanied by a moderate to marked oedema. Microscopic examination showed serous fluid escaping from the

greatly dilated vessels. On the other hand, in vivo studies by these same authors demonstrated that chlorpromazine had no effect on capillary permeability in the guinea pig. However, large doses (12.5 to 50 mg./kg.) decreased capillary permeability in the rat and rabbit.

III. Observations on Autonomic Cardiovascular Control.

a) Anti-adrenergic Action.

EFFECTS ON BLOOD PRESSURE. Courvoisier et al. (1953) first demonstrated the anti-adrenergic action of chlorpromazine in respect to the blood pressure response, following injections of adrenaline and noradrenaline. These authors showed that after a dose of 0.5 to 1 mg./kg. of chlorpromazine, the pressor responses to doses of 0.002 to 0.005 mg./kg. of adrenaline injected intravenously are diminished, abolished or reversed -- with higher doses (5 mg./kg.), these responses were invariably inverted. In regard to the antagonism to noradrenaline, chlorpromazine (1 mg./kg.) diminished the pressor response, but there was no inversion. Marquardt (1953) reported similar results in cats under pernocton anaesthesia. Melville (1954) has studied the antagonism to adrenaline and noradrenaline in both dogs and cats and concludes that under certain conditions (during continuous infusion of adrenaline or noradrenaline), the pressor responses to both drugs can be shown to be inverted. Kopera and Armitage (1954) reported that in the spinal cat preparation following the injection of 10 mg. of chlorpromazine, the pressor response to adrenaline was decreased (75 percent), while that to an equipressor dose of noradrenaline was only slightly diminished. There was no evidence of reversal under these conditions. Huidobro (1954) has shown in cats anaesthetized with pentobarbital sodium that chlorpromazine (8.5 mg./kg. intraperitoneally) reversed the hypertensive effect of adrenaline for a considerable time (more than 26 hours) without affecting the hypertension produced by noradrenaline. It also antagonized the hypertensive effects of

amphetamine and phenylephrine (neosynephrine).

More recently, Pickering et al.(1955) have demonstrated that in anaesthetized dogs, the renal vasoconstriction produced by adrenaline (0.16 µg./kg.) could be blocked by a small dose (25 µg./kg.) of chlorpromazine injected intra-arterially. Further, the decrease in splenic blood flow and the contraction of the spleen following the injection of adrenaline were also blocked by the drug.

In respect to sympathetic reflex control of the circulation and the responses following sympathetic nerve stimulation, Courvoisier et al.(1953) have observed that intravenous injections of chlorpromazine markedly depressed the hypertension due to either occlusion of the carotids or to stimulation of the central end of the vagus nerves. Pocidalo et al. (1954) reported similar observations. In addition, these authors have shown that chlorpromazine diminished the hypertensive effect due to (a) stimulation of the splanchnics, (b) injections of small doses of nicotine, and (c) central stimulation of the medulla by the local application of 30 percent alcohol. Valerenberche et al. (1954) observed that this reduction in the hypertensive effects following splanchnic stimulation in chloralosed dogs occurs both before and after adrenalectomy. However, Kopera et al. (1954) have reported no diminution in the hypertensive response to stimulation of the splanchnic nerves in the adrenalectomized spinal cat.

It is evident from the above that the failure of these complex circulatory reflexes following chlorpromazine administration cannot be ascribed solely to its peripheral anti-adrenergic action. Indeed, the possibility exists that central nervous system depressant effects of the drug may also be involved in modifying these vasomotor responses. Cathala et al. (1952) have reported that injections of chlorpromazine (2 mg./kg.) into the lateral cerebral ventricle of the unanaesthetized dog produces profound narcosis. Under these

conditions, there is a complete lack of circulatory response to stimulation of the central end of the vagus and to occlusion of the carotids. Dasgupta et al. (1954a) have reported that intracisternal injections of chlorpromazine (0.25 to 0.5 mg.) into Rhesus monkeys anaesthetized with allobarbitone cause a suppression of carotid sinus reflexes. They also observed that the pressor responses elicited by electrical stimulation of hypothalamic or medullary pressor areas are completely abolished in decorticated cats, by chlorpromazine (50 to 100 µg./kg., administered intravenously). However, cats under chloralose anaesthesia are considerably less susceptible to these blocking actions of the drug. These observations have been interpreted by the authors to indicate "that in certain circumstances a central depressant effect of chlorpromazine on vasomotor tone and reflex activity can be demonstrated".

EFFECTS ON THE HEART. Finkelstein et al. (1954a), using the papillary muscle of the cat's heart, reported that following chlorpromazine (0.1 to 0.25 mg. percent) the positive inotropic action of epinephrine, l-norepinephrine and N-isopropylnorepinephrine can be partially inhibited (approximately 50 percent). Higher concentrations (0.4 to 0.5 mg. percent) completely antagonized these actions. These authors also state that the inhibitory actions of the drug were reversible.

The electrocardiographic effects following chlorpromazine have been studied by Courvoisier et al. (1953), Melville, (1954) and Huidobro (1954). These workers uniformly reported that following chlorpromazine tachycardia produced by epinephrine, norepinephrine and other pressor amines is not abolished.

b) Anti-cholinergic Action.

Courvoisier et al. (1953) have reported that electrical stimulation of the peripheral end of the vagus nerve still produces cardiac slowing and

a fall in blood pressure in the chloralosed dog following small doses (below 1 mg./kg.) of chlorpromazine injected intravenously. They have also reported that the cardiovascular depression following vagal stimulation is diminished after doses of 2 mg./kg. and abolished completely after doses of 10 mg./kg. under similar conditions. The same authors have also reported that chlorpromazine (1 to 5 mg./kg. intravenously) does not affect the hypotension produced by intravenous injections of acetylcholine (0.01 to 0.05 mg./kg.). However, higher doses of chlorpromazine (10 mg./kg.) can diminish both the slowing of the heart rate and the fall in blood pressure seen after similar injections of acetylcholine.

IV. Miscellaneous Additional Pharmacological Actions of Chlorpromazine.

a) Effects on the Central Nervous System.

DEPRESSANT EFFECTS. Central nervous system depressant effects of chlorpromazine have been repeatedly observed both in experimental animals and in man. Courvoisier et al. (1953) reported that in rats and mice large doses of chlorpromazine (up to 20 mg./kg.) produce a state of central depression that lasts for several hours. In the dog doses of 10 mg./kg. injected subcutaneously produce locomotor disturbances, and with higher doses (10 to 20 mg./kg.) the duration of the depression exceeds four days. As previously stated following injection of chlorpromazine (2 mg./kg.) into the lateral cerebral ventricles of the dog, Cathala and Pocidalo (1952) reported that profound narcosis appeared with complete relaxation and abolition of the corneal reflex. Furthermore, Das et al. (1954) working with Rhesus monkeys found that small doses of chlorpromazine (0.7 to 2 mg./kg.) injected intravenously produced "quietening" of the animals, although all reflexes are present. Higher doses (10 mg./kg.) produced somnolence, but no true anaesthesia was ever seen. The electroencephalogram showed changes that could best be described, according to the authors, by ascribing to the

drug "a suppression on the awakening "upward discharge" of the ascending reticular formation". Dasgupta et al. (1954) have shown that in decorticate and diencephalic cats chlorpromazine can suppress the "sham-rage" of such animals very effectively, and in doses significantly lower than those required to cause somnolence in intact animals. Holzbauer and Vogt (1954) showed in cats that high doses (25 mg./kg.) of chlorpromazine could not inhibit the stimulation of the sympathetic centres produced by morphine. These authors could not confirm the suggestion of Laborit and Huguenard (1951) that the drug exerts an inhibitory action on diencephalic centres.

More recently, Dasgupta (1955), working with female albino rats, reported that chlorpromazine (10 mg./kg.) injected subcutaneously daily could prevent the estrus cycle. Normal rhythmicity reappeared a few days after the drug was discontinued. However, injections of the drug (40 mg./kg.) could not overcome the continuous estrus cycle produced by injections of estrogens. Since the estrus cycle is said to be under hypothalamic control, the effect of chlorpromazine was thought to be a central one. Rutledge and Doty (1955) working with cats trained either to lift a foreleg upon application of a stimulus to the cerebral cortex or trained to lift the foreleg in response to auditory or visual stimulation, have reported that chlorpromazine (4.7 mg./kg.) abolishes these latter reflexes but not the response resulting from cortical stimulation. With higher doses, cortically conditioned responses were however ultimately lost. The authors conclude that their findings are in accord with the interpretation that chlorpromazine acts "somewhere in the afferent mechanism and that direct cortical stimulation to a large degree circumvents this system".

The central depressant action of chlorpromazine has now been widely employed in the field of psychiatry. In the words of Winkelman (1954) "the drug is especially remarkable in that it can reduce severe anxiety, diminish phobias and obsessions, reverse or modify a paranoid psychosis, quiet maniac

or extremely agitated patients and change the hostile senile patient into a quiet easily managed one". Hopkin (1954) states that chlorpromazine "gives a central effect which is characterized by a state of indifference and detachment rather than by a true hypnosis or analgesia. This effect can last for more than 24 hours". Terzian (1952) reports that the overall electroencephalographic effects of chlorpromazine are identical with the pattern obtained during normal sleep. No changes in the electroencephalogram were observed if the state of consciousness is not disturbed. In the state of disinterestedness, there is an accentuation of the "theta" element and other changes. The author concludes that the effects of chlorpromazine represent depression of the reticular formation particularly the sensory and autonomic spheres. Dobkin (1954) also reported that in humans receiving chlorpromazine the electroencephalographic recordings showed a normal sleep pattern and often a hyperactive response to light, touch and noise stimuli.

POTENTIATION OF ANAESTHETICS, ANALGESICS AND HYPNOTICS. Laborit et al. (1952) were the first workers to report that chlorpromazine could potentiate the action of volatile anaesthetics, barbiturates and analgesics. Courvoisier et al. (1953) studied this action of the drug further. They reported that chlorpromazine increase the duration of hexobarbital anaesthesia in the mouse, guinea pig and the dog. In the latter, chlorpromazine (1 to 2 mg./kg., subcutaneously) in conjunction with hexobarbital (50 mg./kg., intravenously) could prolong the anaesthesia from twenty-five (25) to sixty (60) minutes. Higher concentrations of the former (5 mg./kg.) prolonged the anaesthesia for more than two (2) hours. The authors also report that studies performed on mice, rats and guinea pigs show that premedication with chlorpromazine increases considerably the duration of anaesthesia when ether is used. In addition, the time of onset of anaesthesia was also decreased. Chlorpromazine has also been reported to increase the duration of sleep produced by hypnotics such as

butobarbital. In the rabbit, for example, butobarbital (50 mg./kg. orally) produces sleep for approximately one (1) hour. When combined with chlorpromazine (25 mg./kg. subcutaneously), the period of sleep was increased to about five (5) hours. Although chlorpromazine is devoid of analgesic action, Courvoisier and her associates reported that in the mouse, morphine associated with chlorpromazine produces analgesia where the dose of morphine alone has no effect. Chlorpromazine also potentiates the action of pethidine.

Giequel and Schmitt (1954) have stated that in rats chlorpromazine greatly potentiates the onset of action and duration of anaesthesia to chloral hydrate and ethylurethane. Bertrand et al. (1954) have reported that the effects of phenyl-hydantoin are also dramatically potentiated in rats.

In humans, Reckless (1954) reports that preadministration of chlorpromazine potentiates the effects of thiopentone and ether and also the analgesic effects of morphine and pethidine. Wallis (1955) states that in clinical experiments chlorpromazine potentiates or augments the action of hypnotics, narcotics and analgesics. However, this is not substantiated by Houde and Wallenstein (1955) who report that the effect of combination of morphine and chlorpromazine was the same as the analgesic effect of morphine alone in cancer patients. This question of the potentiation of the actions of analgesics by chlorpromazine requires further investigation.

HYPOTHERMIA. Huguenard (1952) described the hypothermic action of chlorpromazine, in connection with "artificial hibernation", induced by a combination of drugs. Courvoisier et al. (1953) further demonstrated that in mice chlorpromazine (2 to 50 mg./kg. subcutaneously) lowers the body temperature from six (6) to ten (10) degrees centigrade. This hypothermia persisted up to twenty-four (24) hours after the administration of the drug. The experiments were repeated on rats and dogs with qualitatively similar results. Various degress of hypothermia have been reported by numerous

workers including Burn (1954), Halpern and Liakopoulos (1954), Giaja and Giaja (1954), Kopera and Armitage (1954) and Dawson and Hiestand (1955). Finally, the extensive literature concerning the application of chlorpromazine in the production of hypothermia and "artificial hibernation" both in surgery and medicine has been reviewed by Laborit and Huguenard (1954), and needs no further comment here.

ANTI-EMETIC ACTION. Courvoisier et al. (1953) observed in dogs that chlorpromazine (2 mg./kg. subcutaneously) completely protected against the vomiting produced by apomorphine (0.1 mg./kg. subcutaneously), but could not prevent vomiting due to copper sulphate administration.

Boyd et al. (1953) and Cook et al. (1954) also working with dogs confirmed the potent anti-emetic action of the drug in respect to apomorphine. The latter workers have also reported that the threshold to veratrum-viride induced emesis was also raised by chlorpromazine, but Lanatoside-C-induced vomiting was not inhibited in any manner. Again, in dogs, Brand et al. (1954) report that chlorpromazine is markedly effective in antagonizing apomorphine induced emesis. In addition, they found the drug also protects against vomiting evoked by morphine and ergot but is ineffective against intravenous copper sulphate, Lanatoside-C, veratrum and oral copper sulphate. These authors conclude that the pattern of anti-emetic action of chlorpromazine in dogs suggests that the mechanism of its action is a "selective depression of the medullary emetic chemoreceptor trigger zone". Glaviano and Wang (1954) have further extended these observations, and conclude that at a dose level of 3 mg./kg. of chlorpromazine the vomiting centre per se is not depressed and the protective action is probably due to its competitive action for the receptors at the chemoreceptive emetic trigger zone. On the other hand, when chlorpromazine was injected intravenously, a higher degree of protection was obtained against certain types of vomiting, and even the response to

large doses of copper sulphate was blocked up to 50 percent. The authors therefore conclude from these results that the more potent anti-emetic effect of intravenous chlorpromazine stems from its "depression of the reticular vomiting mechanism". Finally, the drug has also been used clinically as an effective anti-emetic agent as shown by the reports of Bartis et al. (1954) and Morris et al. (1954).

b) Effects on the Peripheral Nervous System.

EFFECT ON NERVE FIBRES. Chauchard and Chauchard (1952) have shown that application in the dog of chlorpromazine (2.5 percent) close to the preganglionic vagus and cervical sympathetic fibres induces changes in chronaxie. Thus the chronaxie of the vagal fibres increased 3-fold and in 30 to 40 minutes there was complete block. The chronaxie of the preganglionic sympathetic fibres increased less rapidly and was never greater than 5 times its initial value.

EFFECT ON AUTONOMIC GANGLIA. Many authors including Courvoisier et al. (1953) refer to chlorpromazine as a "ganglioplegique" drug apparently without any direct experimental evidence. Valerenberche et al. (1954) ascribe a ganglionic-blocking action to this drug since it was seen to decrease the secretion of adrenaline to stimulation of the splanchnic nerves. However, Bruneaud et al. (1953) have shown that high doses of chlorpromazine can decrease adrenaline secretion to both splanchnic and nicotine stimulation. Thus, they conclude that decreased adrenaline secretion due to stimulation of the splanchnics cannot be taken as evidence for a "synaptic block", since this inhibition could occur at the level of the gland itself. Decourt et al. (1953) in experiments using cat salivary glands and stimulating pre- and post-ganglionic fibres, have shown that chlorpromazine has no ganglionic blocking action at therapeutic levels. At high concentrations (20 mg./kg.), there is a diminution in salivary flow which the authors attribute to the

drug's "narcobiotic" action and to its slight anti-cholinergic effects. Huidobro (1954) concludes that chlorpromazine does not block transmission through the superior mesenteric ganglion when the splanchnic nerves are stimulated. Finally, Holzbauer and Vogt (1954) have investigated the alleged inhibitory action of chlorpromazine on ganglionic transmission, and state that "no such action was found on the superior cervical ganglion".

c) Effects on Striated and Smooth Muscles.

ACTION ON STRIATED MUSCLE. Following injection of chlorpromazine in doses up to 1.35 mg./kg. in the terminal portions of the abdominal aorta in cats, Huidobro (1954) reports that the drug did not modify the intensity of the quadriceps twitch, when the muscle was stimulated indirectly at a frequency of one pulse every 10 seconds. However, in a few instances, a small increase in the twitch was seen. In contrast, Kopera and Armitage (1954) in the sciatic-gastrocnemius preparation of the cat, reported that chlorpromazine (4.6 mg./kg.) caused a slight initial increase in the amplitude of contraction both to direct and indirect stimulation. This was followed by a gradual decrease. The amplitude of contraction to direct stimulation always declined more slowly than that to indirect stimulation. This dose eliminated completely the response to indirect stimulation after 75 minutes. Comparable results were obtained on the phrenic nerve-diaphragm preparation of the rat. Burn (1954) using the cat sciatic-gastrocnemius preparation reported that chlorpromazine (3 mg./kg.) caused a gradual failure of contraction both to direct and indirect stimulation.

ACTION ON SMOOTH MUSCLE. *In vitro* studies on isolated rabbit intestine reported by Courvoisier *et al.* (1953) show that chlorpromazine (5 mg./litre) produces a decrease in the tone and the amplitude of spontaneous intestinal movements. Complete paralysis occurred when the concentration was doubled. At the latter concentration, the stimulating effects of acetyl-

choline (1 mg./litre) were antagonized. On the isolated rabbit uterus (in anestrus), these authors have shown that chlorpromazine is just as active as ergotamine in blocking the spasms produced by adrenaline. The drug was devoid of any antihistamine activity. Lamarche and Arnould (1954) using isolated rat and rabbit intestines reported that chlorpromazine (10^{-6} M) diminishes both the tone and the amplitude of contraction. After 30 minutes, the tone and the amplitude returned to normal levels. The inhibiting effect of adrenaline (10^{-7} M) is the same before, after or during the chlorpromazine inhibition. Chlorpromazine (2×10^{-6} M.) could inhibit reversibly the stimulating effect of acetylcholine (10^{-7} M.).

In the cat, Huidobro (1954) reported that chlorpromazine (1.7 to 6.3 mg./kg.) did not alter the inhibitory effects of adrenaline on the urinary bladder, the intestine or the uterus. The same author also reports that the drug (0.8 to 1.65 mg./kg.) can inhibit the contraction of the nictitating membrane of the cat as induced by electrical stimulation of either the pre- or post-ganglionic fibres. It can also reduce or abolish the contraction of the membrane produced by the intra-carotid injection of adrenaline or acetylcholine. Kopera and Armitage (1954) investigating the anti-cholinergic action of chlorpromazine state that on the mouse pupil doses as high as 20 mg./kg. showed no mydriatic activity. In cats, the drug could inhibit salivary flow and they conclude that chlorpromazine is 1/30 as potent as atropine in this respect. With the guinea pig ileum, a negligible anti-histamine activity was also recorded.

d) Action of Chlorpromazine in the prevention of Induced Shock.

Both Fournel (1952) and Courvoisier et al. (1953) have reported that chlorpromazine (2 mg./kg.) could protect dogs against induced haemorrhagic shock. Some protection against experimental traumatic shock in rats has also been reported by the latter workers. Reilly and Tournier (1953),

Pocidalo and Tardieu (1954) and Hershey and Guccione (1955) have also reported favourable results with the use of chlorpromazine in various types of experimental shock.

e) Actions on Metabolism.

Courvoisier et al. (1953) have measured the oxygen uptake of slices of guinea pig cerebral cortex and found that the addition of chlorpromazine to the medium caused a decrease in the rate of uptake. With concentrations of 175, 350 and 700 mg./litre of the drug, respectively, they recorded a decrease of 21, 43 and 62 percent, respectively, in the rate of oxygen uptake one hour after the addition of chlorpromazine. In view of the excessive concentrations employed, the significance of these findings is questionable. Ganshirt and Brilmayer (1954) employing lower concentrations of the drug on guinea pig cerebral cortex using both brain slices and homogenates, observed that chlorpromazine (10^{-7}, 10^{-6} and 3×10^{-6} M.) decrease the rate of oxygen uptake by 17, 25 and 36 percent, respectively. This decrease in the rate of uptake occurred at reduced temperatures (25°C.). Therefore, the authors conclude that chlorpromazine has "a direct effect on the brain in decreasing oxygen utilization and at these low concentrations the effect cannot be considered a narcotic one". Finkelstein et al. (1954) report experiments in which cat left ventricle heart slices, brain cerebral cortex slices and brain homogenates were employed to determine the influence of chlorpromazine on aerobic metabolism. From their experiments, they conclude that the concentrations of the drug necessary to suppress or diminish oxygen consumption were considerably greater than those which evoke a response in in vivo and in vitro experiments. Thus, they consider it unlikely that the pharmacological effects of chlorpromazine are due to an action on oxidative metabolism. Finally, Bersohn et al. (1955) using brain homogenates have demonstrated that chlorpromazine (10^{-3} M.) can completely inhibit aerobic and anaerobic glycolysis.

The drug had no inhibitory effect on citrate synthesis from pyruvate and oxaloacetic acids. The inhibition of specific and serum cholinesterase was negligible.

PHARMACOLOGICAL APPROACHES EMPLOYED AND EXPERIMENTAL OBSERVATIONS.

I. Studies on the Cardiovascular System as a Whole.

(a) Methods.

Both dogs and cats were employed. In most of the experiments on dogs, the animals were simply anaesthetized with pentobarbital sodium (40 mg./kg.) injected intravenously. In some experiments, the dogs were previously morphinized by subcutaneous injection with 10 mg./kg. of morphine sulphate, 15 to 30 minutes prior to injection of pentobarbital -- a dose of 20 mg./kg. of the anaesthetic sufficed in these cases. In several experiments, the animals were also either vagotomized (the right and left vagus nerves sectioned in the neck) or atropinized (1 to 2 mg./kg. atropine sulphate injected intravenously), at the beginning of the experiment or during its course, as indicated. In the experiments on cats, the animals were always first temporarily lightly etherized and then injected with pentobarbital sodium (20 to 30 mg./kg.) or the brain destroyed and the ether discontinued (spinal animals).

Continuous artificial respiration using a Starling pump was employed throughout all experiments. All animals were heparinized (1 mg./kg. heparin, injected intravenously), and blood pressure was recorded directly from a cannula inserted into the right common carotid artery, using 5% sodium citrate as the anticoagulant in the system. In order to record the electrocardiograms, 3 No. 17 gauge 2 inch hypodermic needles were fused to the lead wires and used as electrodes. These were appropriately placed under the skin. Electrocardiographic changes were observed more or less continuously throughout all experiments using a Sanborn Visocardiette and electrocardiograms (Lead II) recorded at frequent intervals as indicated on the signal line of the kymographic tracings. Some sections of both the electrocardiograms and the blood pressure records are reproduced in the figures. The time intervals of

the latter are marked in minutes throughout.

For continuous infusions, a constant-rate infusion pump was attached to a cannula inserted into a femoral vein. In all experiments, the volume injected was 1 ml. per min., and the desired concentration of the agent made up in 0.9 percent saline solution. All injections of drugs were made directly into an exposed femoral vein in the dog experiments, while in the cat experiments a hypodermic needle was tied into the vein, and the injections washed in with 1 ml. of saline.

In the studies concerning the influence of chlorpromazine on ventricular arrhythmias, chloroform was administered by appropriately inserting a Woulff bottle into the artificial respiration circuit, and following a short period of administration, adrenaline injected, as previously described by Melville (1946).

The following drugs were employed in these studies: Chlorpromazine hydrochloride (Largactil) in powder form;[1] Synthetic l-epinephrine bitartrate and synthetic l-norepinephrine bitartrate monohydrate;[2] These agents are referred to as "adrenaline" and "noradrenaline", respectively, throughout this investigation; Hydergin , a mixture of equal parts of dihydroergocristine, dihydroergokryptine and dihydrocornine.[3] Finally, ephedrine sulphate, atropine sulphate, morphine sulphate and acetylcholine hydrobromide, were also used in different experiments. All solutions of drugs were freshly made up as desired. Seventy (70) different experiments were performed in this series of studies.

(b) Effects of chlorpromazine on blood pressure and associated electrocardiographic changes in pentobarbitalized dogs.

Doses of chlorpromazine, ranging from 0.001 to 10 mg./kg. were tested in several different experiments. Under these conditions, smaller doses of

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1 Kindly supplied by Poulenc, Ltd., Montreal.

2 Kindly supplied by the Sterling Winthrop Research Institute, Rensselear, N.Y.

3 Through the courtesy of Sandoz, Ltd., Montreal.

the drug, ranging from 0.001 to 0.5 mg./kg., were without significant effects on blood pressure and electrocardiograms. Figure 1 shows typical examples of the responses observed when doses of 1 mg./kg. (A), 5 mg./kg. (B and B_1) and 10 mg./kg. (C) of chlorpromazine were injected at short intervals. Comparable responses were observed in several other experiments with such doses. As is evident from the records, following injection (A) of 1 mg./kg., there is only an insignificant dip in blood pressure associated with a slight increase in heart rate and slight depression of the T waves (Nos. 2 and 3). There was no change in either P-R intervals or QRS complexes. Following injection (B) of 5 mg./kg., there is a transient depressor effect associated again with initial tachycardia but there is beginning T wave elevation (Nos. 4 and 5). Repetition of this injection (B_1) induces again a similar depressor response associated however with slowing of the heart and continued T wave heightening with some prolongation of the S-T segments (Nos. 6, 7 and 8). Following injection (C) of 10 mg./kg., there is an intense and sustained fall in blood pressure, associated with marked initial slowing of the heart and T wave elevation (No. 9) followed later by T wave inversion (No. 10). The heart rate rapidly increased again, although the reversal of the T wave persisted (No. 11).

The lower section of the kymograph tracing, which was taken after an interval of 10 minutes, shows that the blood pressure was still below the control level but the normal appearance of the electrocardiogram was now restored (No. 12). At this time, injection of a large dose (0.5 mg.) of adrenaline (E) induces a good reversal effect, associated with marked tachycardia, and heightening of both P and T waves (No. 13). Following injection of a large dose (0.5 mg.) of noradrenaline (N), there was a moderate rise in blood pressure but again a marked associated tachycardia (No. 15). Later in the experiment, following injection of 5 mg./kg. of ephedrine (Eph.), there is only a brief blood pressure inversion but no significant change in the

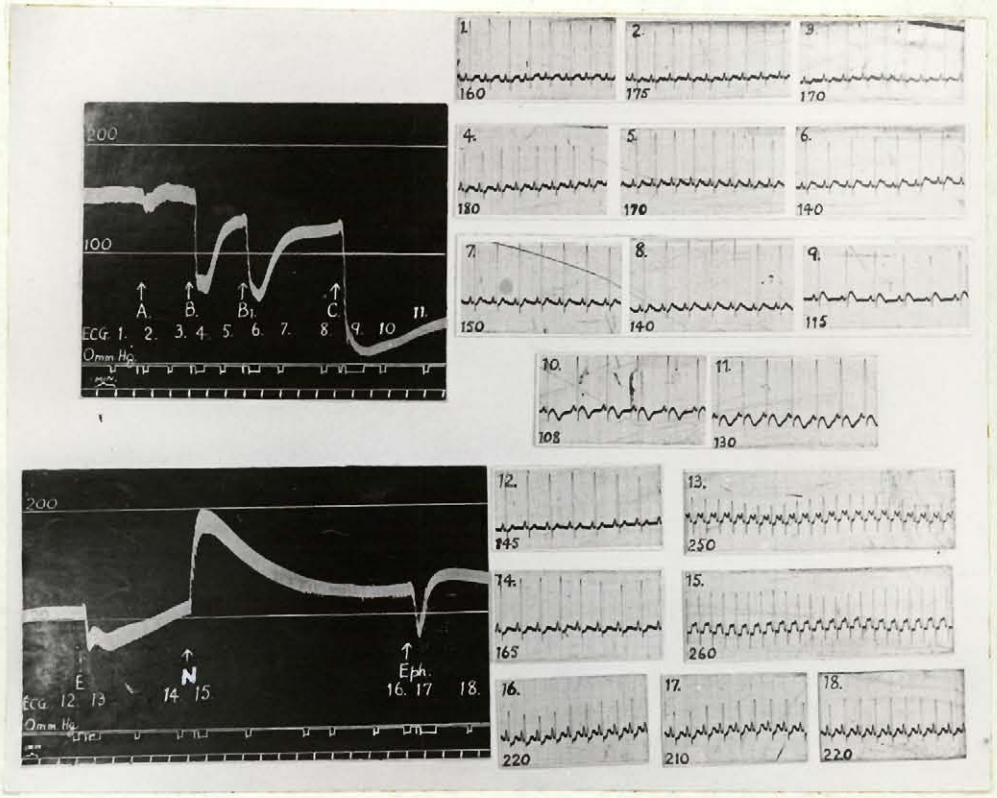


Fig. 1 Dog, Male, 9.0 kg. Pentobarbital.

Blood pressure and electrocardiograms (Lead 2) taken at Nos. 1 to 18. An interval of 10 minutes elapsed between the upper and lower records. At A, chlorpromazine (1 mg./kg.); at B and B_1, chlorpromazine (5 mg./kg.) and at C, chlorpromazine (10 mg./kg.), were injected. At E, adrenaline (0.5 mg.); at N, noradrenaline (0.5 mg.), and ephedrine sulfate (Eph.)(5 mg./kg.), were injected.

electrocardiograms, as shown at Nos. 16, 17 and 18.

Figure 2 shows results of a similar experiment in which the animal was previously vagotomized. As can be seen, the depressor responses obtained throughout are rather similar to those shown in Figure 1, that is in the non-vagotomized animal. However, the associated electrocardiograms show that no significant tachycardia now ensues following the lower doses of the drug, but there was still T wave elevation (Nos. 2 to 9), and following injection of a large dose (10 mg./kg.) of chlorpromazine (C), there is slowing of the heart with flattening and inversion of the T waves (Nos. 10, 11 and 12), as in the non-vagotomized animal.

Figure 3, which is a continuation of the above experiment, shows that after added atropinization, the depressor response to chlorpromazine is not affected (B, between Nos. 1 and 2). Moreover, following injection of hydergin (H), there was a sustained rise in blood pressure, but again the depressor response to chlorpromazine (B, between Nos. 4 and 5) is still in evidence. Indeed, during this latter fall in blood pressure injection of adrenaline (A, between Nos. 6 and 7) led to the pressor-depressor response shown, associated with intense tachycardia. The record also shows that even with the combined "adrenergic blockade" induced by chlorpromazine and hydergin , the blood pressure response to noradrenaline (N) although greatly diminished is not reversed, while that to adrenaline (A, towards end of record) is completely inverted. Again, here the noradrenaline tachycardia is more sustained than that produced by adrenaline. A test injection of a large dose (10 mg.) of acetylcholine (Ac) also shows no evidence of any "ganglionic action", but induces only a fall in blood pressure without significant change in heart rate (No. 11).

It is clear from the above experiments that in the pentobarbitalized dog, chlorpromazine in effective doses (exceeding 1 mg./kg.) induces a fall

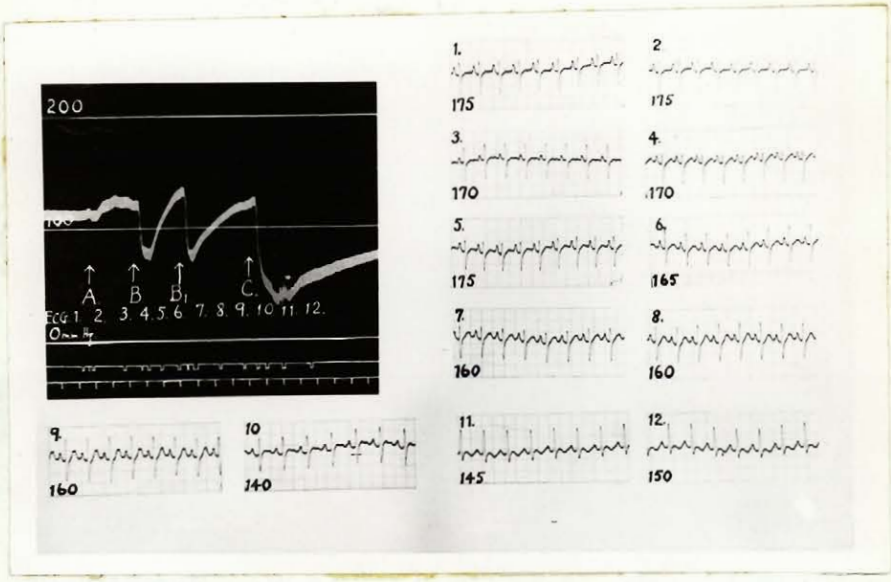


Fig. 2 Dog, Female, 13.5 kg. Pentobarbital. Vagus nerves sectioned in the neck ten (10) minutes earlier. Blood pressure and associated electrocardiograms (Lead 2) taken at Nos. 1 to 12. A, chlorpromazine (1 mg./kg.), B and B_1, chlorpromazine, (5 mg./kg.) and C, chlorpromazine, (10 mg./kg.) were injected, respectively.

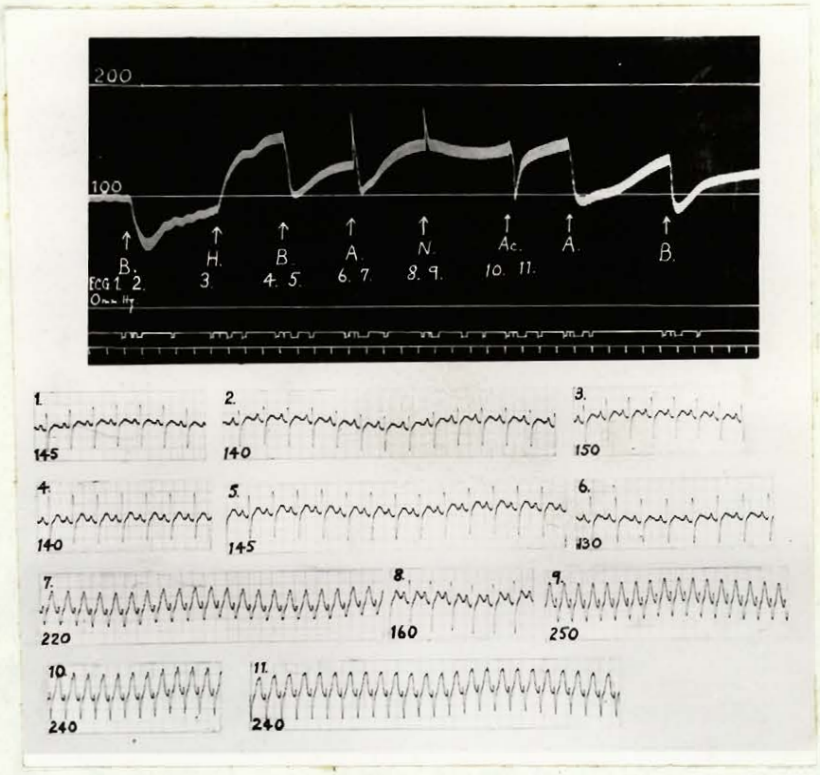


Fig. 3 Continuation of Fig. 2 after an interval of twenty (20) minutes during which atropine sulfate (2 mg./kg.) was injected. Blood pressure and electrocardiograms (Lead 2) taken at Nos. 1 to 11. At each B, chlorpromazine (5 mg./kg.); at H, hydergin (2 mg./kg.); at each A, adrenaline (0.5 mg.); at N, noradrenaline (0.5 mg.), and at Ac., acetylcholine hydrobromide (10 mg.), were injected, respectively.

in blood pressure, which is not significantly affected by either vagotomy, atropinization or injection of hydergin . These depressor responses are rather transitory, except with excessive doses, and are quantitatively repeatable. Lower doses of the drug, however, induce an initial tachycardia which is abolished by previous vagotomy or atropinization. However, with rapidly super-imposed injections or with single large injections, there is a temporary significant slowing of the heart rate. It is also clear that following injection of these doses, there is a consistent alteration of the T waves which are first elevated and later inverted (with large doses only). These T wave changes also occur after atropinization or vagotomy. Finally, the experiments also confirm that injections of chlorpromazine induce a more or less prolonged "anti-adrenergic" action, leading to abolition of the pressor responses to both adrenaline and ephedrine, and characteristic inversion of the blood pressure responses to these agents. However, the pressor response to noradrenaline, although lessened, is not inverted under these conditions. Both adrenaline and noradrenaline also induce sustained tachycardia following previous injections of chlorpromazine.

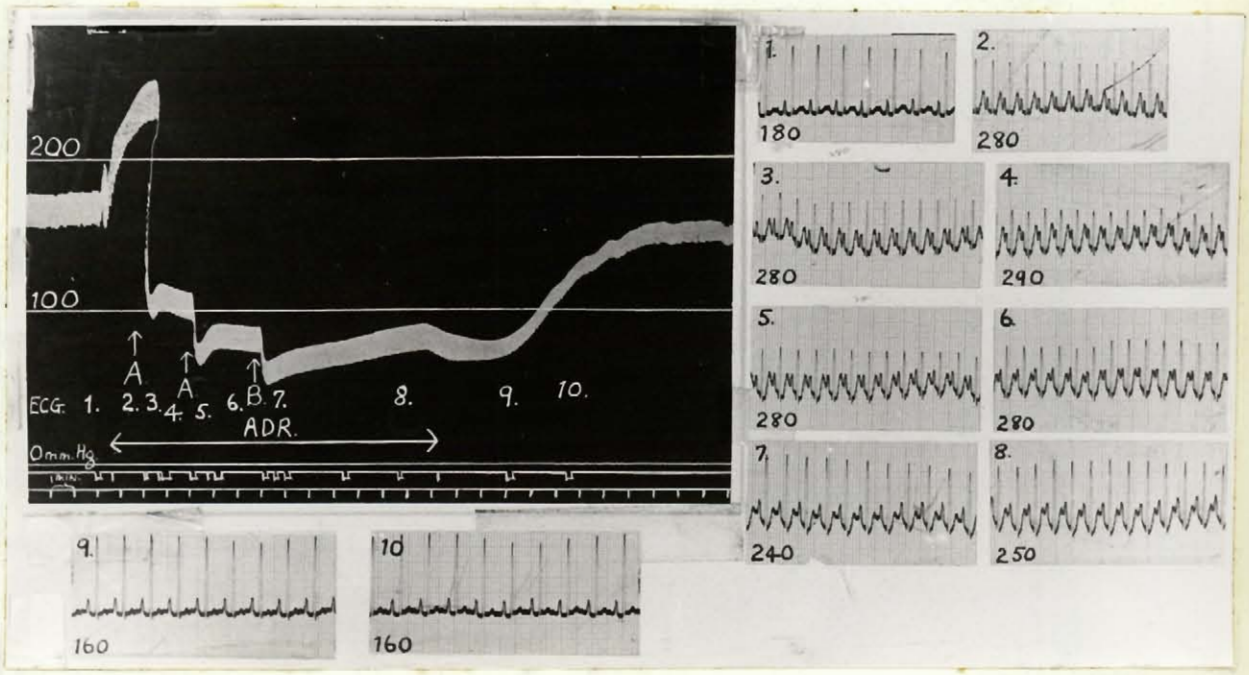
(c) Effects of chlorpromazine when injected during continuous infusion of adrenaline or noradrenaline, or following previous injection of ephedrine, in pentobarbitalized dogs.

In earlier experiments reported by Melville (1951), it was shown that during continuous infusions of adrenaline and noradrenaline, blood pressure reversal effects could be observed with both agents, when superimposed injections of ergotamine or ergotoxine were given. It was suggested that pressor responses, such as are observed with either adrenaline or noradrenaline after previous ergotoxine or ergotamine under certain experimental conditions, might be due mainly to the associated cardiac stimulation induced by the pressor amines. In view of the results described in the previous section (b), it was of interest to investigate the effects of chlorpromazine, when injected

during sustained sympathomimetic action induced by adrenaline, noradrenaline or ephedrine.

The upper record of Figure 4 shows results of an experiment in which doses of 5 mg./kg. (A) and 10 mg./kg. (B) of chlorpromazine were injected during a continuous adrenaline infusion, as shown by the arrow (ADR.). It is clear from the record that the pressor response to adrenaline is completely antagonized and the blood pressure remains at a low level as long as the infusion continues. The electrocardiograms (Nos. 2 to 8) also show that again the adrenaline tachycardia persists throughout the infusion. After the infusion is stopped, there is a prompt slowing of the heart (Nos. 9 and 10) associated with a rise in blood pressure. Ten minutes later, as shown in the lower record, repetition of the adrenaline infusion induces again a sustained depressor effect and concomitant tachycardia (Nos. 2 and 3), as long as the infusion is maintained.

When adrenaline was infused continuously, it was observed that good depressor responses could be obtained with much smaller doses of chlorpromazine than in the absence of adrenaline. Figure 5 shows an example of one such experiment. Thus, it may be seen that during the adrenaline infusion, following injection (A) of a dose of 0.1 mg./kg. of chlorpromazine, there is a definite transient depressor response with no significant change in the electrocardiogram (Nos. 2 and 3). Following a dose of 5 mg./kg. (B), there is a sustained fall in blood pressure. This contrasts strikingly with the effects observed in other experiments, without adrenaline infusion (Figures 1, 2 and 3). When the infusion is stopped, again the blood pressure promptly rises and the heart rate decreases. Thirty minutes later, as shown by the lower records, the effects of the injection of both adrenaline (C) and ephedrine (E) are reversed, while the response to noradrenaline (D) is not, as in the previous experiment.



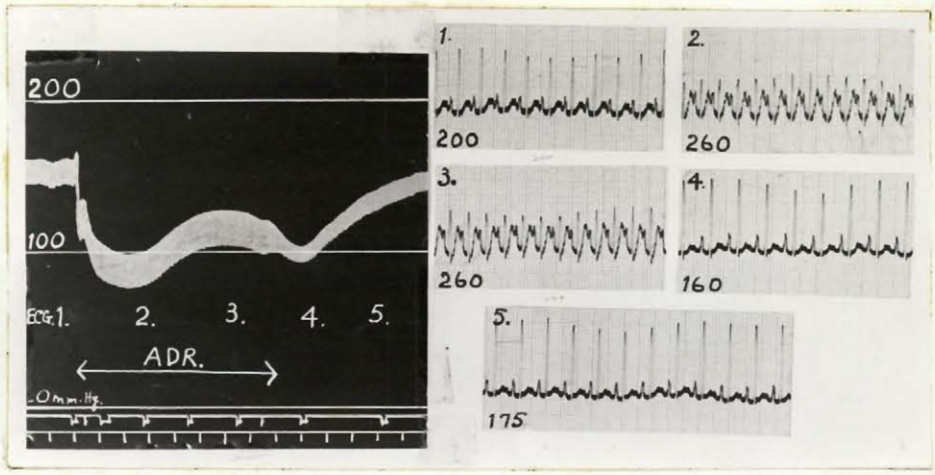


Fig. 4 Dog, Male, 8.7 kg. Pentobarbital.

Blood pressure and electrocardiograms (Lead 2) taken at Nos. 1 to 10 (upper record) and at Nos. 1 to 5 (lower record). An interval of 10 minutes elapsed between the upper and the lower records. At A, chlorpromazine (5 mg./kg.) and at B, chlorpromazine (10 mg./kg.), were injected. Duration of infusions of adrenaline (0.01 mg./kg./min.) shown by the arrows, and marked (ADR.).

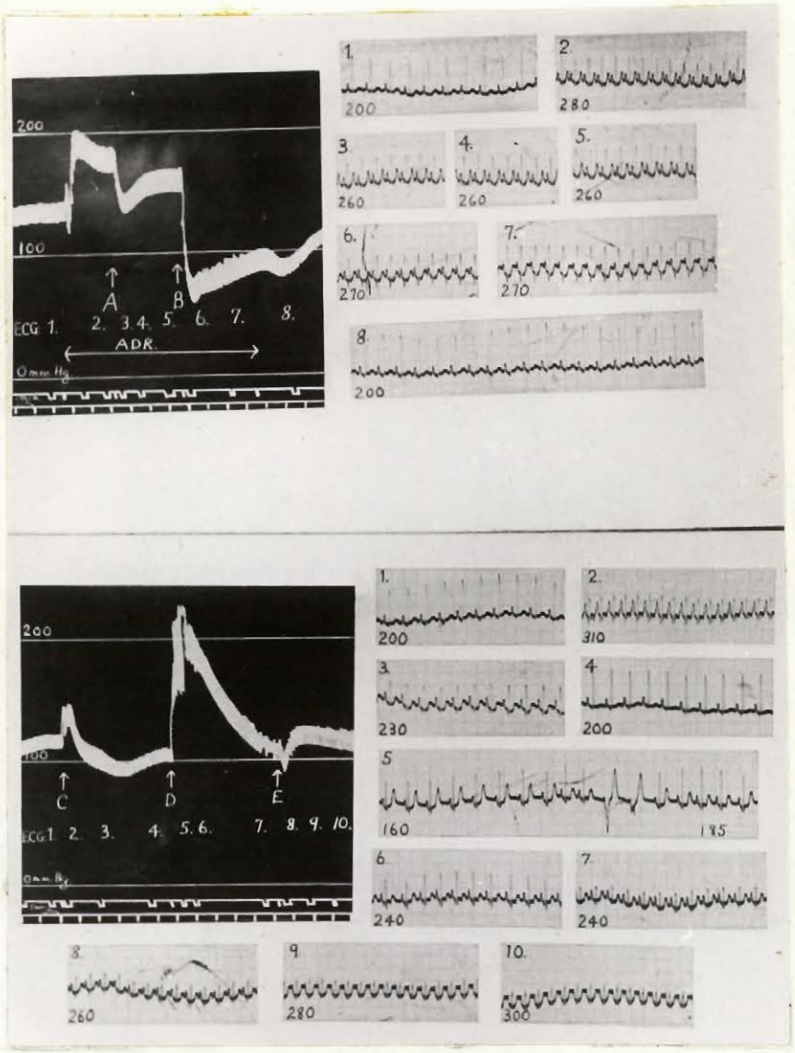


Fig. 5 Dog, Female, 10.9 kg. Pentobarbital. Blood pressure and associated electrocardiograms (Lead 2), as numbered. Upper records. The continuous infusion of adrenaline (0.1 mg./kg./min.) is shown by the arrow (ADR.). At A, 0.1 mg./kg., and at B, 5.0 mg./kg., respectively, of chlorpromazine were injected. Lower records. At C, 0.5 mg. of adrenaline; at D, 0.5 mg. of noradrenaline, and at E, 25 mg. of ephedrine, were injected. An interval of thirty minutes elapsed between the upper and lower records.

Figure 6 shows the effects observed in a somewhat similar experiment in which again small and usually ineffective depressor doses of chlorpromazine were injected during adrenaline infusion. Thus, at A, following injection of 0.01 mg./kg. during the adrenaline infusion, there is a definite depressor effect. Concomitantly, the heart rate also increased slightly (Nos. 2 and 3). It is also clear from the record that after injection of a dose of 1 mg./kg. (B) a marked and sustained adrenaline reversal occurs under these conditions. The electrocardiograms (Nos. 5 and 6) show that this is associated with further cardiac acceleration. It must be emphasized that similar doses of chlorpromazine exert little or no effect on the blood pressure in the absence of adrenaline (Figures 1 and 2).

It was also of some interest in these experiments to ascertain to what extent the vagal control of the heart was affected by such doses of chlorpromazine. As shown on the kymograph tracing (Figure 6) cutting the vagus nerves (vagi cut) following these relatively small doses of chlorpromazine and during the adrenaline, produced a progressive rise in blood pressure, but no further acceleration of the heart (Nos. 7 and 8). Furthermore, stopping the infusion now leads to a fall in blood pressure (unlike the rise uniformly observed in otherwise non-vagotomized animals), and later reinfusion of the adrenaline produced the sustained rise in blood pressure shown with associated tachycardia (Nos. 11 and 12). It may also be seen that injections of larger doses (5 mg./kg.) of chlorpromazine at C, almost completely antagonized this pressor response.

Figure 7 shows the results of an identical experiment in which noradrenaline was infused instead of adrenaline. As can be seen, under these conditions, no significant depressor responses were observed following superimposed injections of either 0.01 mg./kg. (A) or 1 mg./kg. (B) of chlorpromazine. Subsequent cutting of the vagus nerves in the course of the infusion (vagi cut) led to no rise in blood pressure nor any significant

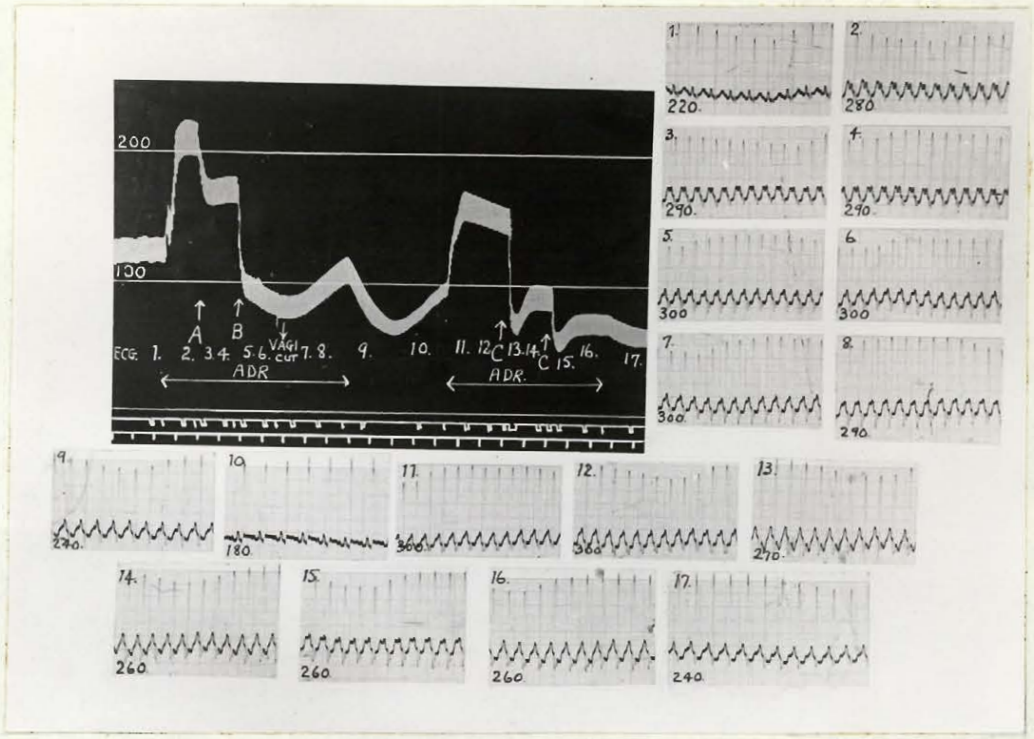


Fig. 6 Dog, Female, 6.8 kg. Pentobarbital.

Blood pressure and associated electrocardiograms (Lead 2) taken at Nos. 1 to 17. Continuous infusions of adrenaline (0.01 mg./kg./min.) are shown by the arrows (ADR). The vagus nerves were cut towards the end of the first infusion, as marked (Vagi cut). At A, 0.01 mg./kg., at B, 1 mg./kg., and at each C, 5 mg./kg. of chlorpromazine, were injected, respectively.

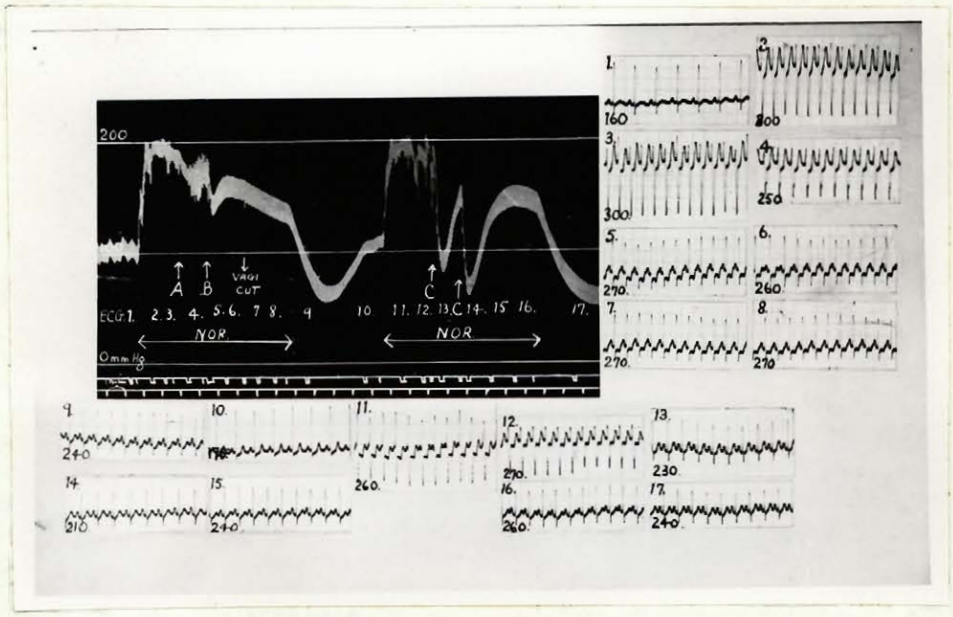


Fig. 7 Dog, Female, 5.7 kg. Pentobarbital.

Blood pressure and associated electrocardiograms (Lead 2) as numbered. Continuous infusions of noradrenaline (0.01 mg./kg./min.) are shown by the arrows, (NOR). The vagus nerves were cut towards the end of the first infusion as marked (Vagi cut). At A, 0.01 mg./kg., at B, 1 mg./kg., and at each C, 5 mg./kg., respectively, of chlorpromazine were injected.

change in heart rate. However, when the infusion was stopped, again the blood pressure fell rapidly, and an almost identical pressor response was observed following the reinfusion of the noradrenaline. As can be seen at C, following injections of large doses (5 mg./kg.) of chlorpromazine, there were only brief depressor responses observed, and no sustained noradrenaline antagonism demonstrated.

In connection with the earlier observations, it was of interest to study the responses to chlorpromazine after previous injection of ephedrine in the pentobarbitalized dog. Figure 8 shows an example of the results obtained in such an experiment. As can be seen, following the injection of 5 mg./kg. of ephedrine (E), there is a sustained rise in blood pressure and the usual vagal reflex slowing of the heart rate from 155 per min. in the control period to 80 per min. shortly after the injection. At this time a dose of 0.01 mg./kg. (C) of chlorpromazine induced little change in blood pressure or heart rate. An injection of 1 mg./kg. of chlorpromazine at C_1, however, produced now a marked and sustained depressor response associated with tachycardia (270 per min.).

When the vagus nerves were now sectioned (vagi cut), as can be seen there is a rapid and intense pressor response with no significant change in heart rate, indicating that the chlorpromazine had not blocked reflex vagal control. Repetition of the chlorpromazine injection at C_1 now induced again a somewhat similar depressor effect -- the heart rate being maintained at a high level. More will be said of this type of response later.

From the above observations, it appears that during infusion of adrenaline or following previous injection of ephedrine, the pressor responses to these agents can be antagonized by much smaller doses of chlorpromazine than can be shown to lead to a depressor effect in the normal pentobarbitalized dog without adrenaline or ephedrine. On the other hand, no significant

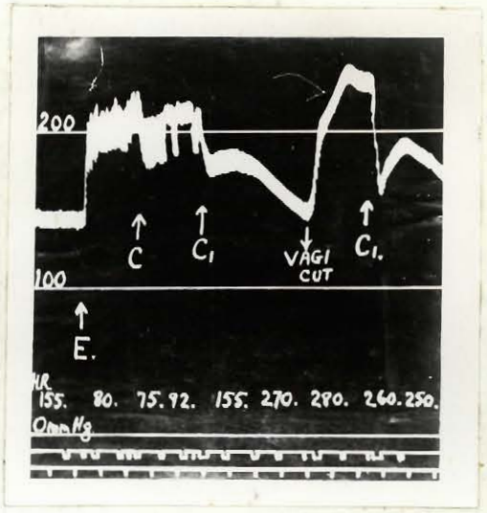


Fig. 8 Dog, Male, 7.6 kg. Pentobarbital. At E, 5 mg./kg. of ephedrine; at C, and at each C_1, 0.01 mg./kg., and 1.0 mg./kg., respectively, of chlorpromazine were injected. The vagus nerves were cut as shown (Vagi cut).

difference in sensitivity to chlorpromazine depressor response is observed in similar experiments during infusions of noradrenaline.

(d) Effects on morphinized-pentobarbitalized dogs.

In view of the well-known central nervous system depressant action of chlorpromazine, and its alleged potentiating effect on various anaesthetics and analgesic drugs, it was of interest to study the cardiovascular actions of the drug in the morphinized animal. Figure 9 shows results of an experiment of this type. As can be seen from the upper record, injection of a dose of 0.5 mg./kg. of chlorpromazine (A) during the initial adrenaline infusion, leads to a more or less complete antagonism of the pressor response to adrenaline but no significant change in heart rate. It is to be noted that the control heart rate in this experiment is quite low (65 per min.) due to the previous morphinization. When the adrenaline was discontinued, there was a slight, transient fall in blood pressure associated with a marked tachycardia. This latter effect disppeared within 10 minutes, and the re-infusion of adrenaline now induced little pressor response, although the heart slowed definitely from 96 per min. to 50 per min. -- three minutes after the infusion was restarted. Repetition of the chlorpromazine at this time (A) produced only a slight further decline of the blood pressure but again some increase in heart rate. Cutting the vagus nerves now elicited only a slight rise in blood pressure but a marked tachycardia.

The lower record (Figure 9) shows in contrast the marked pressor responses observed following similar short infusions of adrenaline, and the lessened antagonism of these pressor responses by similar doses of chlor-promazine (A) after the vagi were cut. Moreover, as can be seen from the records, when the adrenaline infusion is stopped, the blood pressure falls rapidly to a low level.

Figure 10 shows a somewhat similar experiment in which chlorpromazine

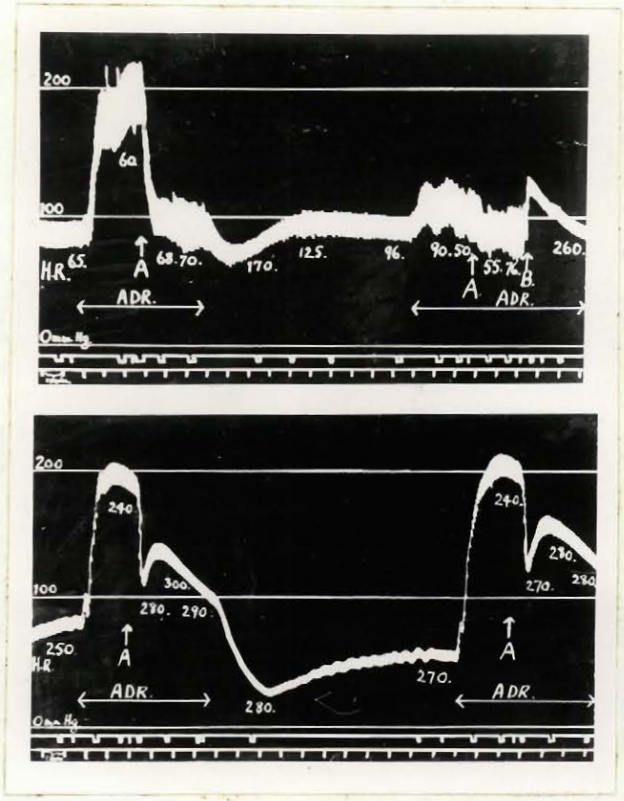


Fig. 9 Dog, Female, 8.5 kg. Morphine and Pentobarbital. Blood pressure and associated heart rates per minute (H.R.). Continuous infusions of adrenaline (0.01 mg./kg./min.) are shown by the arrows (ADR). At each A, 0.5 mg./kg. of chlorpromazine was injected (At B, Vagi cut). An interval of ten (10) minutes elapsed between the upper and the lower records.

was injected during noradrenaline infusions before and after vagotomy. Again, it may be seen that during the first infusion of noradrenaline, injection of 0.01 mg./kg. of chlorpromazine (A), produced a slight transient dip in the blood pressure curve. Definite antagomism of the noradrenaline rise occurs following injection of 0.5 mg./kg., as shown at B. When the noradrenaline infusion is stopped, there is a progressive acceleration of the heart (Nos. 7, 8 and 9) more or less similar to that shown in the adrenaline experiments (Figure 9). When the noradrenaline infusion was restarted, there was a good vagal reflex slowing of the heart (No. 10) which was progressively blocked by injection (B) of 0.5 mg./kg. of chlorpromazine, and the heart rate now accelerated (No. 11). Following vagotomy the usual sustained tachycardia ensued, as shown at the end of the tracing and reinfusion of noradrenaline produces a good pressor effect with associated tachycardia after previous vagotomy.

It appears from the above experiments that the sensitivity to the adrenaline and noradrenaline reversal is much greater in morphinized pentobarbitalized dogs than in non-morphinized pentobarbitalized animals. It is also evident that doses of chlorpromazine which antagonize the hypertensive actions of adrenaline and noradrenaline do not abolish the reflex vagal slowing of the heart under these conditions. However, the continued presence of the adrenaline or noradrenaline in the circulation seems to be in some way or other involved, since when the infusions are stopped, the heart quickly accelerates without much change in the blood pressure. There is some possibility that the drug might increase the central vagal inhibition due to morphine. However, with higher doses, some peripheral vagal block might occur, as indicated by the progressive tachycardia observed.

(e) Effects on spinal cats.

In contrast to the more or less variable responses observed in the

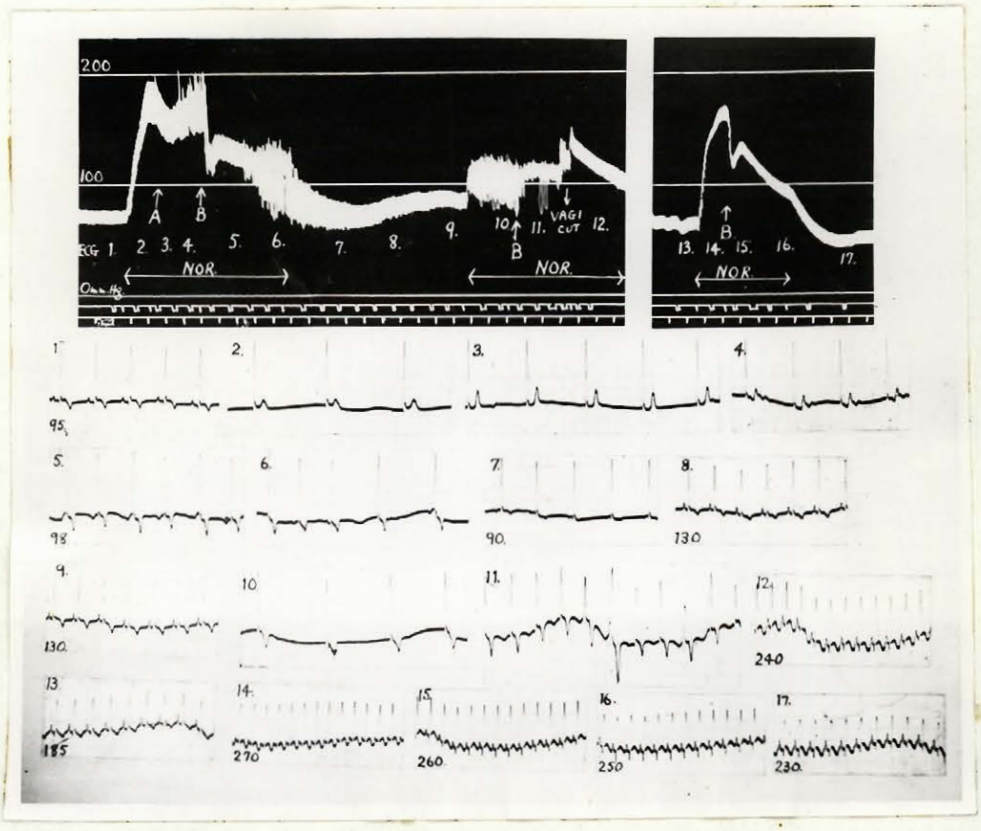


Fig. 10 Dog, Male, 11.4 kg. Morphine and Pentobarbital. Blood pressure and associated electrocardiograms (Lead 2) as numbered. Continuous infusions of noradrenaline (0.01 mg./kg./min.) are shown by arrows (NOR). At A, 0.01 mg./kg., and at each B, 0.5 mg./kg. of chlorpromazine were injected, respectively. The vagus nerves were cut towards the end of the infusion of noradrenaline, as marked (Vagi cut). An interval of fifteen (15) minutes elapsed between Nos. 12 and 13.

above-described experiments, the responses to injections of chlorpromazine, during infusion of either adrenaline or noradrenaline, were rather similar and uniform in the spinal cat. Figure 11 shows examples of these results. Thus, as shown comparatively in the upper and lower records, the effects of repeated injections of small doses (0.02 to 0.04 mg./kg.) of chlorpromazine injected during continuous infusions of adrenaline (upper record) and noradrenaline (lower record), were essentially similar. Again, in Figure 12, are shown good reversal effects following injections of larger doses (1 mg./kg.) of chlorpromazine (A) during infusion of either adrenaline (Exp. 2) or noradrenaline (Exp. 3) in spinal cats. There is little doubt from the above records that in the spinal cat the hypertensive response to adrenaline and noradrenaline can be definitely antagonized or inverted by chlorpromazine. The latter records also show that superimposed injections of both adrenaline (B) and noradrenaline (C) are rather similar when given following chlorpromazine and during either type of infusion. These results would suggest that chlorpromazine is not only a peripheral anti-adrenergic agent but that its cardiovascular effects in the intact animal might be influenced by its central actions.

(f) Effects of chlorpromazine on chloroform-adrenaline arrhythmias and ventricular fibrillation.

It has previously been shown (Melville, 1948) that following intravenous injection of a dose of 0.02 mg./kg. of adrenaline during brief periods (3 to 5 minutes) of chloroform inhalation in pentobarbitalized artificially respired dogs, ventricular fibrillation invariably ensues. Figure 13 shows some examples of the results obtained in some experiments in which the effect of chlorpromazine upon this response was tested. As can be seen from the upper records, when the adrenaline injection (A) is preceded (C) by an injection of 0.5 mg./kg. of chlorpromazine, there is a moderate pressor response associated with marked tachycardia (Nos. 5a and 5b) but no evidence of ventricular extrasystoles or

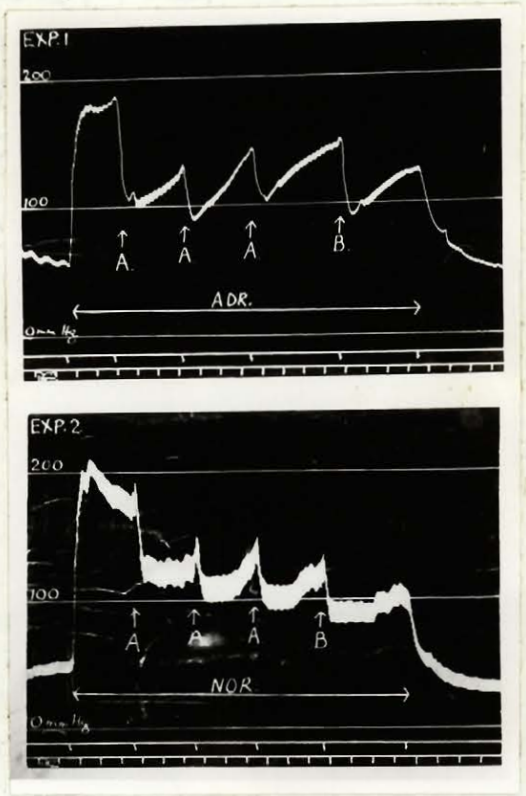


Fig. 11 Blood pressure records. Exp. 1 Spinal cat, Male, 2.65 kg. The continuous infusion of adrenaline (0.01 mg./kg./min.) is shown by the arrow (ADR). Exp. 2 Spinal cat, Male, 2.25 kg. The continuous infusion of noradrenaline (0.1 mg./kg./min.) is shown by the arrow (NOR). In each experiment, at each A, 0.02 mg./kg., and at each B, 0.04 mg./kg., respectively, of chlorpromazine was injected.

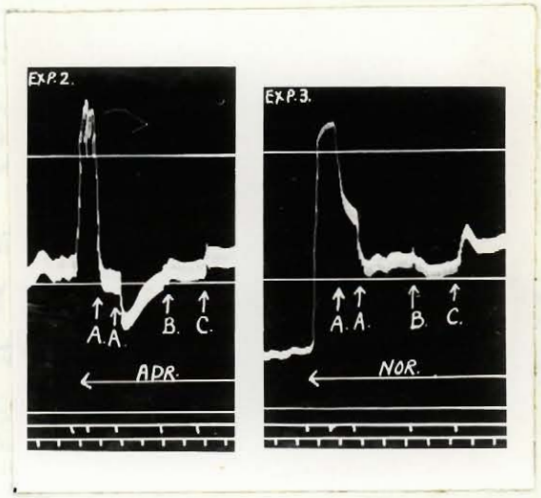
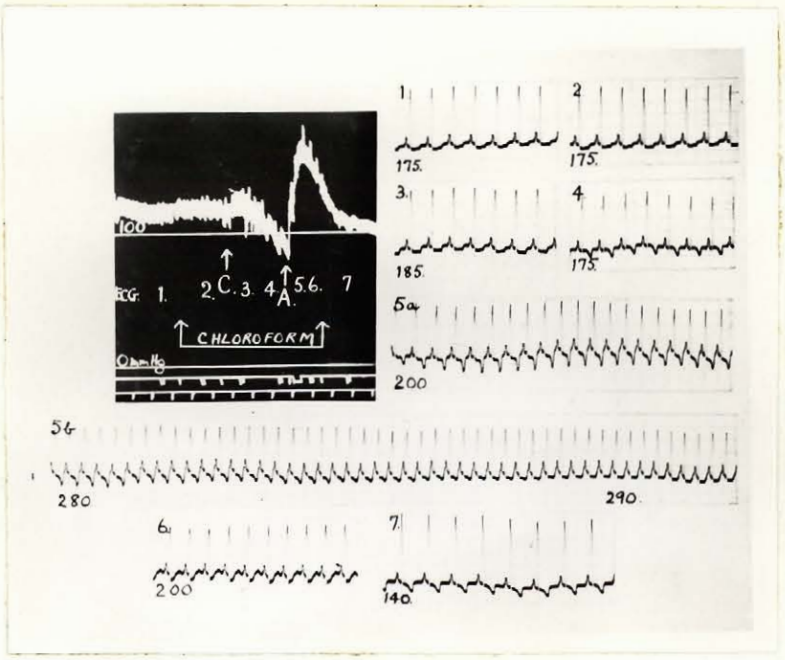


Fig. 12 Exp. 2 Blood pressure records. Spinal cat, Male, 3.9 kg. Atropinized. The continuous infusion of adrenaline (0.2 mg./kg./min.) is shown at the arrow (ADR).

Exp. 3 Spinal cat, Male, 3 kg. Atropinized. The continuous infusion of noradrenaline (0.2 mg./kg./min.) is shown at the arrow (NOR).

In each experiment, at the arrow marked A, 1 mg./kg. of chlorpromazine, was injected; at each arrow marked B, superimposed test injections of adrenaline (0.1 mg.), and at each C, superimposed test injections of noradrenaline (0.1 mg.), were given, respectively.



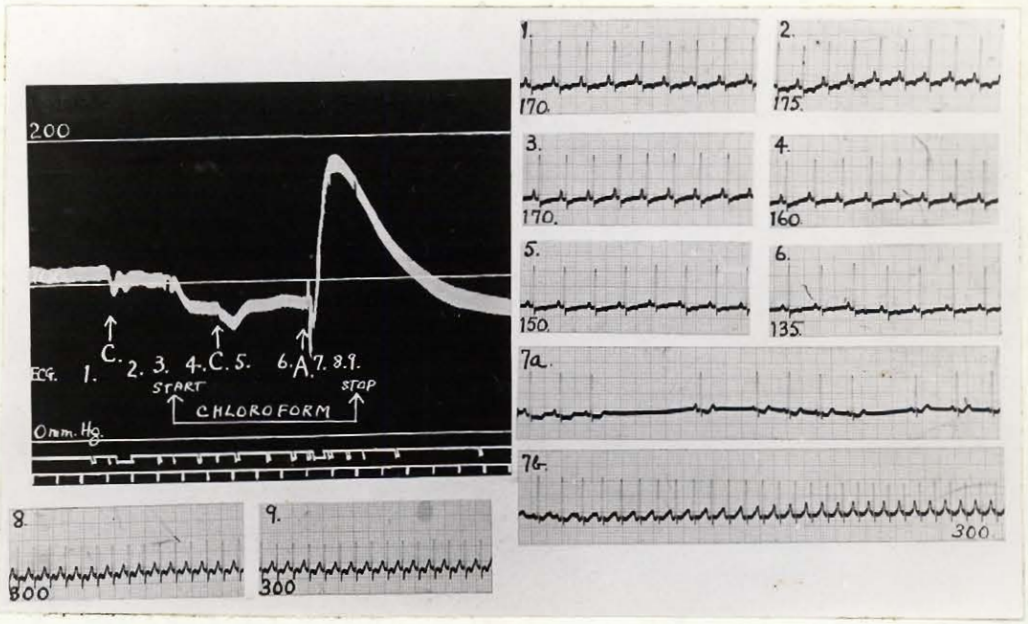


Fig. 13 Blood pressure and associated electrocardiograms (Lead 2), as numbered. In both records, the duration of chloroform inhalation is shown by the arrows. Pentobarbital anesthesia.

Upper record. Dog, Female, 11.1 kg. At A, 0.02 mg./kg. of adrenaline was injected. At C, 0.5 mg./kg. of chlorpromazine was injected. Lower record. Dog, Female, 11.6 kg. At each C, 1 mg./kg. of chlorpromazine, and at A, 0.4 mg./kg. of adrenaline, respectively, were injected. Cardiogram records Nos. 7a and 7b are continuous.

fibrillation. The lower record shows a similar type of result and as shown at C (between Nos. 1 and 2) an initial injection of a large dose (1 mg./kg.) of chlorpromazine induces little change in blood pressure or heart rate, and repetition of this injection during chloroform inhalation induces only a slight transient fall in blood pressure. At this point, a dose of 0.4 mg./kg. of adrenaline (A), that is twenty (20) times the usual fibrillating dose, was injected and as can be seen from the record there was only a slight initial dip in the blood pressure followed by a good rise. These changes were also associated with an initial slowing of the heart (No. 7a) followed by marked and sustained tachycardia (Nos. 7b, 8 and 9). It is therefore clear that moderate doses of chlorpromazine (0.5 to 1.0 mg./kg.) can effectively prevent cardiac arrhythmias and ventricular fibrillation under these conditions. More will be said about this later.

(g) Summary.

1. Chlorpromazine (1 to 10 mg./kg.) injected intravenously into pentobarbitalized dogs induces a fall in blood pressure which is not significantly affected by vagotomy, atropinization or injection of hydergin . This depressor response is transitory except with excessive doses.

2. Associated with the depressor response, after small doses, there is an initial cardiac acceleration and slight depression of T wave, but no change in either P-R interval or QRS complexes. These effects are abolished by previous vagotomy or atropinization.

3. Associated with the depressor response, after large doses (10 mg./kg.), there is conversely slowing of the heart rate, with initial elevation of the T wave followed by inversion. These effects are not prevented by either atropinization or vagotomy.

4. Following single injections of small doses of chlorpromazine (1 mg./kg.), the pressor responses to both adrenaline and ephedrine are reversed, while

that to noradrenaline, though diminished, is not inverted. The cardiac acceleration induced by adrenaline and noradrenaline is not prevented by chlorpromazine.

5. During continuous infusion of adrenaline or following injection of ephedrine, small doses (0.01 to 0.5 mg./kg.) of chlorpromazine induce immediate significant depressor responses in pentobarbitalized dogs. These responses appear to be potentiated by morphinization, and are conversely lessened by vagotomy or atropinization.

6. During continuous infusion of noradrenaline, chlorpromazine (0.01 to 5 mg./kg.) induces no significant sustained reversal effect, although the usual transient depressor responses of the drug are still demonstrated. In previous morphinized animals, chlorpromazine (0.1 to 0.5 mg./kg.) produces sustained reversals of noradrenaline infusions. This effect is lessened by vagotomy or atropinization.

7. In the spinal cat, chlorpromazine (0.02 to 0.04 mg./kg.), when injected during continuous infusion of either adrenaline or noradrenaline, produces almost identical depressor responses.

8. Finally, chlorpromazine (0.5 to 1 mg./kg.) effectively prevents ventricular extrasystoles and fibrillation after adrenaline (0.02 to 0.4 mg./kg.) during chloroform, but marked tachycardia still ensued.*

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* Part of the preceding results were presented at the Research Psycho-Pharmacological Conference, Montreal, March 26, 1955.

II. Studies on the Heart-Lung Preparation.

(a) Methods.

The dog heart-lung preparation, as described by Starling and Knowlton (1912), was used. The apparatus was modified in order to permit continuous recording of the cardiac output. Two dogs were employed in each experiment. The first and larger animal was bled from a common carotid artery under light ether anaesthesia. The blood was defibrinated and strained through several layers of cheesecloth and kept at 38°C. until ready for use, that is for 1 to 2 hours generally. The second dog, weighing 8 to 12 kilograms, was anaesthetized either with chloralose (100 mg./kg.) or with pentobarbital sodium (40 mg./kg.), injected intravenously. Following which a midline incision was made in the neck; the right and left common carotid arteries tied off; and the vagus nerve sectioned. The trachea was cannulated and artificial respiration maintained with a Starling pump. The incision was then extended through the skin over the full length of the sternum, and any bleeding from the intercostals checked with haemostats. The thorax was opened by sawing through the middle of the sternum, care being taken to avoid injuring the lungs. The internal mammary arteries were tied off and cut. The thoracic cavity was maintained opened, as widely as possible, by retracting the chest walls with strong twine. The phrenic nerves were sectioned and the thymus gland tissue tied off and excised between ligatures. The azygos vein and the left subclavian artery (S.A.) were tied off. The superior vena cava and the innominate artery were freed of connective tissue and two loose ligatures placed around each, in order to facilitate cannulation later. Loose ligatures were also placed around the inferior vena cava and the descending aorta, to permit tying them off as quickly as possible, when necessary.

The general arrangement of the apparatus as employed is shown diagrammatically in Figure 14. The reservoir (A) is filled with defibrinated

blood, and the venous cannula (B) completely filled with blood. The venous cannula also contains a thermometer passing through a rubber stopper, as shown, ~~at Y.~~ The blood reservoir (A) as well as the coils (L) conveying the blood from the heart back to the reservoir, are immersed in a large circulating water-bath, maintained thermostatically at 39°C. The distal ligature on the superior vena cava (S.V.C.) is first tied and a bull-dog clamp placed on the proximal side of the vein near the atrium. The vein is then cannulated between the ligature and the clamp. The distal ligature on the innominate artery (I.A.) is also tied off, and the artery cannulated (C). Following insertion of the arterial cannula, the animal's blood is allowed to flow through the side-arm (D), collected and defibrinated. Concurrently the venous inflow clamp is removed and the inferior vena cava and descending aorta tied off, as quickly as possible. When the heart and lungs have been flushed out with sufficient defibrinated blood, the side-arm (D) is closed, and the blood from the innominate artery now passes through the artificial "peripheral resistance" (I), thence by (J), either through the coil (L) and back to the reservoir, or through (N) to the Weese Stromuhr (P), and thence, through (M) and the coil (L) back to the reservoir. In all experiments, the volume of blood in the reservoir (A) was kept at a constant volume of 700 cc.

The "peripheral resistance" consisted of a 10 cm. length of thin-walled rubber tubing (I), interposed between the inlet and outlet of a larger glass cylinder (F). The desired degree of pressure on the rubber tubing is obtained by increasing the air pressure in the glass cylinder with the aid of the air pump (X), and reading the pressure on a suitably attached mercury manometer. The "peripheral resistance" was adjusted to a pressure of 80 to 100 mm. of mercury in all experiments. The so-called "blood pressure" in the preparation is recorded by a mercury manometer connected at E.

The cardiac output was recorded by a signal magnet connected with

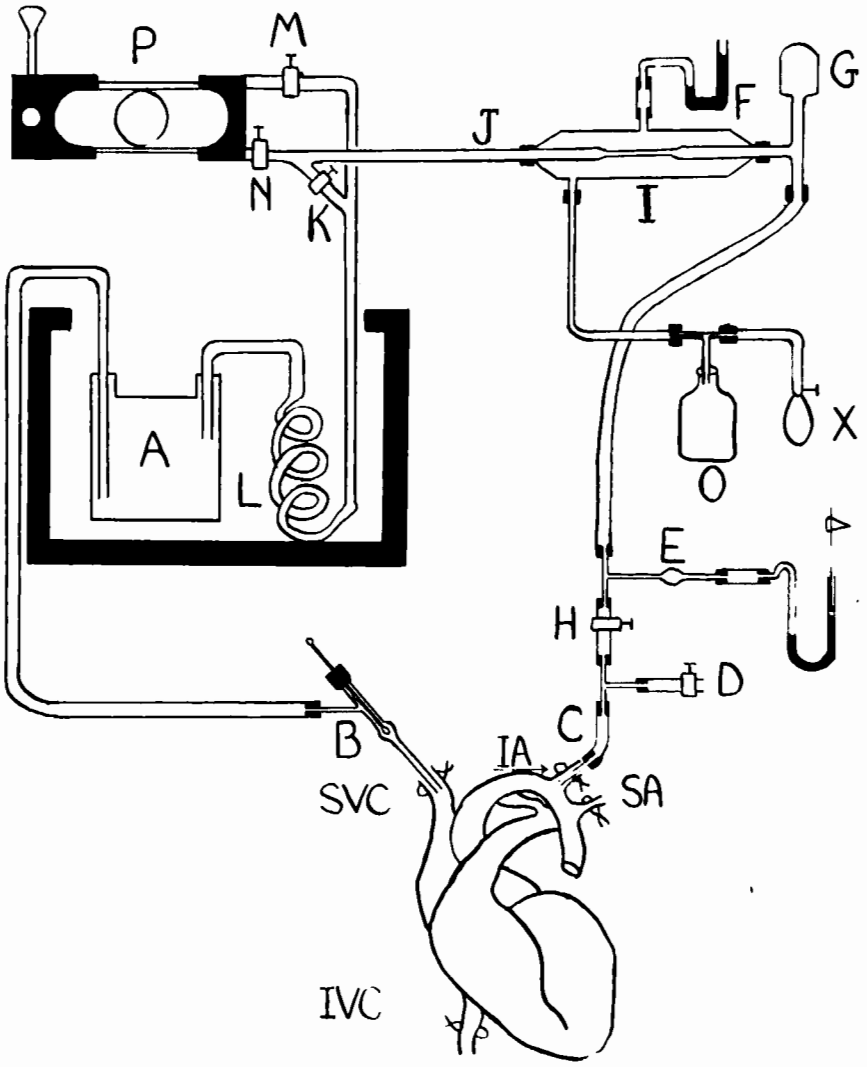


Fig. 14 Diagram of the Heart-Lung Apparatus.

the stromuhr (P), a devise originally described by Weese (1932). The instrument used was manufactured by Karl Heuwing, Munich, Germany.

Figure 15 shows diagrammatically some details of the instrument. It consists essentially of a glass cylinder (A) within which is a closely-fitting but freely movable plastic ball (B). The direction of flow through the outlet at G is determined by the movement of the lever (I), which can be pulled back or forth by the electromagnets (K and H). These magnets are connected to a 10 volt battery. The cylinder and its connections must be completely freed of air bubbles at the beginning of the experiment.

As the blood enters through C (following the arrow), it pushes the ball (B) towards D, the blood originally contained in the cylinder now passes through the tube (E) and out at F. When the ball reaches the end of the cylinder (D), it closes an appropriately arranged electrical contact so that the electromagnet H is energized. This pulls on the lever (I) and reverses the direction of flow at the outlet (G). As a result, the blood entering at C now passes through E and pushes the ball towards J, the blood finally escaping through F. When the ball reaches J, electromagnet (K) is now energized, and the lever (I) pulled out so that the direction of flow through the outlet (G) is again reversed. Thus, the ball moves back and forth continuously. The electromagnets are connected with a signal magnet, which records on a kymograph at each change in direction of flow. From the record obtained the cardiac output can be calculated (see legend of Figure 16).

Throughout all experiments, electrocardiograms (Lead II) were taken at frequent intervals with a Sanborn Visocardiette. All solutions of drugs for injections were freshly made up in isotonic saline, and injected through the rubber tubing connecting the reservoir and the venous cannula, close to the insertion in the superior vena cava. Chlorpromazine, adrenaline, noradrenaline, and acetylcholine, as previously referred to, were used.

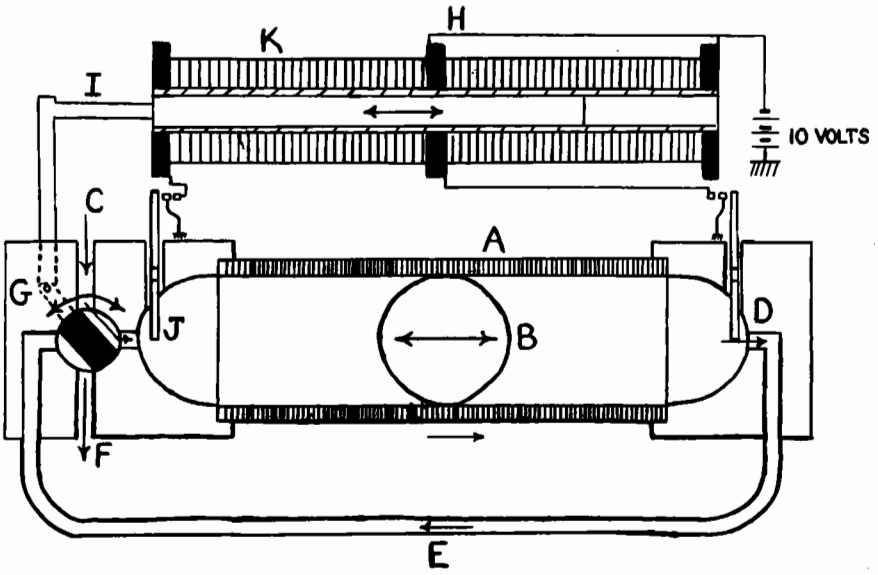


Fig. 15 Diagram of the Weese Stromuhr.

(b) Effects of chlorpromazine upon cardiac output and heart rate and its influence upon the responses to adrenaline, noradrenaline and acetylcholine.

Since the effects of chlorpromazine upon the dog heart-lung preparation have not been previously investigated, so far as we are aware, in most of these experiments the responses to varying doses of the drug were first tested. Thirteen different experiments were performed. Some typical examples of the results obtained are presented in Figures 16, 17 and 18. These figures are taken from six different experiments. They all show injections which were made at the beginning of each experiment. The upper record of Figure 16 shows examples of the effects observed following injections (C) of 5 and 10 mg. of chlorpromazine. There was no change in either heart rate, blood pressure or cardiac output. There appears to be slight increase in the amplitude of the record, suggesting some dilatation of the heart.

Slight depressant effects to chlorpromazine became apparent when the dose was increased to 20 mg. (Figure 16, lower record). This concentration usually produced a small increase in heart rate and also in cardiac output. The level of the blood pressure, however, remained about the same.

With higher concentrations of chlorpromazine (40 mg.) (Figure 17), a more pronounced depression occurred. On the average (3 experiments) the heart rate decreased 12 percent. The maximum effect took place about 60 seconds after the injection. The average cardiac output also was reduced approximately 44 percent, and this, too, occurred between 60 to 80 seconds following the injection. However, the blood pressure did not show any marked corresponding decrease. Recovery of both rate and cardiac output was in evidence about 120 seconds after these injections.

In this preparation, a single injection of 50 mg. of chlorpromazine (Figure 18) appeared to be toxic to the heart. In general, within 40 to 50

seconds, the rate was depressed 20 percent (average of 3 experiments) and the cardiac output was greatly reduced. After 100 seconds, the latter was decreased by 77 percent; and fell almost to zero within an average of 125 seconds. The blood pressure also was markedly and rapidly diminished. If no attempt was made to resuscitate the heart, it usually continued to beat in a depressed state for some time and then stopped.

Chlorpromazine could not inhibit the stimulating effects of adrenaline or noradrenaline on the heart rate, cardiac output or "blood pressure", whether the amines were given either immediately or as long as 10 minutes after the drug. Figures 16 and 17 show injections of either adrenaline or noradrenaline (100 μg.) after chlorpromazine (5 to 40 mg.). It is evident from the records that the stimulating effects of the amines are still well marked.

Adrenaline (100 μg.) given after 50 mg. of chlorpromazine (Figure 18, lower record) produced a slight stimulation of the heart rate, but did not increase the blood pressure. However, a tenfold increase (1 mg.) of adrenaline resulted in a prompt recovery of the heart; the rate returned to control values whereas the blood pressure and cardiac output exceeded control values.

Low concentrations of chlorpromazine (10 and 20 mg.) had no effect on the acetylcholine response in this preparation. This can be seen in Figure 16. However, by comparing the response to acetylcholine before and after chlorpromazine in Figure 17, it can be seen that the response to the former (40 mg.) though not abolished is diminished. The decrease in the output is about the same, but the depressant effect on the heart rate and blood pressure is less after chlorpromazine than before. This was seen consistently in all experiments when chlorpromazine was employed at a concentration of 40 mg. or more.

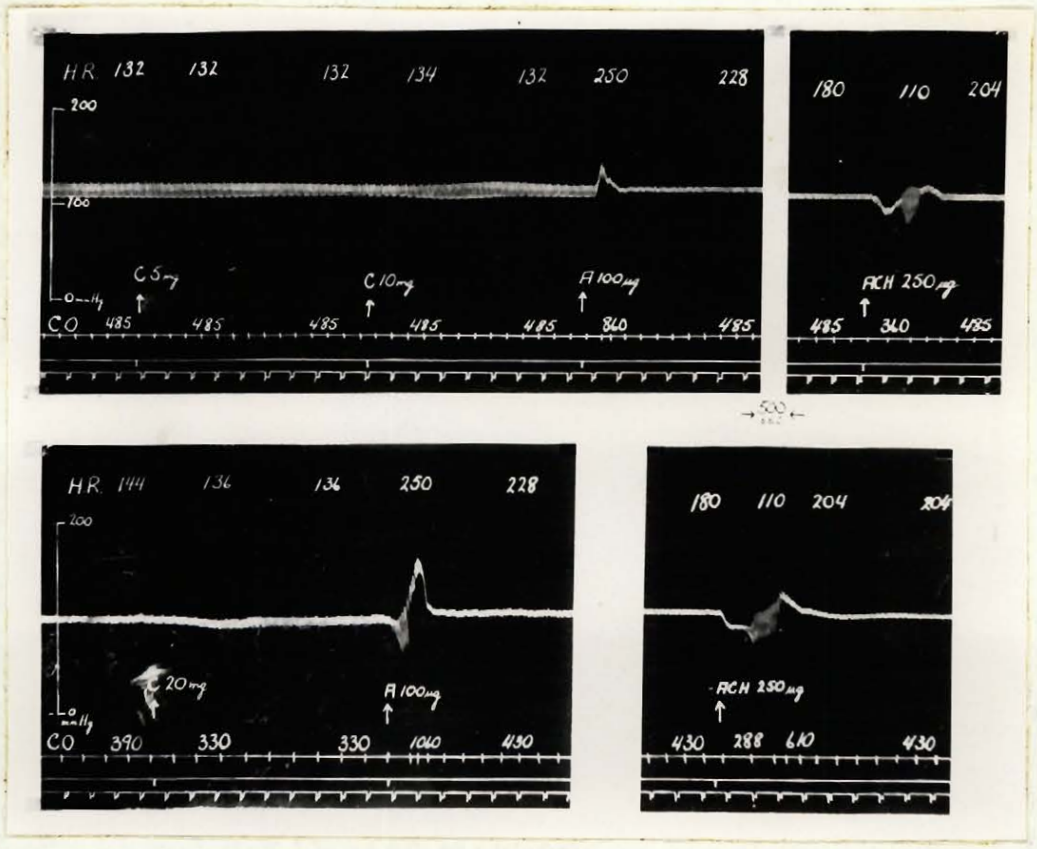

HR 132 132 132 134 132 250 228
200
100
0 mm Hg
C 5 mg
C 10 mg
A 100 mg
CO 485 485 485 485 485 840 485
180 110 204
ACH 250 mg
485 340 485
HR 144 136 136 250 228
200
0 mm Hg
C 20 mg
A 100 mg
CO 390 330 330 1040 430
180 110 204 204
ACH 250 mg
430 288 610 430

FIG. 16

Fig. 16 Heart-Lung preparation. Records from two (2) experiments. In this, and all the succeeding figures, the records are all arranged similarly from above downwards.

1. Figures at the top are the heart rate per minute counted from the electrocardiogram (H.R.).
2. Blood pressure recording.
3. Cardiac output (C.O.) in c.c./min. Each mark corresponds to the passage of the ball from one end of the Stromuhr to the other, and the interval represents a measured output of 55 c.c. Figures above this line are the calculated rates of the cardiac output in c.c./min.
4. Marker.
5. Time intervals of 10 seconds.

At A, adrenaline (100 µg.) injection; at ACh, acetylcholine (250 µg.) injection; at C, top record, chlorpromazine (5 and 10 mg.) injection; lower record, chlorpromazine (20 mg.) injection.

In the lower record, the time between the two tracings is 210 seconds.

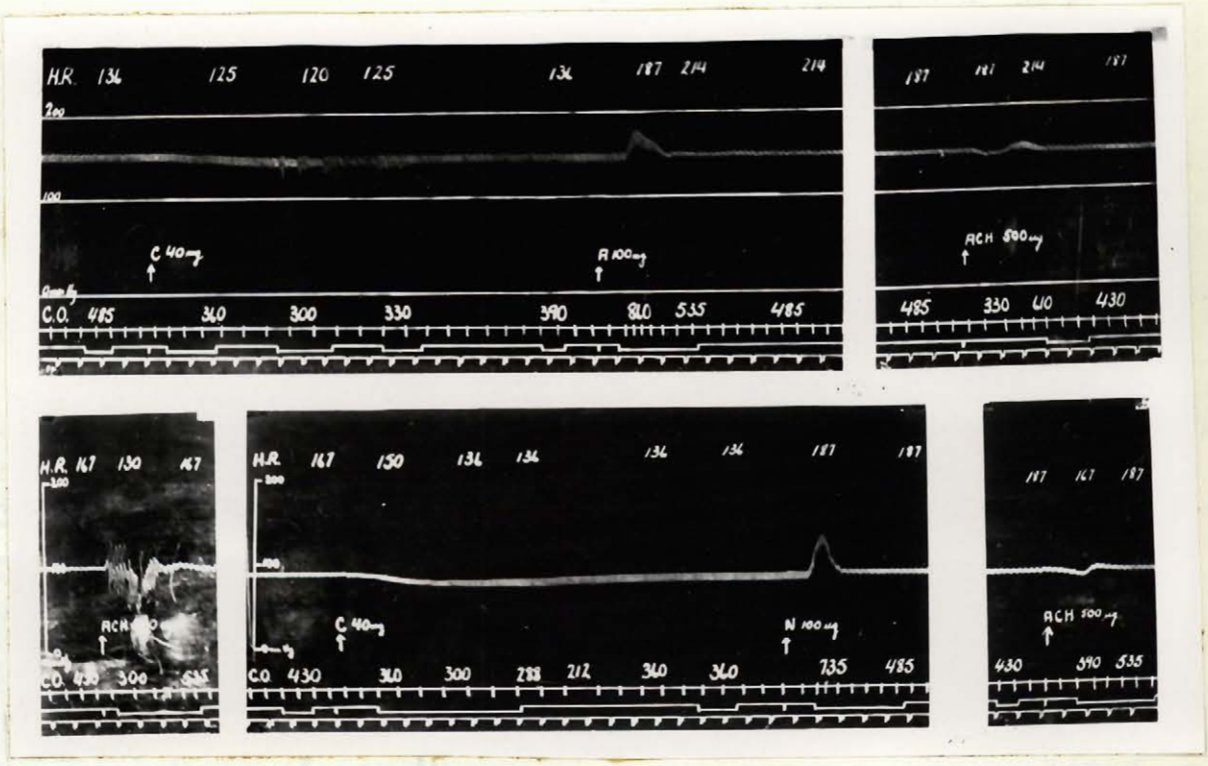


Fig. 17 Heart-Lung preparation. Records from two (2) experiments (see legend, Fig. 16). In the above records, the injections are as follows: at A, adrenaline, 100 µg., at ACH, acetylcholine, 500 µg., at N, noradrenaline, 100 µg., and at C, chlorpromazine, 40 mg. In the top record, the time between the two tracings is 600 seconds. In the lower record, the time between the first two tracings is 100 seconds, and between the second two tracings 300 seconds.

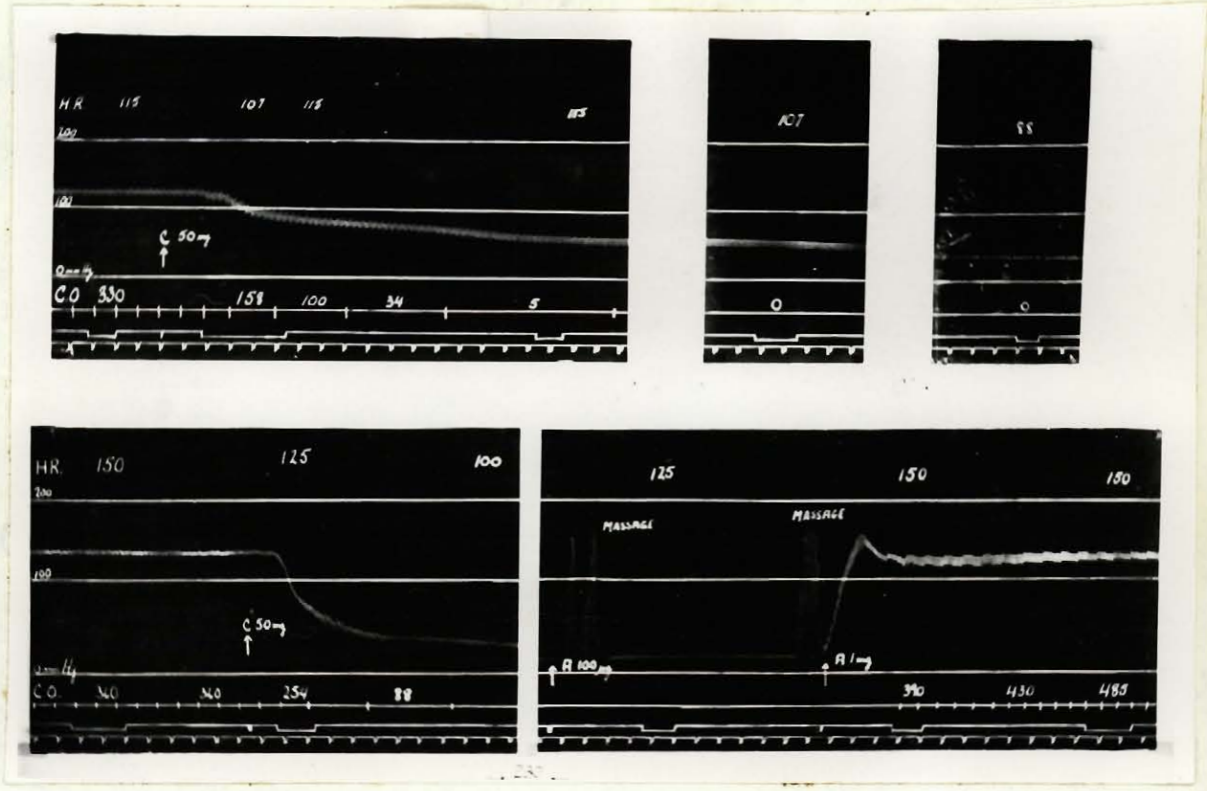


Fig. 18 Heart-Lung preparation. Records from two (2) experiments (see legend, Fig. 16). In the above records, the injections are as follows: at C, chlorpromazine, 50 mg., and at A, adrenaline, 100 µg. and 1 mg., respectively. In the top record, the time between the first two tracings is 70 seconds and between the second two tracings 200 seconds. In the lower record, the time between the tracings is 230 seconds.

(c) Summary.

1. Chlorpromazine in doses of 5 to 10 mg. exerts no effect on cardiac output and heart rate in the dog heart-lung preparation, under the conditions employed. In higher doses (20 to 40 mg.) the drug leads to progressive depression of both cardiac output and heart rate, but complete recovery ensues spontaneously. Following excessive doses (50 mg.) there is marked depression and ultimate complete cessation of cardiac activity.

2. Chlorpromazine in doses ranging from 5 to 50 mg. does not prevent the stimulating actions of adrenaline and noradrenaline (100 µg.) in this preparation. Following excessive chlorpromazine (50 mg.) restoration of the circulation can often be attained by injection of large doses of adrenaline.

3. Chlorpromazine in doses of 5 to 20 mg. does not affect the cardiac responses to acetylcholine (250 µg.). Higher doses (40 mg.) diminish or partially antagonize the response to acetylcholine (250 to 500 µg.). It is therefore clear that the dog heart-lung preparation is relatively resistant to the effects of chlorpromazine (see isolated rabbit heart experiments).

III. Studies on Isolated Perfused Rabbit Hearts.

(a) Methods.

In all of these experiments the isolated rabbit's heart preparation (Langendorff method) was used. The animal was first injected intravenously with heparin (1 mg./kg.), then killed by a sharp blow on the back of the neck region. As soon as possible the thorax was opened and the excised heart placed in Locke's solution at room temperature. The extraneous tissues were quickly removed and the heart attached to the perfusion apparatus, previously suitably arranged. This whole procedure usually did not require more than 2 to 3 minutes. The glass cannula of the perfusing system was inserted into the aorta close to the heart. The perfusion apparatus employed was that described previously by Lu and Melville (1950). This is a modification of a "moving globule method" described by Stehle (1932). The apparatus used permitted simultaneous and continuous registration of coronary inflow and of heart contractions on a moving kymograph.

The apparatus and its general method of operation.

The actual apparatus used was slightly modified from that described by Lu and Melville. Figure 19 shows diagrammatically its essential features. Locke's solution is put in the reservoir which is placed 60 to 80 cm. above the heart, maintained at a temperature of 38°C., and oxygenated continuously. Tube A leads from this reservoir and is attached to a three-way stopcock at B. This stopcock permits, as necessary, connection of the reservoir either directly with the heart through tube C, or indirectly through tube D, via the channels shown by the arrows, and which lead finally to the side arm attached to C. In both instances the perfusing fluid, after leaving tube C, passes through a condenser maintained at the temperature of the reservoir, and thence to the aortic cannula and the heart, in the usual manner of the Langendorff method. These latter attachments are not included in the diagram

in order to avoid complicating it unnecessarily. When a record of the coronary flow changes is to be made, stopcock B is turned so as to connect tube A to tube D, while the connection to tube C is blocked. The perfusion fluid thus flows from the reservoir to tube D as shown in the diagram. From this point the perfusion fluid either passes through one system controlled by tubes E and G or another controlled by tubes F and H. These four tubes are intercepted with short segments of rubber tubing which are either opened or closed in pairs by two electromagnetic clamps I and I'.

The perfusing fluid (following the arrows) then passes down through the horizontal tube K, in which is placed an indicator, made of a short section of a blue-coloured glass plunger taken from a tuberculin syringe. This indicator was designed to fit snugly into K, so that it could be easily moved along this tube by the perfusing solution. The closeness of the fitting was checked so that no solution could pass over the sides. This was verified by injection of a solution of methylene blue into the fluid on one side of the indicator, and opening the outlet of the apparatus without having the heart attached. The methylene blue solution remained on the same side of the indicator during its complete passage in one direction. This test was repeated in the other direction with similar results. At M and M' on the diagram, that is at either end of the horizontal tube K, two photo-electric cells are installed. L and L' are the light sources of these cells. When the solution is coming in through tube D and down through E, the indicator is moved to one end and it casts a shadow on the photoelectric cell M' in this case. This sets up a current which through a relay energizes the electromagnetic clamp I to close tubes G and E and simultaneously the electromagnetic clamp I' is de-energized to open tubes, F and H. The perfusion fluid now flows from D through F and out H to the heart.

During this time G and E remain closed and as soon as the indicator reaches in front of the photoelectric cell M, as shown in the diagram, the processes are reversed. Thus the shadow of the indicator now activates photocell M to set up a current which energizes electromagnetic clamp I', thus closing tubes F and H and at the same moment opening G and E. Again the perfusion fluid comes down D through E. It is thus possible to have a continuous inflow of the perfusing fluid into the coronary vessels during this back and forth movement of the indicator in tube K.

Other information concerning the apparatus.

The photocells M and M', their light sources, L and L' with their control housing (not shown in diagram) are two standard Photoswitch control units No. 20 DJI made by Photoswitch Inc., Cambridge, Mass. Each light source is placed at a distance of approximately 40 cm. from each cell.

In order that the moving indicator may cut off the light beam completely the face of the photoelectric cell is shielded except for a slit of about 15 mm. x 5 mm. Supports on the shield hold tube K in position. The whole apparatus is made in glass except for the rubber tubing under the electromagnetic clamps, and the two small lengths of rubber tubing at either end of K. These facilitated filling of the apparatus and were closed off during the experiment.

Kymographic registration of coronary flow.

By connecting the photoelectric cells to a signal magnet, the coronary flow can be registered on a kymograph. The heart contractions are recorded, pari passu from the left ventricle. The coronary flow rate is calculated from the known volume of fluid displaced by each passage of the indicator from one photoelectric cell to the other -- the rate of movement of the drum being known. The heart rate during the same period of time is calculated by counting the number of beats on the tracing. Full details of the arrangements

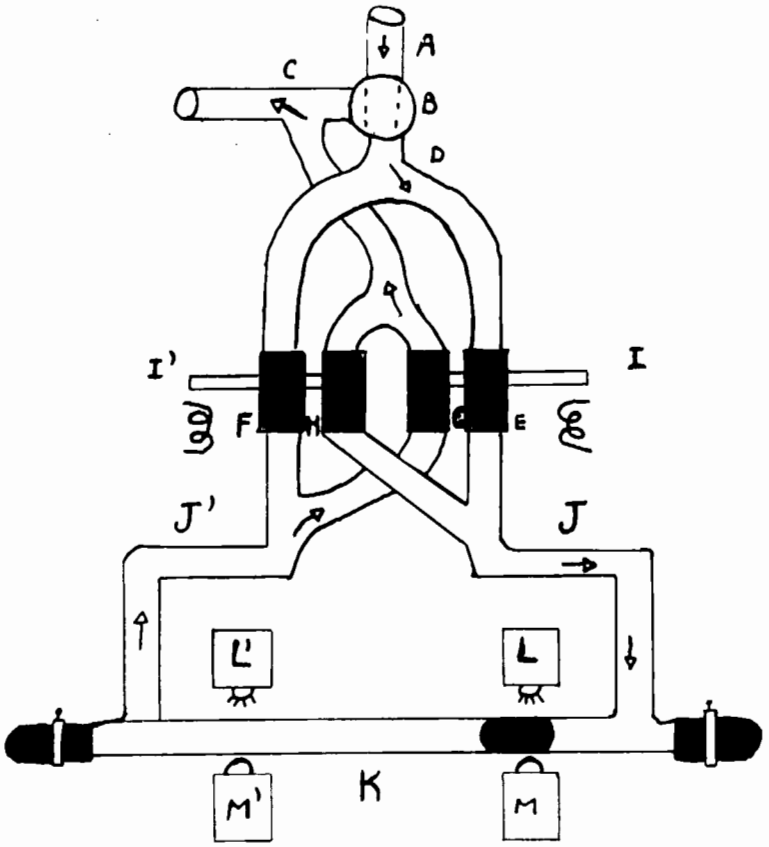


Fig. 19 Diagram of the apparatus employed for the perfusion of the isolated rabbit heart.

of the records in the various figures are given in the legend of Figure 20.

The original apparatus of Lu and Melville (1950) differed from the one described above in that two thin rubber membranes were inserted at J and J' in such a manner that the fluid in tube K is separated from the fluid perfusing the heart. The operation of the apparatus is essentially the same as that seen in Figure 19. The movement of the fluid in tube K being through the compression of the rubber membranes. This separation of the fluids is necessary when defibrinated blood is being used as the perfusion solution. Also the indicator employed by these workers was a globule of toluene dark-coloured with iodine (2% solution). The globule was injected by means of a small hypodermic needle.

Other details of experimental procedure.

The perfusion pressure employed was 60 cm. H_2O and the temperature 37°C. maintained by a thermostat. Both perfusion pressure and temperature were kept constant throughout the experiment.

The perfusion solution was normal Locke solution and contained:

NaCl	0.92%
KCl	0.042%
$CaCl_2$	0.024%
$NaHCO_3$	0.015%
Glucose	0.1%

NaCl, KCl and $CaCl_2$ solutions were diluted from a stock solution. Stock solutions of KCl and NaCl contained 0.84 percent of KCl and 18.4 percent of NaCl. $CaCl_2$ stock solution was 4.8 percent. $NaHCO_3$ and glucose solutions were made freshly every day.

In all experiments the heart was perfused with Locke solution for 15 minutes before commencing the recording. At first the coronary flow was very rapid, and if at the end of this 15 minute period the flow had not decreased definitely, and established at a constant rate, the heart was discarded.

All drugs used were made up freshly in aqueous solutions each day.

These were all diluted with Locke solution prior to injection. In order to prevent precipitation of chlorpromazine in Locke solution, it was necessary to add 1 drop of dilute HCl per 100 c.c. Locke.

The dose of any drug to be injected was always made up in a final volume of 1 c.c. The drugs used in the following experiments are the same as previously described (methods, I and II).

(b) Effects of chlorpromazine on coronary flow and heart contractions in the isolated perfused rabbit's heart.

The effects of injections of chlorpromazine were studied with doses ranging from 0.1 μg. to 1 mg. As stated above (Methods), it was necessary to lower somewhat the pH of the Locke's solution in order to render the chlorpromazine soluble. This decreased pH of Locke's solution could be shown to cause brief depression of the heart, and slight increase in the coronary inflow even in the absence of the drug. Indeed, when low doses (0.1 μg. to 1 μg.) of chlorpromazine were employed, the actions observed were so slight that it was difficult to determine whether the effects were due to the chlorpromazine or to the decreased pH. The lowest clearly effective dose of the drug in this preparation was of the order of 10 μg., and there is little doubt that with this and higher doses the effects observed were due to chlorpromazine and not to any change in pH. It is evident also that with increasing doses of chlorpromazine increasing effects on coronary flow and heart contractions were observed (Table I).

In Table I, an analysis of the changes observed in coronary inflow and amplitude and rate of heart contractions, as recorded in eleven (11) different experiments is presented. This table shows average values obtained when three different dose levels of chlorpromazine were studied. Some examples of the records obtained in such experiments are also shown in Figure 20.

As can be seen from these data, chlorpromazine causes a marked increase in coronary inflow. Following injection of 10 μg., within the initial 10

seconds there was an average increase of 107 percent over the control. The coronary inflow then decreased progressively with time, and two (2) minutes after the injection, had almost completely worn off. With a higher dose (100 μg.), the maximum average increase observed was 248 percent over the control values and this occurred 20 seconds after the injections. This intense increase in coronary inflow declined relatively slowly and had almost completely worn off five (5) minutes following the injection. With 500 μg. of chlorpromazine, the coronary inflow increased over the controls an average of 463 percent within the first 20 seconds. Even after 5 minutes the increased inflow was still maintained as high as 225 percent above the controls. Indeed, after 15 minutes, a detectable increase (21 percent) was still apparent. Figure 20 shows some typical examples of these effects, as produced by doses of 10, 100 and 500 μg. of chlorpromazine (upper, middle and lower records, respectively) and needs no detailed comment.

Chlorpromazine could also be shown to exert invariably a depressant effect on the amplitude of contraction. Following 10 μg., this depressant action was rather slight (Figure 20, upper record) and averaged only a decrease of 8 percent of the controls in 4 experiments. Moreover, the amplitude returned to normal within 2 minutes after the injection. With a higher dose (100 μg.) the depression was more intense and in 4 experiments the amplitude of contraction showed a maximum average decrease of 28 percent (Table I). It is apparent that the degree of the depressant response was not directly proportional to the concentration of drug used. After 5 minutes, the effect had diminished, but the initial amplitude of contraction was not restored. With 500 μg. of chlorpromazine the maximum average decrease in amplitude was 54 percent within 10 seconds, and again even after 15 minutes following the injection, the contractions were still quite markedly depressed (Figure 20, lower record).

Since in the perfused rabbit's heart preparation, there is usually a slowly progressive decrease of amplitude with time, it is difficult to know whether or not the continued depressant effect observed with these high concentrations of chlorpromazine is due entirely to the action of the drug. However, the normal decrease in amplitude after 15 or 20 minutes was never as great as that seen in these experiments. Therefore, it seems reasonable to assume that high concentrations of chlorpromazine have a long-lasting depressant effect on the amplitude of contraction.

In regard to the heart rate changes, Table I and Figure 20 demonstrate that chlorpromazine (10 µg.) causes an initial slight decrease in heart rate. This is followed, however, by a slight increase which reaches its maximum 50 seconds after the injection. At the end of 2 minutes, the rate is but slightly above the control values. Essentially comparable results were noted when the dose was increased to 100 µg. and even to 500 µg. -- recovery was slightly more delayed with the increasing dose, and the average increased rate was only slightly greater than that observed with the smaller dose.

A few experiments were also performed in which doses of 1 mg. and 2.5 mg. respectively of chlorpromazine were injected. In one experiment, the increase in coronary flow exceeded 600 percent above the control value. Ordinarily, there was also an associated increase in heart rate, but the amplitude was invariably very greatly depressed.

In all experiments even with the highest doses used, the heart always remained regular; no auricular or ventricular arrhythmias were ever seen in the isolated rabbit heart with chlorpromazine alone.

(c) Influence of chlorpromazine upon the responses to adrenaline and noradrenaline.

The objective of these experiments was to determine whether or not chlorpromazine could inhibit the effects of adrenaline and noradrenaline in the isolated perfused rabbit's heart. The actions of these latter drugs on

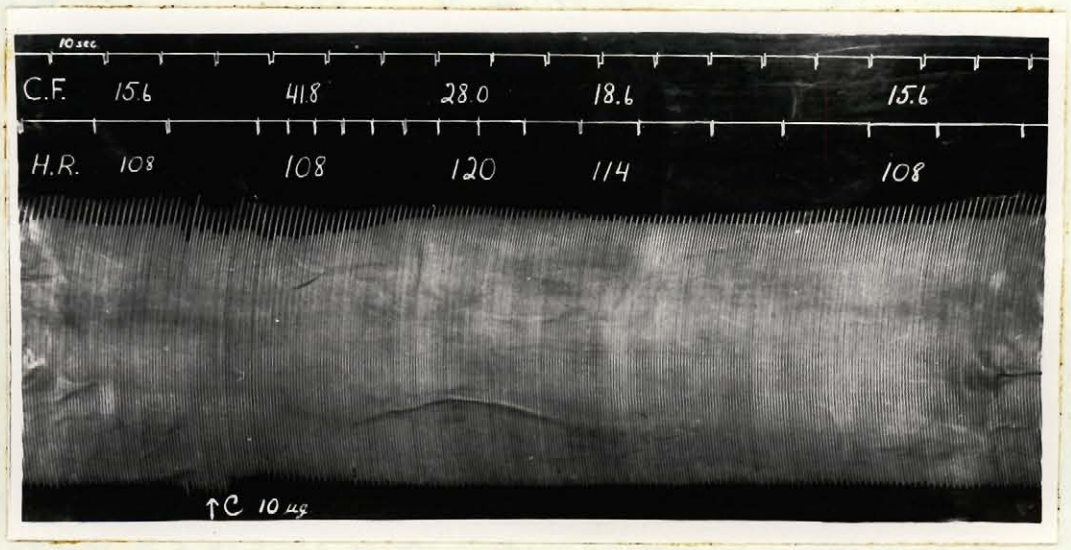
Table I.

Effects of injections of increasing doses of chlorpromazine upon coronary inflow and heart contractions (amplitude and rate) in isolated perfused hearts of rabbits.

Time after injection in seconds	% Increase (+) or % Decrease (-) in coronary inflow per min.			% Increase (+) or % Decrease (-) in amplitude of contractions			% Increase or % Decrease (-) in heart rate per min.		
	Dose injected 10 µg.	Dose injected 100 µg.	Dose injected 500 µg.	Dose injected 10 µg.	Dose injected 100 µg.	Dose injected 500 µg.	Dose injected 10 µg.	Dose injected 100 µg.	Dose injected 500 µg.
10	+107	+220	+365	-8	-15	-54	-4	-1	-4
20	+106	+248	+463	0	-21	-51	0	0	+2
30	+ 79	+230	+418	-1	-20	-46	+3	+7	+10
40	+ 61	+199	+391	-3	-10	-32	+5	+12	+13
50	+ 48	+172	+367	-2	-22	-36	+8	+13	+14
60	+ 17	+130	+311	-3	-14	-36	+8	+13	+14
120 (2 min.)	+ 2	+ 62	+225	0	-27	-38	+1	+6	+14
180 (3 min.)		+ 42	+136		-28	-47		+4	+10
240 (4 min.)		+ 22	+ 89		-24	-44		+3	+8
300 (5 min.)		+ 8	+ 72		-12	-44		0	+8
480 (8 min.)			+ 20			-46			0
900 (15 min.)			+ 21			-46			0
	(1)	(2)	(3)						

(1) and (2) Average figures for 4 experiments each.
(3) Average figures for 3 experiments.

49a.


10 sec.
C.F. 15.6 41.8 28.0 18.6 15.6
H.R. 108 108 120 114 108
↑C 10 μg

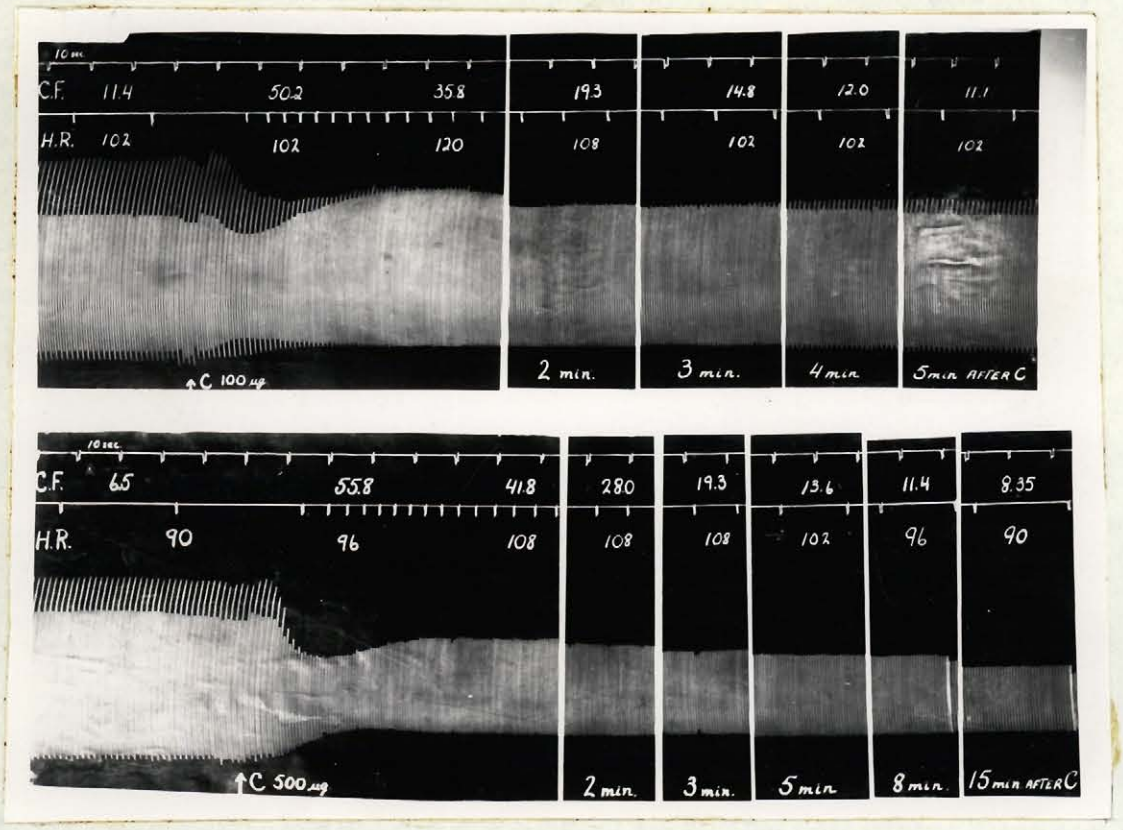

10 sec.
C.F. 11.4 50.2 35.8 19.3 14.8 12.0 11.1
H.R. 102 102 120 108 102 102 102
↑C 100 μg
2 min.
3 min.
4 min.
5 min AFTER C
10 sec.
C.F. 65 55.8 41.8 28.0 19.3 13.6 11.4 8.35
H.R. 90 96 108 108 108 102 96 90
↑C 500 μg
2 min.
3 min.
5 min
8 min.
15 min AFTER C

FIG. 20

Fig. 20 Isolated rabbit heart perfusion. Records from three (3) experiments. In this, and all the succeeding figures, the records are all arranged similarly from above downwards as follows:

1. Time intervals of 10 seconds.
2. Coronary inflow (C.F.) in c.c./min. Each mark corresponds to the passage of the indicator in front of each photoelectric cell alternately, and each interval represents an inflow of 3.7 c.c. The figures above this line are calculated rates of coronary inflow (c.c./min.). The figures below this line are the calculated heart rates per minute (H.R.).
3. Heart contractions with systole (above) and diastole (below) as recorded from a lever attached to the apex of the left ventricle.

In the above records at C, injections of chlorpromazine as follows: upper record, 10 μg., middle record, 100 μg., and the lower record, 500 μg.

this preparation have been studied extensively by Melville and Lu (1950) and Lu and Melville (1951), and need no repetition here. The general procedure employed was to give an initial injection of a certain dose of adrenaline or noradrenaline, allow the response to wear off, and then administer chlorpromazine. The adrenaline or noradrenaline injections were repeated at various intervals of time following the chlorpromazine. In these experiments, doses of one (1) microgram of adrenaline or of noradrenaline were used throughout.

It must be stated at the outset that although the qualitative responses to a given dose of adrenaline or noradrenaline are generally similar the effects of these drugs on the isolated rabbit heart preparation are not quantitatively repeatable from one preparation to the other and even fron one time to the next in the same preparation. Therefore minor differences in the responses to adrenaline or noradrenaline before and after chlorpromazine are of no significance.

Eight (8) different experiments were performed in which chlorpromazine was employed in dosages of 100 and 250 μg., and with both of these dosages the results obtained were similar. Table II gives an analysis of the data found with the higher dose (250 μg.).

In a general way the percentage increase in heart rate after the adrenaline was slightly greater following chlorpromazine than during the control period, in five experiments, and slightly less in the other three. With regards to the amplitude of contraction the percentage increase after the adrenaline was greater following chlorpromazine, in four experiments, but in the other four was less. None of the above mentioned differences in the adrenaline responses appeared significant. Some typical examples of the responses to adrenaline before and after chlorpromazine (100 and 250 μg.) can be seen in Figure 21 (upper and middle records).

Table II

The effect of adrenaline on contraction (rate and amplitude) and coronary inflow in the isolated rabbit heart before and after chlorpromazine (250 μg.)

Adrenaline (1 μg.) before chlorpromazine					Adrenaline (1 μg.) after chlorpromazine (250 μg.)				
Expt. no.	Time after adrenaline injection sec.	%Increase(+) %Decrease(-) in heart rate per minute	% Increase(+) % Decrease(-) in amplitude of contraction in cms.	% Increase(+) % Decrease(-) in coronary inflow c.c. per min.	Time of adrenaline injection after previous chlorpromazine	Time after adrenaline injection sec.	% Increase(+) % Decrease(-) in heart rate per minute	% Increase (+) % Decrease (-) in amplitude of contraction in cms.	% Increase(+) % Decrease(-) in coronary inflow c.c. per min.
1	20	+72	+62	+36	20 sec.	20	+73	+110	-18
	60	+11	+1	+27		60	+33	+47	-25
2	20	+42	+103	+29	30 sec.	20	+42	+53	0
	60	+10	+12	0		60	+10	-7	-41
3	20	+40	+97	-	40 sec.	20	+32	+246	-
	60	+10	+8	-		60	+26	+17	-
4	20	+45	+97	+14	2 min.	20	+52	+110	+23
	60	+13	+13	+5		60	+33	+40	+23
5	20	+69	+73	+10	5 min.	20	+84	+120	+54
	60	+19	+7	+2		60	+57	+33	+35

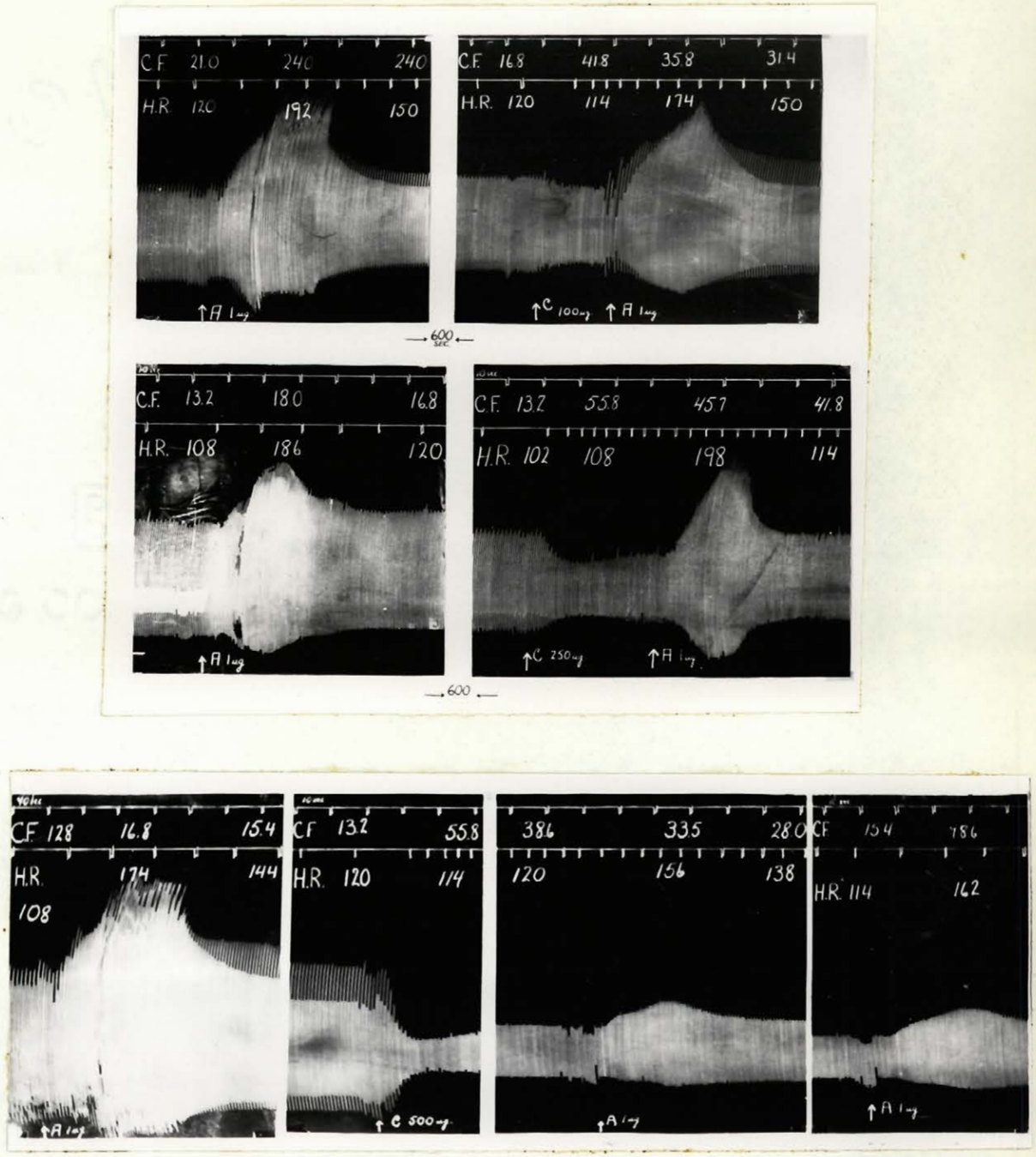


Fig. 21 Isolated rabbit heart perfusion. Records from three (3) experiments (see legend, Fig. 20). In the above records, at A, adrenaline (1 µg.) injection, and at C, injection of chlorpromazine as follows: upper record, 100 µg., middle record, 250 µg., lower record, 500 µg. In the lower record, the second injection of adrenaline was given 70 seconds after C, and the third injection was given 340 seconds after C.

As can be seen from the data contained in Table III and from Figure 21 (lower record), the responses (amplitude and rate of contractions) to adrenaline when given immediately after a high dose (500 µg.) of chlorpromazine were consistently diminished to approximately 50 percent of the responses observed to the control injection of adrenaline. However, this chlorpromazine depressant effect apparently did not persist and a second injection of adrenaline (5 minutes after the first) produced relatively normal stimulating responses. With higher doses (1.5 mg. and 2 mg.) of chlorpromazine in other experiments similar results were obtained. The responses to adrenaline (1 microgram) after the drug were greatly diminished, but still demonstrable. In two other experiments, when a higher dose of adrenaline (100 µg.) was used following these high doses of chlorpromazine, ventricular fibrillation ensued. This is rather curious, since as already pointed out, chlorpromazine can be shown to exert anti-fibrillatory action with the heart in situ.

Identical results with those described above were obtained when the responses to noradrenaline (1 µg.) were tested before and after injections of chlorpromazine. Figure 22 shows some typical examples of results obtained in three (3) different experiments in which doses of 100 µg. (upper record), 250 µg. (middle record) and 500 µg. (lower record) of chlorpromazine were employed. It is evident again that doses of 100 µg. and 250 µg. of the drug do not significantly influence the responses to noradrenaline (upper and middle records). Finally, as can be seen from the lower record, following 500 µg., the responses to noradrenaline although diminished are still demonstrable.

In respect to the changes in coronary flow, Figures 21 and 22 (upper and middle records) show clearly that during the stimulation of the heart

Table III

The effect of adrenaline on contraction (rate and amplitude) and coronary inflow in the isolated rabbit heart before and after chlorpromazine (500 μg.)

	Adrenaline (1 μg.) before chlorpromazine				Adrenaline (1 μg.) after chlorpromazine (500 μg.)				
Expt. no.	Time after adrenaline injection sec.	%Increase(+) %Decrease(-) in heart rate per minute	% Increase(+) % Decrease(-) in amplitude of contraction in cms.	% Increase(+) % Decrease(-) in coronary inflow c.c. per min.	Time of adrenaline injection after previous chlorpromazine	Time after adrenaline injection sec.	% Increase(+) % Decrease(-) in heart rate per minute	% Increase(+) % Decrease(-) in amplitude of contraction in cms.	% Increase(+) % Decrease(-) in coronary inflow c.c. per min.
1	20	+52	+171	+23	20 sec.	20	+35	+73	+12
	60	+33	+ 40	+23		60	+20	+33	-24
					3 min.	20	+45	+109	+30
						60	+30	+30	+25
2	20	+55	+104	+10	40 sec.	20	+31	+40	-29
	60	+25	+ 8	+10		60	+15	+33	-29
					4 min.	20	+52	+104	-16
						60	+45	+33	- 5
3	20	+61	+62	+ 7	1 min.	20	+30	+38	-20
	60	+22	+ 4	+77		60	+15	+19	-25
					5 min.	20	+62	+103	+13
						60	+26	+44	- 2
4	20	+40	+76	+23	1 min.	20	+ 4	+76	- 4
	60	+22	+24	+73		60	+ 4	+60	-13
					8 min.	20	+61	+127	+37
						60	+52	+ 72	+ 5

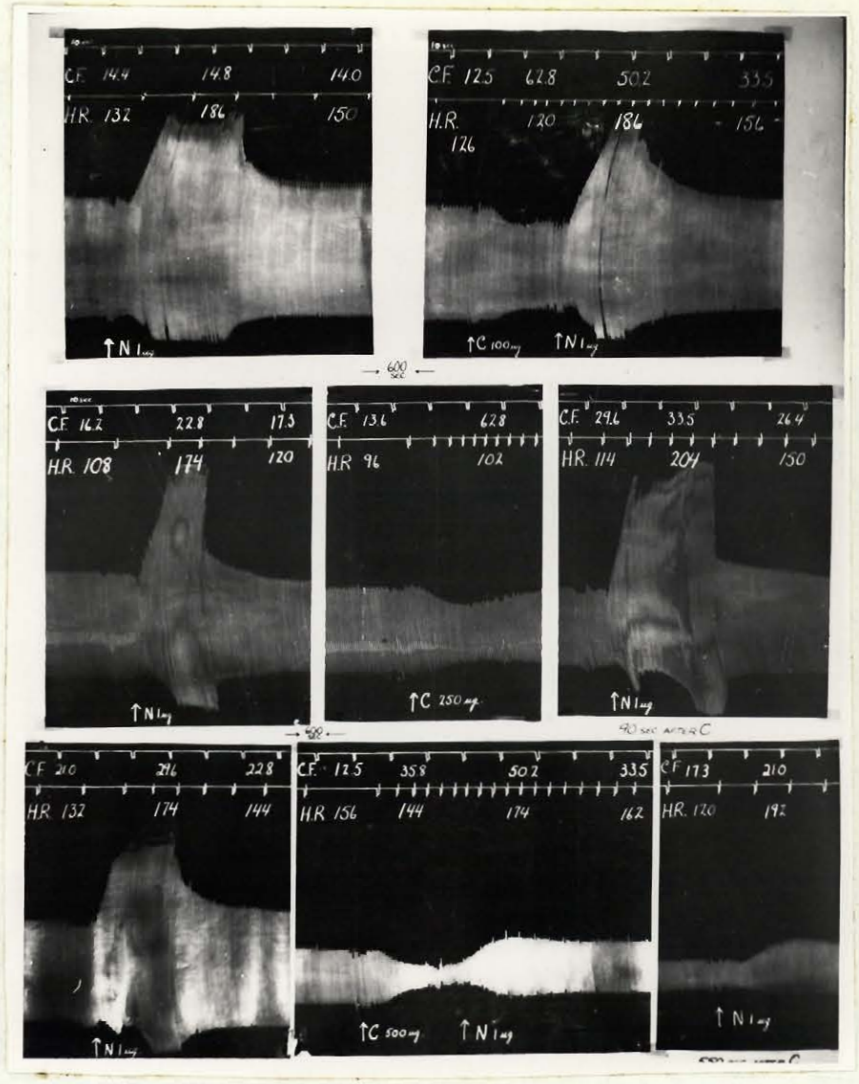


Fig. 22 Isolated rabbit heart perfusion. Records from three (3) experiments (see legend, Fig. 20). In the above records, at N, noradrenaline (1 µg.) injection, and at C, injection of chlorpromazine as follows: upper record, 100 µg., middle record, 250 µg., lower record, 500 µg. In the lower record, the interval of time between the first and second tracing is 15 minutes, and the third injection of noradrenaline was given 550 seconds after C.

contractions induced by adrenaline or noradrenaline, after previous chlorpromazine there is no significant inhibition of the coronary flow. Indeed in all instances it is still well maintained. In view of the intense coronary dilatation produced by the chlorpromazine, exact interpretation of these changes in coronary flow is rather difficult. It is evident however that chlorpromazine changes significantly the effects of adrenaline and noradrenaline on the coronary circulation under these conditions.

(d) Influence of chlorpromazine upon the responses to acetylcholine.

The influence of chlorpromazine upon the responsiveness of the isolated heart to acetylcholine has not been previously studied, so far as we are aware. Courvoisier (1953) has reported an atropine-like action with large doses of chlorpromazine in anaesthetized animals, and Huidobro (1954) states that the drug can inhibit the muscarinic action of acetylcholine. It was therefore of interest to investigate whether chlorpromazine could prevent the inhibitory effects of acetylcholine on the isolated perfused rabbit's heart.

Ten (10) experiments were performed employing doses of chlorpromazine ranging from 50 to 500 µg. Acetylcholine was used in doses of 10 and 30 µg. A test dose of acetylcholine was first injected, and after the response had worn off chlorpromazine was given. The same dose of acetylcholine was then repeated at varying intervals following the chlorpromazine. The results obtained were rather variable.

In several different experiments, it was observed that following injection of a dose (50 µg.) of chlorpromazine, the usual cardiac depressant and coronary dilatory action of acetylcholine (10 µg.) were unaffected. When a dose of 100 µg. of chlorpromazine was tested, in only one of four experiments performed was there any evidence of anti-acetylcholine action -- doses of both 10 and 30 µg. of acetylcholine were used. When the dose of chlorpromazine was further increased to 250 µg., there was definite evidence of an anti-

cholinergic action when a 10 μg. dose of acetylcholine was injected. In one out of three experiments no response at all to acetylcholine took place; in the other two, the response was diminished but still in evidence. Figure 23 shows an example of one of these experiments. The progressive diminishing response to acetylcholine can be clearly seen.

This "anti-cholinergic action" of chlorpromazine was not seen immediately after the drug was injected but only developed within 3 to 5 minutes. Since it is well known that the responses to acetylcholine are quantitatively repeatable, this delayed acetylcholine-blocking action of the drug cannot be attributed to any "tachyphylaxis" to repeated acetylcholine injections. This has been confirmed in two control experiments in which acetylcholine was injected at five minute intervals during a 60 minute period — the responses to acetylcholine were almost identical.

It is evident that even in high doses (250 μg.) chlorpromazine is not a potent anti-cholinergic drug. Indeed, in a similar experiment to that shown if Figure 23, the cardio-inhibitory responses to repeated acetylcholine (30 μg.) were still quite definite. Apparently therefore this blocking action can be overcome by larger amounts of acetylcholine. It is not clear whether or not this is a true "anti-cholinergic action", since chlorpromazine itself in large doses induces intense depression of the heart contractions, thus precluding any evaluation of the subsequent responses to acetylcholine.

(e) Summary.

1. Following injection of chlorpromazine (in doses exceeding 10 μg.) produces a marked increase in the rate of coronary inflow in the isolated perfused rabbit heart. This effect is associated with some increase in rate and some decrease in the amplitude of contractions.

2. Chlorpromazine in doses of 100 to 250 μg., injected into the perfused isolated rabbit heart could not be shown to alter in any way the responses

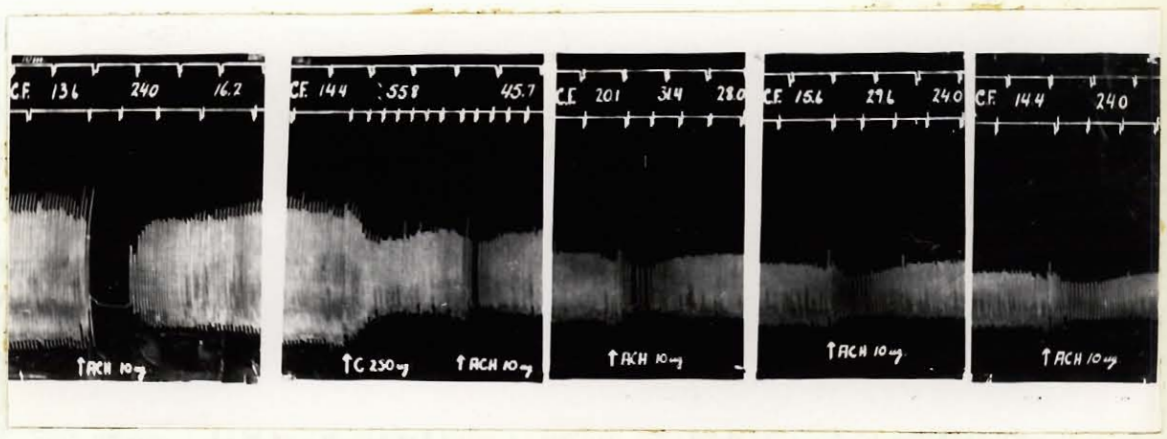


Fig. 23 Isolated rabbit heart perfusion. Record from one (1) experiment. At ACH, acetylcholine (10 μg.) injection, and at C, chlorpromazine (250 μg.) injection. The third, fourth, and fifth injection of acetylcholine was given 100, 200 and 340 seconds after C, respectively.

to adrenaline or noradrenaline (1 μg. doses). With higher doses of chlorpromazine the responses to adrenaline and noradrenaline are uniformly diminished but never completely abolished.

3. Chlorpromazine appears to have a slight "anti-acetylcholine" like action, but even with the high doses tested this action is not very potent.

IV. Studies on the Isolated Perfused Frog Heart.

It is evident from the preceding investigations that chlorpromazine produces a direct depressant effect on the myocardium. With the hope of elucidating this action further a considerable number of different types of pharmacological experiments were performed using the isolated perfused frog heart. With this preparation problems concerned with the effects of the drug on the coronary circulation could be eliminated, and the simplicity of the method facilitated a wider variety of pharmacological studies to be accomplished. Thus, in addition to studying the effects of chlorpromazine and its influence on the responses to adrenaline and noradrenaline (as in the previously described experiments) the actions of the drug were compared with those induced by a number of other types of myocardial depressants. Since the antagonism between chlorpromazine and ouabain had not previously been investigated in this preparation, it was also of interest to study this problem. It was hoped that as a result of these studies it might be possible to establish more clearly the basic mechanism of action of the drug on the myocardium.

(a) Methods.

In this series of experiments, isolated frog hearts of the Rana pipiens were used. The heart was perfused *in situ* through the vena cava in the usual manner. The frog was first pithed (brain and spinal cord destroyed) and fastened back downward on a board. The heart was exposed and a small hook with a silk thread attached was fixed into the ventricular apex.

Figure 24 shows diagrammatically the apparatus used. The tip (A) of the perfusing cannula was tied in the vena cava and the aortae were cut. The cannula was provided with a stand pipe (B) which served as a manometer for the perfusion pressure. The arms (C and D) of the cannula were connected with Mariotte bottles containing the perfusion solutions. These also provided for the aeration of the solution. By means of a two-way stopcock at (E), the

perfusing solution could be changed without interrupting the record. The Mariotte bottles were arranged to give a pressure of 6 cms. of water. After this pressure was obtained, the stand pipe at (B) was closed off with plasticine and kept closed during the experiment.

For the perfusion, Clark's solution at room temperature was used. The solution had the following composition:

NaCl	0.70%
KCl	0.014%
$CaCl_2$	0.024%
$NaHCO_3$	0.020%
pH	7.5 - 7.7

During the experiment, the exterior surface of the heart was constantly irrigated with Clark's solution dropping from a Mariotte bottle at the rate of 8 to 10 drops per minute. The thread from the hook in the ventricular apex was attached to an isotonic lever, the tip of which recorded on a slow moving kymograph -- systole above, diastole below.

All drugs to be administered were freshly dissolved in Clark's solution. In the case of chlorpromazine, a drop of dilute HCl was added to every 100 ml. of Clark's solution. This lowered the pH slightly (7.2 to 7.4) and prevented precipitation of the drug in the perfusing solution. This decrease in pH did not in any way affect the actions of the heart. At the beginning of each experiment, the heart was always perfused for 15 minutes with Clark's before the introduction of any drug solution.

Chlorpromazine, adrenaline and noradrenaline, as previously described Ia), were employed. In addition, for reasons already stated, Promethazine (Phenergan) powder (Poulenc), procaine, cocaine and nupercaine hydrochlorides (Merck), Pontocaine hydrochloride (Winthrop Chemical Co.) and ouabain (S.B. Penick and Company) were used.

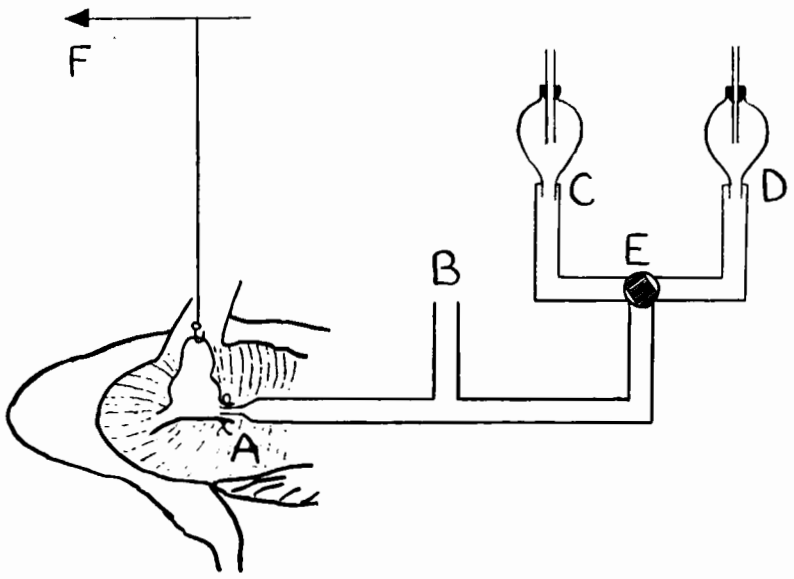


Fig. 24 Apparatus for the perfusion of the isolated frog heart in situ.

(b) Effects of chlorpromazine upon the frog heart contractions and its influence upon the responses to adrenaline and noradrenaline.

In these experiments the hearts were perfused with concentrations of chlorpromazine, ranging from 1 µg./ml. to 100 µg./ml., and in order to assess the influence of the drug upon the responses to the amines or vice versa, a similar series of perfusions were carried out with either adrenaline (10 µg./ml.) or noradrenaline (10 µg./ml.) freshly added to the perfusing fluid.

In Table IV, a analysis of results obtained with a low concentration of chlorpromazine (2 µg./ml.) is presented. As can be seen, during perfusion with chloropromazine alone, there was a slow progressive depression in both the heart rate and the amplitude of contractions. Thus, after 28 minutes of perfusion the average heart rate had decreased 36.2 percent and the average recorded height of the amplitude of contractions decreased 82.8 percent (3 experiments). During perfusions with similar concentrations of chlorpromazine with added adrenaline, there was a definite initial stimulation of the heart (both rate and amplitude) for the first 5 minutes of perfusion, but depression subsequently ensued and again, after 28 minutes of perfusion, there was an average decrease in heart rate of 23.6 percent (3 experiments) and an average decrease in amplitude of 55.1 percent (3 experiments). Similar results were observed when instead of adrenaline, noradrenaline was added to the perfusion fluid. These can be readily seen from the table. Moreover, after the perfusion fluids were changed to normal Clark's solution, in all three types of experiments, there was a slow progressive recovery, characterised in general by early improvement in the heart rate and only later by restoration of the amplitude of contractions. Thus, in all three types of experiments, as can also be seen from the table, after 50 minutes of Clark's solution, the average heart rate had recovered to almost normal, while the average size of the amplitude of contractions had recovered to only approximately half the normal.

Table IV

The effects of chlorpromazine (2 µg./ml.) on the isolated frog heart (rate and amplitude) and its influence on the adrenaline and noradrenaline response.

Time after start of perfusion in mins.	Changes in auricular rate % Increase (+) % Decrease (-)			Changes in ventricular contraction % Increase (+) % Decrease (-)		
	Chlorpromazine (2 µg./ml.)	Chlorpr.(2µg./ml.) + Adrenaline(10µg./ml.)	Chlorpr.(2µg./ml.) + Noradr.(10µg./ml.)	Chlorpromazine (2µg./ml.)	Chlorpr.(2µg./ml.) + Adr. (10µg./ml.)	Chlorpr.(2µg./ml.) + Noradr.(10µg./ml.)
2	-18.8	+57.8	+62.5	-17.6	+16.8	+20.7
5	-45.4	+49.1	+31.8	-31.7	+ 8.3	+15.4
15	-41.0	+11.1	- 4.7	-64.4	-33.1	- 4.5
25	-36.2	-23.6	-33.7	-82.8	-55.1	-41.8
	Perfusion fluid changed to normal Clark's					
2	-38.3	-26.7	-40.1	-90.0	-57.3	-50.0
25 - 30	-16.0	- 9.2	-11.4	-68.7	-47.0	-61.0
40 - 50	- 8.6	+ 5.8	-	-53.1	-46.2	-
80 - 90	-	-	+16.1	-	-	- 52.3
	Average 3 expts.	Average 3 expts.	Average 3 expts.			

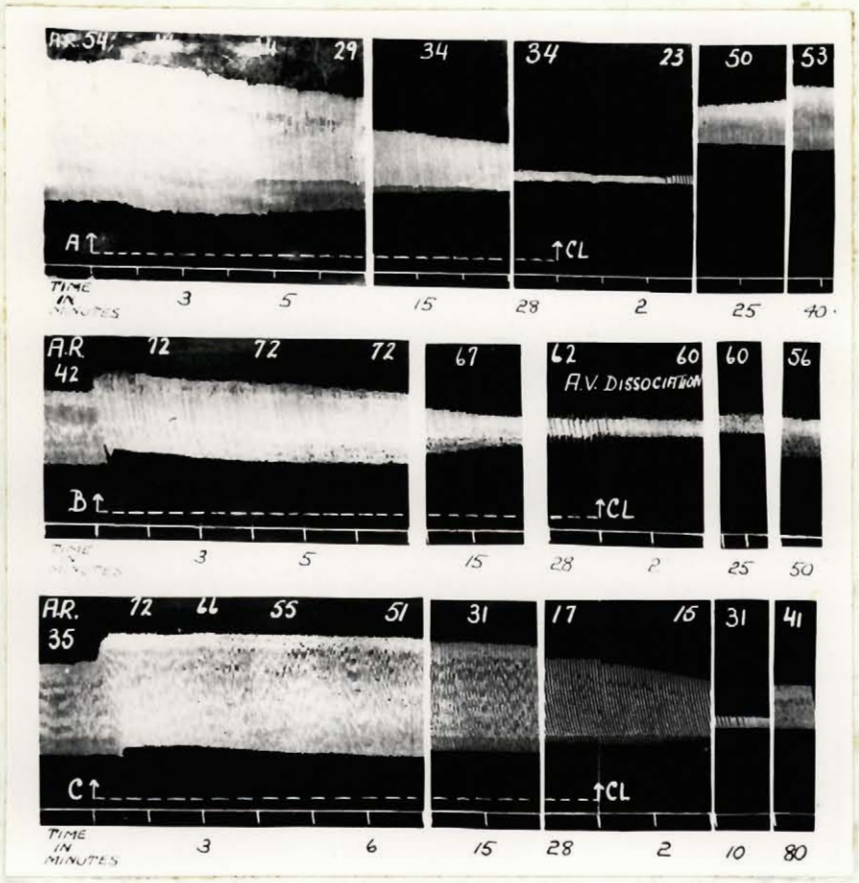

A.R. 54
29
34
34
23
50
53
A↑
↑CL
TIME IN MINUTES
3
5
15
28
2
25
40
A.R. 42
72
72
72
67
62
60
A.V. DISSOCIATION
60
56
B↑
↑CL
3
5
15
28
2
25
50
A.R. 35
72
66
55
51
31
17
16
31
41
C↑
↑CL
TIME IN MINUTES
3
6
15
28
2
10
80

FIG. 25

Fig. 25 Isolated perfused frog heart. Record of three (3) experiments. In this, and in all the succeeding figures, the records are all arranged similarly from above downwards as follows:

1. The auricular rate (A.R.) per minute, as counted.
2. Ventricular contractions with systole (above) and diastole (below) as recorded from a lever attached to the apex of the ventricle.
3. Duration of perfusion, in minutes, shown below.

Upper record, A to CL, chlorpromazine (2 µg./ml.) perfusion as shown by the broken line. CL to the end perfusion with normal Clark's solution. Middle record, B to CL, perfusion with chlorpromazine (2 µg./ml.) together with adrenaline (10 µg./ml.)as shown by the broken line, CL to the end perfusion with normal Clark's solution. Lower record, C to CL, perfusion with chlorpromazine (2 µg./ml.) together with noradrenaline (10 µg./ml.) as shown by broken line. CL to the end perfusion with normal Clark's solution.

It is therefore clear that when either adrenaline or noradrenaline was contained in the perfusion fluids, the recovery of heart action was not significantly enhanced.

Figure 25 shows some typical examples of the comparative effects of perfusions of the frog heart with a low concentration (2 μg/ml.) of chlorpromazine alone (upper record), the same with added adrenaline (middle record) and the same with added noradrenaline (lower record). These results demonstrate clearly the changes described above and need no further comment.

In contrast to the above findings, some examples of the results obtained in control experiments are shown in Figure 26. As can be seen (upper record) during as long as 300 minutes of continuous perfusion with Clark's solution alone there is no significant change in either heart rate or heart contractions. The figure also shows the sustained stimulation of the heart induced by either 30 minutes of adrenaline (10 μg./ml.) perfusion (Middle record), or 60 minutes of noradrenaline (10 μg./ml.) perfusion (lower record). It is clear therefore that the progressive cardiac depression induced by low concentrations of chlorpromazine cannot be antagonized by either adrenaline or noradrenaline, in otherwise effective cardio-stimulating concentrations.

When higher concentrations of chlorpromazine were studied, the depressant effects on the heart developed somewhat more rapdily. Figures 27 and 28 show typical examples of repetitions of the above-described experiments when concentrations of 10 μg./ml. and 100 μg./ml. respectively of chlorpromazine were employed.

Similar results to those shown in Figure 27 were obtained in 6 different experiments of this type, and it is clear that with moderate concentrations of chlorpromazine (10 μg./ml.) definite depression of the amplitude of the ventricular contractions occurs within 5 to 6 minutes; auriculo-ventricular dissociation follows within 8 to 10 minutes; and usually there is complete

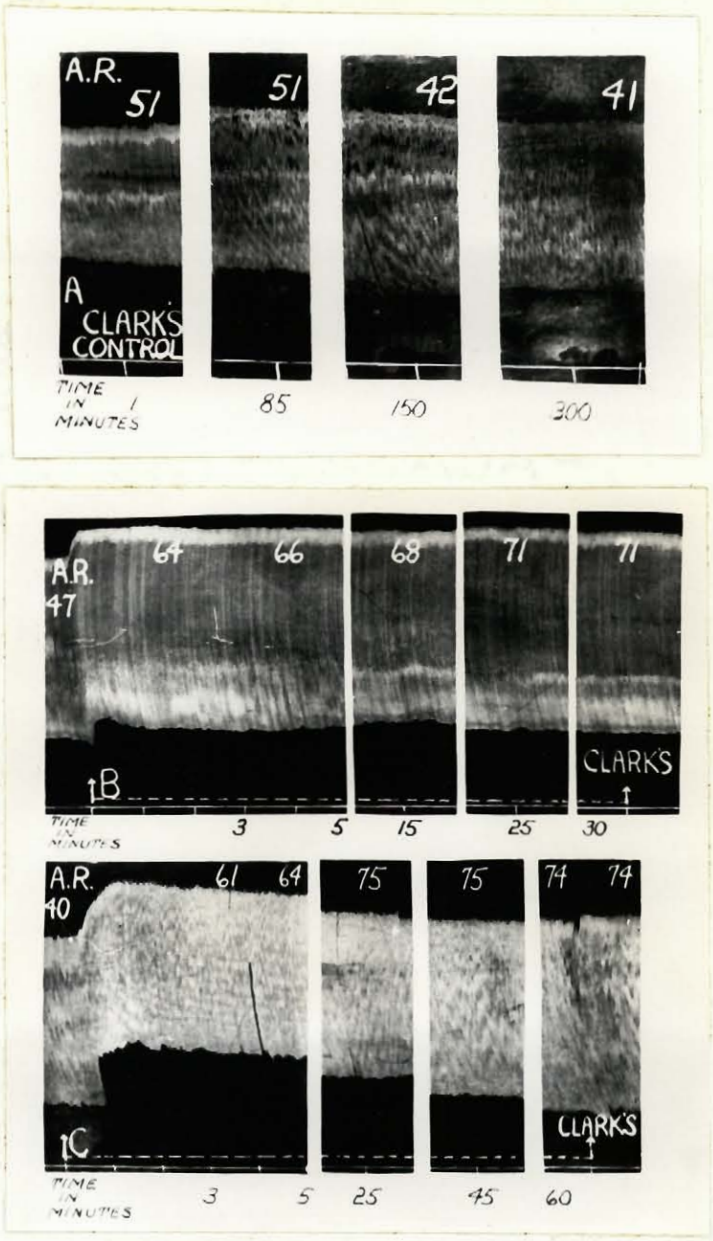


Fig. 26 Isolated perfused frog heart. Records from three (3) experiments (see legend, Fig. 25). Upper record, control experiment heart perfused with normal Clark's solution for 300 minutes. Middle record, B to Clark's, adrenaline (10 µg./ml.) perfusion as shown by the broken line. Clark's to the end perfusion with normal Clark's . Lower record, C to Clark's, noradrenaline (10 µg./ml.) perfusion as shown by the broken line. Clark's to the end perfusion with normal Clark's solution.

ventricular arrest within 12 minutes but the auricles continued to beat longer. In 4 out of 6 experiments performed, there was complete heart arrest after 15 minutes of perfusion whereupon the perfusion solution was changed back to Clark's, and although the cardiac arrest persisted for an additional 20 to 30 minutes, subsequent recovery of the heart ensued. This was characterized first by auricular and then by ventricular recovery with A-V dissociation occurring before complete restoration. After 80 to 100 minutes of Clark's solution, the auricular rate had recovered almost completely (on the average to 88 percent of the normal) and the amplitude similarly to approximately 76 percent of the normal. Completely normal rhythmicity of the heart also occurred.

When chlorpromazine was perfused along with adrenaline (Figure 27, middle record), except for a brief initial stimulation, the response to chlorpromazine was not altered and again depression ensued within 3 to 5 minutes, followed by A-V dissociation, ventricular arrest with the auricles feebly beating and finally complete cardiac stoppage. Despite the fact that this arrest lasted for 20 to 30 minutes after the perfusion fluid was changed to Clark's, progressive recovery ultimately ensued.

When noradrenaline was added to the chlorpromazine (lower record, Figure 27), again there is an initial transitory increase in heart rate but gradual depression of the amplitude of the heart contractions occurred followed by complete arrest within 12 minutes. As in the other experiments, changing the perfusing fluid to normal Clark led to the usual slow recovery of the heart.

Finally, when a concentration of 100 µg./ml. of chlorpromazine was employed (Figure 28), arrest of the heart occurred within 4 to 6 minutes, and in some experiments A-V dissociation could be detected. However, even with this high concentration of the drug, when the perfusing fluid was changed to normal Clark's solution, progressive recovery ensued and after 150 to 180 minutes

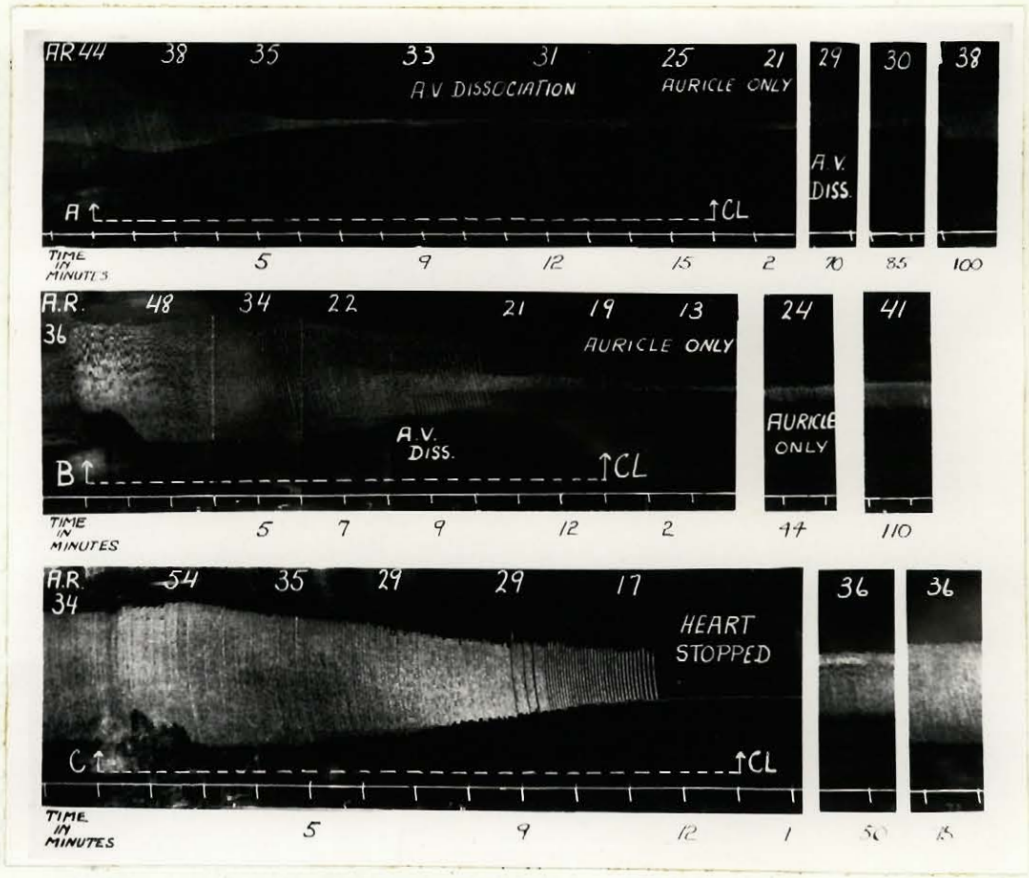


Fig. 27 Isolated perfused frog heart. Records from three (3) experiments (see legend, Fig. 25). Upper record, A to CL, chlorpromazine (10 µg./ml.) perfusion as shown by the broken line. CL to the end perfusion with normal Clark's. Middle record, B to CL, chlorpromazine (10 µg./ml.) and adrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's. Lower record, C to CL, chlorpromazine (10 µg./ml.) and noradrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's solution.

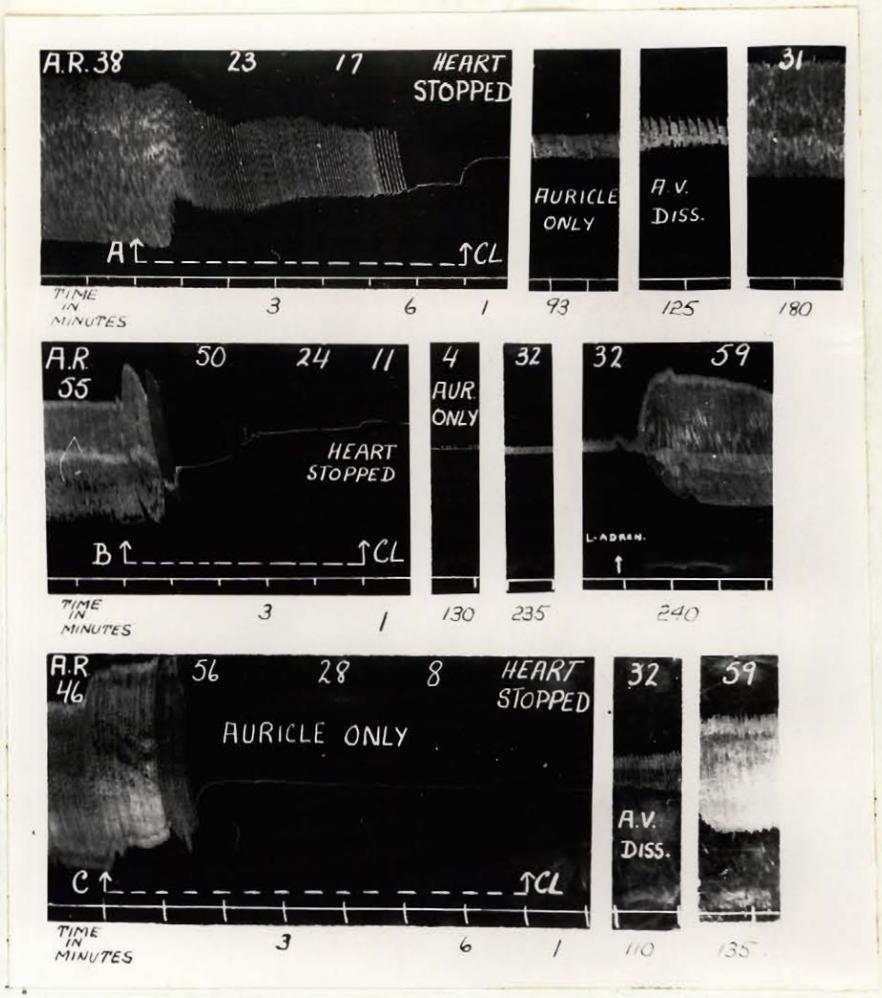


Fig. 28 Isolated perfused frog heart. Records from three (3) experiments, (see legend, Fig. 25). Upper record, A to CL, chlorpromazine (100 µg./ml.) perfusion as shown by the broken line. CL to the end perfusion with normal Clark's. Middle record, B to CL, chlorpromazine (100 µg./ml.) and adrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's. At arrow, adrenaline (10 µg.) injection. Lower record, C to CL, chlorpromazine (100 µg./ml.) and noradrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's solution.

of continued perfusion complete or almost complete restitution of heart activity occurred (upper record). When either adrenaline (middle record) or noradrenaline (lower record) was added to this high concentration of chlorpromazine, in both instances the ventricular contractions stopped, within 2 minutes, but the auricular rate was accelerated for some time and the auricle continued beating for 6 to 7 minutes. Again, following complete arrest of the heart in both instances, progressive recovery followed with prolonged perfusion with normal Clark's solution. It is rather astounding that, although the heart has been completely arrested for as long as 50 to 60 minutes during the perfusion with normal Clark's solution, the auricular and then the ventricular contractions were restarted. This occurred with varying degrees of A-V dissociation, before a coordinated heart beat developed.

In conclusion, therefore, chlorpromazine (2µg/ml) produces slow progressive depression of the isolated frog heart - both rate and amplitude. With higher concentrations (10 µg/ml to 100 µg/ml), these effects develop more quickly and are more marked. The ventricle appears to be most sensitive to the drug, and invariably stops before the auricles - varying degrees of heart-block also occur. This depression is completely reversible although recovery of the heart is rather slow - in some experiments, normal contractions were only restored after 2 to 3 hours of perfusion. During simultaneous perfusion of the heart with chlorpromazine and either adrenaline or noradrenaline, there is an initial cardiac stimulation, but again, progressive depression ensues. The recovery was not affected by the previous perfusion with the amines. These agents, therefore, do not protect the heart against the deleterious effects of chlorpromazine.

(c) Comparative effects of promethazine (Phenergan) and its influence upon the responses to adrenaline and noradrenaline.

In view of their close chemical similarity, it was of interest to compare the effects of promethazine with those of chlorpromazine on the isolated frog heart. Twelve (12) different experiments were performed ~~in~~ with promethazine (10 μg/ml) alone or with added adrenaline (10 μg/ml) or noradrenaline (10 μg/ml). Some examples of the results obtained in these experiments are shown in Figure 29.

In a general way, as can be seen from the record, this concentration of promethazine induces a rapid depression of the heart with complete arrest of the ventricle within 4 minutes, and in some experiments, complete cardiac arrest after 6 minutes. When the perfusion fluid was changed to normal Clark's solution, complete recovery ensued much more promptly in these experiments than in similar chlorpromazine experiments. Thus, within 8 to 10 minutes after promethazine (upper record), there was good recovery while it required 80 to 100 minutes (10 times as long) for recovery of the heart after a similar concentration of chlorpromazine. A-V dissociation was rarely seen during promethazine action or during the subsequent recovery period.

When adrenaline or noradrenaline (10 μg/ml) was mixed with phenergan (10 μg/ml) and perfused, the response was somewhat similar to that seen with chlorpromazine. Thus, there was at first stimulation of the rate and amplitude which lasted for 2 to 3 minutes followed by a gradual depression. However, the presence of these amines seemed to delay the onset of cardiac arrest, and the perfusion could now be maintained for 11 to 12 minutes and sometimes even longer before cardiac arrest occurred. When the perfusing solution was changed to Clark's, an increase in the auricular rate over the control value was customary and the amplitude usually returned to near control values in 10 to 12 minutes.

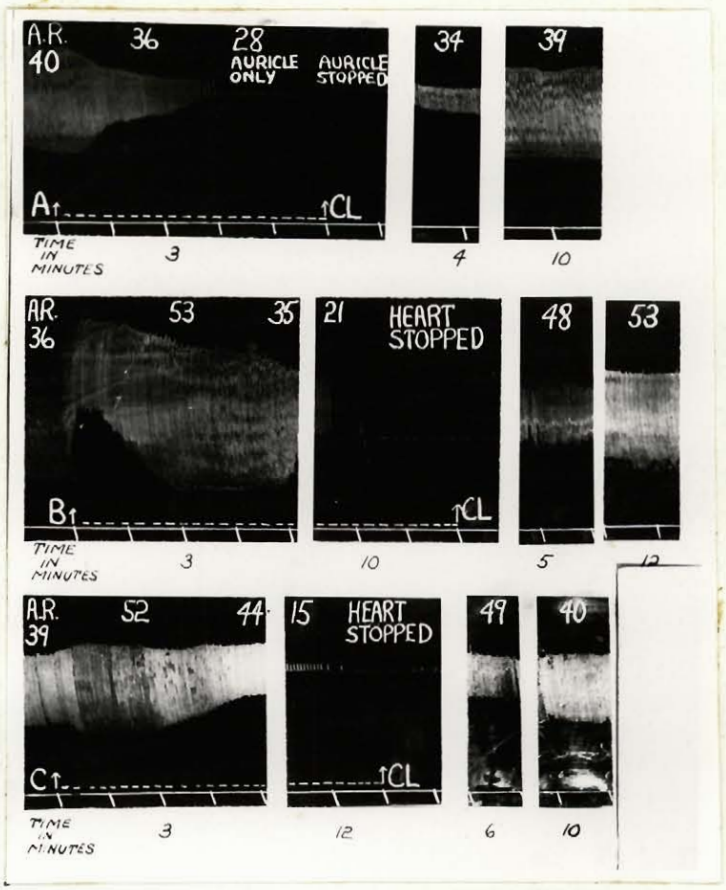


Fig. 29 Isolated perfused frog heart. Records from three (3) experiments (see legend, Fig. 25). Upper record, A to CL, promethazine (10 μg./ml.) perfusion as shown by the broken line. CL to the end perfusion with normal Clark's. Middle record, B to CL, promethazine (10 μg./ml.) and adrenaline (10 μg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's. Lower record, C to CL, promethazine (10 μg./ml.) and noradrenaline (10 μg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's solution.

It is therefore apparent that although promethazine appears to be a more potent myocardial depressant, its effects are not as long lasting as those of chlorpromazine. It is also apparent that adrenaline and noradrenaline are more potent antagonists to the depressant action of promethazine than to that of chlorpromazine. The results obtained indicate that the myocardial depressant effect of chlorpromazine is not a specific action of this phenothiazine derivative.

(d) Comparative effects of some local anesthetics on the isolated frog heart and their influence upon the responses of adrenaline and noradrenaline.

Since it has been demonstrated that chlorpromazine has a strong local anesthetic action, it was thought worthwhile to compare its effects with those produced by some well-known local anesthetics under similar conditions to those described above. Four local anesthetic agents; namely, cocaine, procaine, pontocaine and nupercaine were studied. The latter three drugs all showed qualitatively similar effects. Therefore, the results from the procaine experiments only will be shown here as representative of these three local anesthetics. Average figures from twelve (12) experiments with procaine can be seen in Table V, and typical recordings in Figure 30.

In low concentrations (procaine, 200 and 300 µg./ml.; pontocaine, 20 and 30 µg./ml.; nupercaine, 2 and 3 µg./ml.), these agents caused a depression of both the rate and amplitude of contraction. In addition, A-V dissociations were always seen. When the perfusion fluid was changed to Clark's, there was prompt recovery with procaine and pontocaine, but less so with nupercaine. With the latter, recovery was more prolonged. When higher doses were used (procaine, 400 µg./ml.)(pontocaine, 40 µg./ml.) (nupercaine, 4 µg./ml.), there was a rapid depression and complete ventricular arrest ensued within 2 to 3 minutes. However, the auricles kept beating at a slower rate than normal throughout the entire duration of perfusion. Upon returning to Clark's,

Table V

The effect of procaine hydrochloride on the isolated perfused frog heart (rate and amplitude) and its influence on the adrenaline and noradrenaline response.

Time after start of perfusion in mins.	Changes in auricular rate % Increase (+) % Decrease (-)			Changes in amplitude of contraction % Increase (+) % Decrease (-)		
	Procaine (400 µg./ml.)	Procaine (400µg./ml.) + Adrenaline(10µg./ml.)	Procaine(400µg./ml.) + Noradr.(10µg./ml.)	Procaine (400µg./ml.)	Procaine(400µg./ml.) + Adrenaline(10µg./ml.)	Procaine (400µg./ml.) + Noradrenaline (10µg./ml.)
1	-52.3	-	-	-49.3	-	-
5	-33.3	+14.4	+6.5	auricle only	+34.2 *	+45.9 *
20	-38.9	+11.4	+6.5	auricle only	+32.4 *	+34.5 *
30	-35.7	+11.3	+5.5	auricle only	+33.3 *	+33.3 *
40	-38.9	- 1.9	+2.6	auricle only	+22.7 *	+16.2 *
	Perfusion fluid changed to normal Clark's					
2	-33.3	+23.8	+21.6	-53.3	+22.8	+ 6.6
5	-19.0	+20.2	+14.3	normal	+15.2	+ 1.9
8	+ 9.	+23.1	+11.5	+8.0	+ 7.3	-15.0
	3 expts.	3 expts.	3 expts.	3 expts.	3 expts.	3 expts.

* Auriculo-Ventricular dissociations.

6½a.

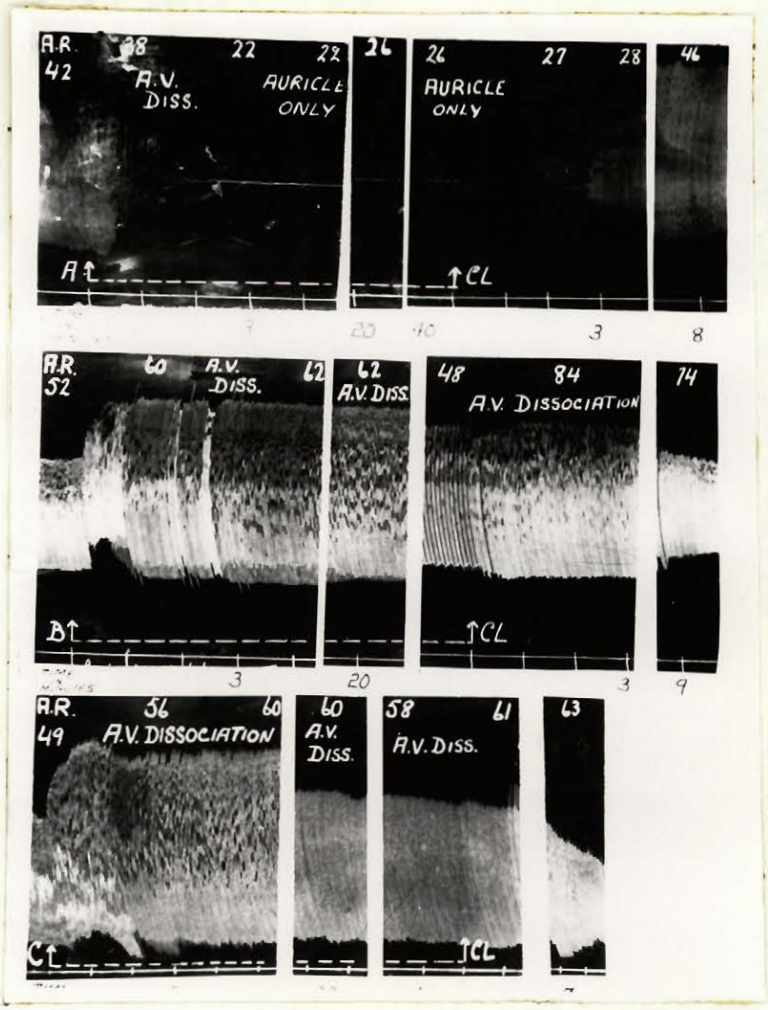


Fig. 30 Isolated perfused frog heart. Records from three (3) experiments (see legend, Fig. 25). Upper record, A to CL, procaine hydrochloride (400 µg./ml.) perfusion as shown by the broken line. CL to the end perfusion with normal Clark's. Middle record, B to CL, procaine hydrochloride (400 µg./ml.) and adrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's. Lower record, C to CL, procaine hydrochloride (400 µg./ml.) and noradrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's solution.

there was again prompt recovery except as noted earlier with nupercaine. An example of these results can be seen in Fig. 30 (upper record) and average figures are shown in Table V.

When adrenaline or noradrenaline was added to the local anesthetic, the results obtained were rather similar. In general, for the entire period of perfusion, a stimulation of auricular rate occurred (middle or lower record, Fig. 30 - also Table V). In a few experiments, there was a slight decrease in rate but this was never as marked as with the local anesthetic alone. Whereas the latter produced complete ventricular arrest in 2 to 3 minutes, with adrenaline and noradrenaline, the ventricle continued to beat during the entire period of perfusion. However, A-V dissociation was always still seen, and in the majority of experiments, was of the order of 2:1. This stimulation usually persisted for some time after the perfusing fluid was changed back to Clark's. The amplitude of contraction was always well maintained above the control value when adrenaline or noradrenaline was added to the perfusion fluid. The stimulation usually lasted some minutes after the return to Clark's before a control state was resumed, and in some cases, a slight depression followed later.

In regard to cacaine, a fairly large number of experiments (30) were performed with either cocaine alone or in combination with adrenaline and noradrenaline. The concentrations of cocaine used ranged from 50 to 490 µg./ml. The only observed difference between the effects of cocaine and those of procaine, as previously described, were 1) the ventricular arrest was not maintained during the entire period of perfusion, and irregular ventricular beats were seen altefnating with periods of complete arrest, and 2) in all instances, there was mark difference in response when cocaine was perfused together with either adrenaline or noradrenaline. This can be seen in Table VI and Fig. 31 B,C. It is apparent that adrenaline and noradrenaline cannot

Table VI

The effect of cocaine hydrochloride on the isolated perfused frog heart (rate and amplitude) and its influence on the adrenaline and noradrenaline response.

Time after start of perfusion in mins.	Changes in auricular rate % Increase (+) % Decrease (-)			Changes in amplitude of ventricular contraction % Increase (+) % Decrease (-)		
	Cocaine (400µg./ml.)	Cocaine(400µg./ml.) + Adrenaline(10µg./ml.)	Cocaine(400µg./ml.) + Noradr.(10µg./ml.)	Cocaine (400µg./ml.)	Cocaine(400µg./ml.) + Adrenaline(10µg./ml.)	Cocaine (400µg./ml. + Noradr. (10µg./ml.
1	-31.5	+42.8	+42.7	-81.6	+6.6	+4.2
5	-35.2	+30.4	+45.5	-	-20.3 *	-25.1 *
20	-28.2	+29.2	+49.8	-82.4 *	-26.1 *	-46.1 *
30	-19.8	+28.5	+49.0	-	-36.8 *	-50.0 *
40	-27.1	+27.5	+49.0	-76.19 *	-41.1 *	-55.3 *
	Perfusion fluid changed to normal Clark's					
1	-19.5	+33.3	+49.8	-76.19	-25.1	-14.5
5	- 5.0	+49.4	+60.0	-76.6	+17.3	+21.8
8	+11.9	+44.7	+53.42	+4.9	+25.9	- 9.9
	4 expts.	4 expts.	4 expts.	4 expts.	4 expts.	4 expts.

* A uriculo-Ventricular dissociation

63a.

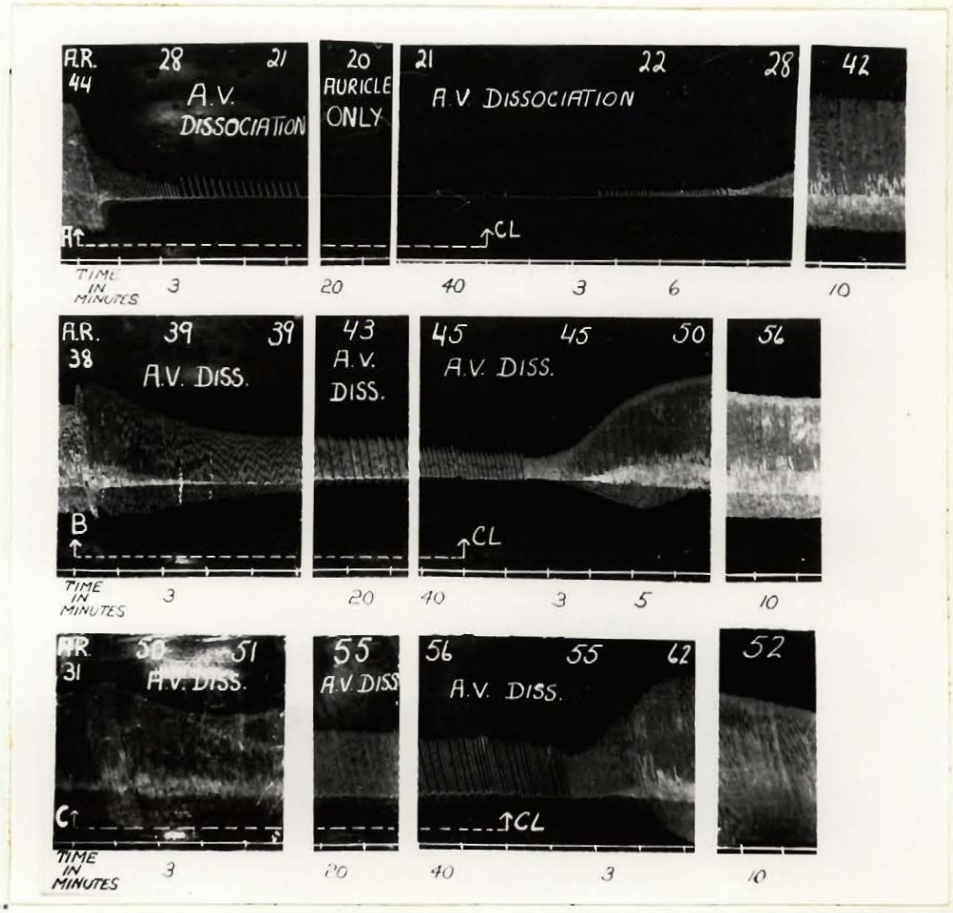


Fig. 31 Isolated perfused frog heart. Records from three (3) experiments (see legend, Fig. 25). Upper record, A to CL, cocaine hydrochloride (400 µg./ml.) perfusion as shown by the broken line. CL to the end perfusion with normal Clark's. Middle record, B to CL, cocaine hydrochloride (400 µg./ml.) and adrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's. Lower record, C to CL, cocaine hydrochloride (400 µg./ml.) and noradrenaline (10 µg./ml.) perfused together as shown by the broken line. CL to the end perfusion with normal Clark's solution.

completely overcome the depression of cocaine on the amplitude. An increase in the amplitude was usually seen for the first few minutes of perfusion. This was followed by progressive depression. As with the other 3 local anesthetics, adrenaline and noradrenaline could overcome the depressant effect of cocaine on the auricular rate. The latter was maintained above normal for the duration of the perfusion. In all other respects, cocaine did not materially differ from procaine or pontocaine.

Thus, in summary, the results obtained in this series of experiments render it unlikely that chlorpromazine exerts an effect on the heart which is comparable to the effects of the local anesthetics. This deduction is warranted for the following reasons:

Firstly, the mode of depression is different. In general, high concentrations of chlorpromazine produce ventricular arrest followed by auricular arrest whereas with very high concentrations of the local anesthetics auricular arrest was never seen.

Secondly, the depression by the local anesthetics is readily reversible with Clark's solution (2 to 5 minutes) whereas with chlorpromazine reversal only occurs after a very prolonged period of time (100 to 180 minutes). Even with the most potent anesthetic, nupercaine, the recovery period is still considerably shorter than that obtained with chlorpromazine.

And finally, whereas adrenaline or noradrenaline can afford a considerable degree of protection against the depressant effects of the local anesthetics, this is not so in the case of chlorpromazine.

(e) Effect of ouabain upon the response to chlorpromazine.

Since, as previously shown, chlorpromazine can prevent ventricular fibrillation following adrenaline during chloroform infusion (1b) as stated by Melville (1954) but the drug could not prevent the ventricular irregularities produced by ouabain in the anesthetized animal, it was of interest to investigate the effects of ouabain upon the responses to chlorpromazine in this preparation. Fig. 32 shows typical examples of the results obtained.

Ouabain, at a concentration of 5 μg./ml., was used throughout these experiments. This concentration stopped the heart uniformly between 18 to 20 minutes of perfusion. When the heart was previously depressed with chlorpromazine(10 μg./ml.), as shown by the upper record (A-B), changing the perfusing fluid to ouabain improves the amplitude of the heart contraction much more quickly than when Clark's solution was used as shown in Fig. 27 (upper record). However, cardiac irregularities and A-V dissociation occurred. Indeed, with the continued perfusion of ouabain for 15 minutes, the amplitude of contraction decreased and in most experiments, the hearts stopped between 18 to 20 minutes. Under these conditions, recovery does not occur when the heart is subsequently exposed to Clark's solution.

When chlorpromazine and ouabain were perfused together (lower record, Fig. 32), the depressant effect of the chlorpromazine on the amplitude was usually overcome for the initial 15 minutes, but the heart rate was irregular, and A-V dissociations were again seen. At the end of about 20 minutes, all heart activity ceased and could not be restored by perfusing with normal Clark's solution. Similar results to those described above were observed in 3 other similar experiments. It may be concluded, therefore, that the depressant effect of chlorpromazine on the myocardium can be temporarily antagonized by ouabain.

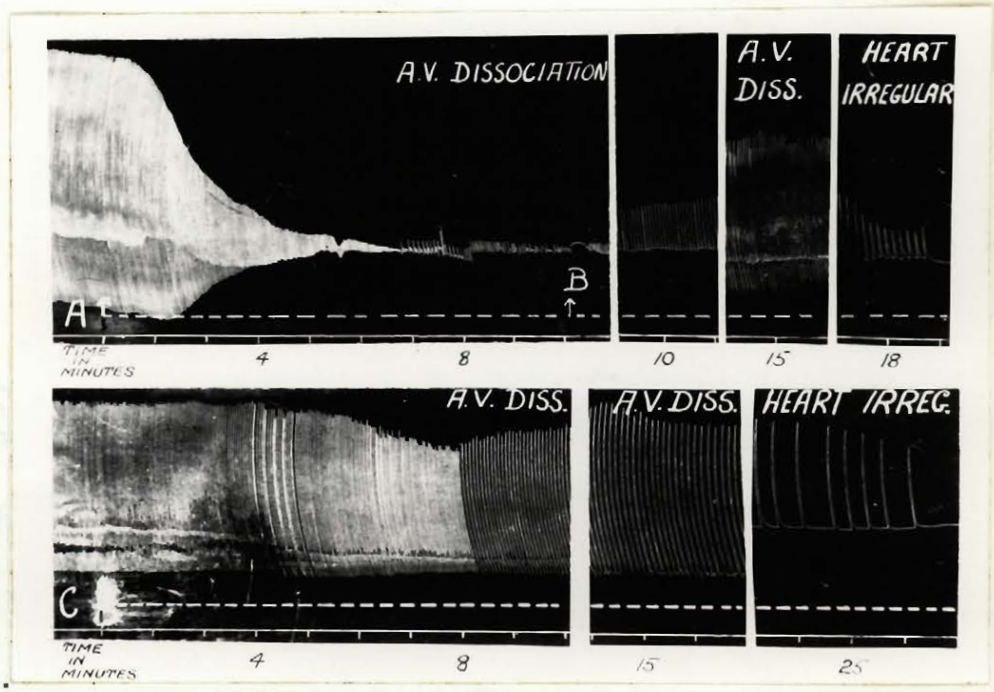


Fig. 32 Isolated perfused frog heart. Records from two (2) experiments (see legend, Fig. 25). Upper record, A to B, chlorpromazine (10 μg./ml.) perfusion; B to the end, ouabain (5 μg./ml.) as shown by the continued broken line. Lower record, C to the end, chlorpromazine (10 μg./ml.) perfused together with ouabain (5 μg./ml.) as shown by the broken line.

(f) Summary.

1) Chlorpromazine in concentrations ranging from 1 to 100 µg./ml. produces progressive depression of both ventricular and auricular contractions, associated with auriculo-ventricular dissociation, in the perfused frog heart. This depression is slowly reversible. Simultaneous continued perfusion with either adrenaline or noradrenaline cannot prevent the development of these depressant effects.

2) Promethazine, a more potent anti-histamine, in concentrations of 10 µg./ml., produces a relatively intense depression, but customarily, a more rapid recovery of the heart. Simultaneous perfusion with either adrenaline or noradrenaline again did not prevent these depressant effects.

3) The local anesthetics - procaine, cocaine, pontocaine and nupercaine - all induce a prompt and intense depression which can be partially antagonized by simultaneous adrenaline or noradrenaline administrations and the recovery from these agents is also rapid.

4) The depressant effect of chlorpromazine on the frog heart is only temporarily antagonized by ouabain. On the other hand, chlorpromazine cannot prevent the ultimate toxicity of ouabain on this preparation.

5) In comparing the above findings, it can be stated: a) that the observed depressant actions of chlorpromazine appear to be rather similar to those shown by the phenothiazine derivative, phenergan, except that this action of the former is much more prolonged; b) that although chlorpromazine is a potent local anesthetic, its myocardial depressant action appears to be rather different from that observed with the different local anesthetics studied. Even with excessive concentrations of the local anesthetics, complete cardiac arrest was not seen, and recovery occurred very rapidly; c) that chlorpromazine does not appear to exert any specific anti-adrenergic action in the isolated frog heart since a similar antagonism to these amines was

also observed with phenergan and d) the temporary antagonism of the depressant effect of chlorpromazine by ouabain might suggest that the effect of the former is directly on the myocardium.*

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* A part of the preceding results were presented at the Eighteenth Annual Meeting of the Canadian Physiological Society, Toronto, October 22, 1954.

DISCUSSION

The data presented above show clearly that chlorpromazine can exert striking and important pharmacological actions upon the circulatory system. In view of the multiplicity of physiological functions which have been demonstrated (see Historical Review) to be affected by the drug, the question naturally arises, to what extent the above-described cardiovascular changes are "primary," that is to say, direct effects of the drug upon the heart or blood vessels, or "secondary," that is to say, indirect effects, resulting from actions upon the central or autonomic nervous systems, or through reflex mechanisms. In addition, with the increasing widespread usage of chlorpromazine as a therapeutic agent, the question also arises, to what extent these experimentally-observed cardiovascular changes might be of significance in the clinical usefulness of the drug?

In regard to the first of these questions, the initial observations reported above concerning the effects of chlorpromazine on the blood pressure and the electrocardiograms, show clearly that in small and moderate doses (1 to 5 mg./kg.), chlorpromazine leads to a transient reversible hypotension. This effect is not prevented by vagotomy, previous atropinization or following adrenergic blockade induced by hydergin. It is, however, associated with an initial elevation of T wave and tachycardia, both of which are abolished by vagotomy or atropinization, and therefore, appear to be "reflex vagal" effects. It is evident from these findings that this transient depressor action of chlorpromazine is probably a non-autonomic effect, and might be due to either a diminished cardiac output or to some peripheral vasodilator action of the drug.

When a single large dose of chlorpromazine (10 mg./kg.) is injected, the intense depressor response observed is, on the contrary, associated with transient cardiac slowing and marked T wave changes (both depression and

elevation). These effects are not prevented by vagotomy or previous atropinization, and leave little doubt that high doses of chlorpromazine can induce transient impairment of cardiac function. These findings would rather favour the view that the slight depressor effects as observed with small doses of the drug might be due to some cardiac depression which occurs without any siggificant change in the electrocardiogram. It is also noteworthy that on repeated injections of chlorpromazine, even with high doses of the drug, no evidence of any cumulative toxic action was ever observed. Usually in such experiments, the blood pressure remained at a low level (20 to 40 mm. Hg.) while the heart action returned to relative normal, that is, there was some increase in heart rate, but the form of the electrocardiogram remained essentially normal. It may be concluded, therefore, that chlorpromazine is not a highly toxic agent to the heart under these conditions, and that the fatal termination in such experiments resulted apparently from the sustained low blood pressure level leading to a shock-like state. It is not too clear whether or not repeated injections of chlorpromazine in anesthetized dogs lead to some tolerance or tachyphylaxis in the heart, as curiously enough, with repeated and prolonged administration of the drug, there is a progressive increased heart rate associated with the sustained depressor response. This cardiac action might be due to some type of vagal blocking effect associated with prolonged administrations of the drug. More will be said about this later. There is little doubt, however, that with repeated injections of large doses of the drug, peripheral vasodilatation rather than cardiac depression is the important factor in maintaining the hypotension.

In regard to the results obtained on the dog heart-lung preparation, again, it is evident that relatively high concentrations of chlorpromazine (20 to 40 mg. in 700 to 800 c.c. of blood) are required to lead to any decrease in the cardiac output, and with such concentrations, spontaneous and

complete recovery of the heart always occurs. Indeed, even when higher doses of chlorpromazine (50 mg.) are employed in such experiments, the intense depression of the heart which ensues although not spontaneously reversible, can be antagonized by injections of large doses of adrenaline. In the heart-lung experiments, the heart rate was in general not too markedly reduced, and it would appear therefore that the diminished cardiac output is due mainly to the diminished contractile force of the muscle, that is, a reduction in the stroke volume of the heart. It is evident also that this cardiac depression is not an irreversible process. The heart-lung experiments showed also that chlorpromazine can exert some slowly-developing anti-acetylcholine action in the dog heart, and this might be responsible for the progressive cardiac acceleration seen with repeated injections of large doses of the drug, as stated earlier.

In the isolated perfused rabbit heart, the most striking action of chlorpromazine is its intense coronary dilator effect. This was already mentioned by Courvoisier et al.(1953), but these authors found that doses of chlorpromazine of the order of 0.5 to 1 mg. increase the coronary flow 60 to 100 percent. These authors used a Condon drop recorder and observed only the coronary outflow. In our experiments, however, with similar doses, increases in the coronary inflow of the order of 400 to 600 percent were recorded. The coronary dilator action of the drug is associated with a slightly increased heart rate and a decrease in the amplitude of contraction. It might be argued that this decrease in amplitude could be responsible for the increased coronary flow observed. However, with low doses (10 µg.) of the drug, the decrease in amplitude is slight and could hardly account for the intense observed increase in coronary flow (100 percent). With higher doses (500 µg.), the associated intense depression of the amplitude, of course, by decreasing the extravascular compression on the coronary vessels, might be partly responsible for the increased coronary inflow. In conclusion, the potent

coronary dilator action of the drug would suggest that it might be worthwhile to investigate more thoroughly its clinical usefulness in the prevention of attacks of angina pectoris. No systematic study on this question has yet been undertaken, so far as we are aware.

From the experiments on the isolated frog heart, it is apparent that chlorpromazine exerts a progressive and prolonged depression of the heart contractions and the heart rate. Even with the highest concentrations of the drug studied, and with which there was complete cessation of all visible or mechanical heart action, this depression of the heart could be reversed by continued and prolonged perfusion of the preparation with normal Clark's solution, and invariably, complete restoration of normal heart action achieved. Indeed, in some of these experiments, heart action was only restored after 2 to 3 hours of perfusion with normal Clark's solution. These findings donfirm the observations reported above and leave little doubt that the depressant effects of chlorpromazine upon the frog heart is one which is only very slowly reversible. In the case of the isolated frog heart, the weakened and depressed contractions can be promptly overcome by the positive inotropic action of ouabain. However, this effect is only transitory. In contrast, the cardiac depression induced by the drug in this preparation is not significantly altered by either adrenaline or noradrenaline, as will be shown later. Why this is so is not clear. It is evident, however, that chlorpromazine produces a progressive, reversible type of non-specific myocardial depression, which requires further investigation.

Anti-adrenergic action.

a) On the blood pressure.

Our experiments on the blood pressure confirm in general the original observations of Courvoisier et al. (1953) that chlorpromazine can

antagonize the pressor actions of both adrenaline and noradrenaline, and that the response to adrenaline is also inverted. It is apparent, however, that this antagonism to the amines is variable depending upon the experimental conditions employed. Thus, it has been shown that during continuous infusion of either adrenaline or noradrenaline in the previously morphinized-pento-barbitalized dog, injections of small doses of chlorpromazine can produce a depressor effect in both cases. Moreover, under these conditions, the antagonism to adrenaline or noradrenaline is quite prolonged, as indicated by the lack of pressor responses to repeated infusions of the amines. However, following either atropinization or vagotomy, this increased sensitization to the vasomotor reversal effect is abolished. These latter observations would rather suggest that the vagus control of the circulation might be involved in some way or other in this vasomotor antagonism. Since chlorpromazine is a central nervous system depressant, the possibility arises that this vagus action might be a central one. In some experiments on the pithed or spinal cat, it has been observed that during continuous perfusion of either adrenaline or noradrenaline, the depressor responses to superimposed test injections of chlorpromazine are rather similar. These findings would also suggest that the adrenaline or noradrenaline blood pressure antagonism induced by the drug might conceivably be modified by its central nervous system actions. It was also noted in our experiments that the pressor responses to large doses of ephedrine were consistently antagonized and even reversed by the previous injection of chlor-promazine. In this respect, the anti-adrenergic action of the drug appears to be somewhat different from that of the ergot alkaloids, which as is well-known, seldom lead to a reversal of ephedrine effect (Salter, 1952). Whether or not this effect is related to the fact that ephedrine in large doses is also a central nervous system stimulant, cannot be stated. In view of the multiple factors which have been shown to influence the vasomotor reversal phenomena,

further experimental study of this problem is necessary before any definite conclusion can be drawn.

b) On the heart.

In connection with the blood pressure experiments, it was shown that the cardiac stimulating actions of adrenaline or noradrenaline were not antagonized by doses of chlorpromazine which induced good adrenaline or noradrenaline vasomotor reversal effects. It is evident, therefore, that under these conditions, there was no evidence of any anti-adrenergic action in the heart.

In the heart-lung preparation also, no anti-adrenergic action could be ascribed to chlorpromazine. Even when very high concentrations were used, subsequent injections of adrenaline or noradrenaline always produced marked tachycardia associated with increased cardiac output. Similarly, in the isolated rabbit heart, no definite anti-adrenergic action was demonstrated with chlorpromazine. In fact, following doses of 250 μg., the responses to 1 μg. of adrenaline or noradrenaline remained unchanged. Following doses of 500 μg. or more, the responses to adrenaline or noradrenaline although diminished were still apparent, and it would appear that this latter effect might be due only to the non-specific myocardial depressant effect of the drug evident with high doses or concentrations. Several publications in the literature, as reviewed by Nickerson (1949), show that other known adrenergic blocking agents also do not antagonize the effects of the sympathomimetic amines in the mammalian heart. However, Nickerson (loc. cit.) also reported that in the isolated amphibian heart, dibenamine and other anti-adrenergic agents can antagonize the stimulating action of adrenaline. When chlorpromazine was studied on the isolated frog heart, as already indicated, it was difficult to arrive at a definite answer to this question. This was so because neither anti-adrenaline nor anti-noradrenaline action could be observed with concentrations

of chlorpromazine which in themselves did not lead to depression of heart contractions. However, the fact that, even with the highest doses of chlorpromazine employed, there was always an initial stimulation of the heart by the amines, that is, prior to the onset of the depression, would rather indicate that the drug also exerts no specific anti-adrenergic action in this preparation.

Anti-fibrillatory action of chlorpromazine.

As previously stated, chlorpromazine has been shown to antagonize the cardiac arrhythmias induced by adrenaline, aconitine and adenosine phosphoric acid (Courvoisier et al.(1953)). Melville (1954) has also reported that the drug prevents chloroform-adrenaline ventricular fibrillation, but not the ventricular fibrillation induced by ouabain or posterior pituitary extracts in dogs under pentobarbital anesthesia. From the data presented, it is clear that in the anesthetized dog, small doses of the drug (1 mg./kg.) can antagonize the cardiac lethal effects of considerable doses of adrenaline (0.4 mg./kg.) administered during chloroform inhalation. Curiously enough, effective anti-fibrillatory doses of the drug do not lead to any significant change in form of the electrocardiogram, and following the injection of adrenaline, marked cardiac stimulation and some increase in the blood pressure level still occurred. The question arises whether or not some central action of the drug might not have again been involved. However, from the above described observations on the heart-lung preparation, the isolated rabbit heart and the isolated frog heart, it would rather appear that the anti-fibrillatory action of the drug is not due to any specific anti-adrenergic action. This conclusion is, however, in disagreement with that of Dilalme and Catenocci (1955). These authors using the anesthetized cat also observed that chlorpromazine could protect the heart against petroleum ether-adrenaline ventricular fibrillation, but, since the drug did not raise the threshold to electrical atrial fibgillation, as was observed with both quinidine and procainamide, it was concluded that "chlorpromazine exerts its protective action by virtue of an anti-epinephrine effect

rather than a direct muscle action." This conclusion appears to us to be unwarranted since, in all the isolated experimental heart preparations tested, the sensitivity of the ventricle to the depressant action of chlorpromazine was invariably greater than was that of the auricles. Indeed, in both the isolated rabbit and isolated frog heart preparations, ventricular contractions were depressed and often arrested, while the auricles were still beating well. Moreover, no specific antagonism to adrenaline or noradrenaline could be noted in these preparations. It is suggested, therefore, that the anti-fibrillatory action of chlorpromazine is associated with the direct myocardial depressant action of the drug as referred to above rather than with its anti-adrenergic action. The basic mechanism of this myocardial depressant action of chlorpromazine warrants further investigation.

GENERAL SUMMARY

1. The object of this research was to study the pharmacological effects of chlorpromazine on the cardiovascular system. Four (4) different preparations were used, namely, the animal as a whole, the heart-lung preparation of the dog, and the isolated perfused hearts of both the rabbit and the frog. With this diversified approach, we hoped to achieve a greater understanding of the complex cardiovascular actions of this drug.

2. In the intact animal, chlorpromazine in small doses (1 to 10 mg./kg.) induces a transitory fall in blood pressure associated with an initial cardiac acceleration, a slight depression of T wave, but no change in either P-R interval or QRS complex. This hypotension is not significantly affected by vagotomy, atropinization or the injection of hydergin. However, the cardiac acceleration and T wave changes on the electrocardiogram are abolished by vagotomy or atropinization. With large doses (10 mg./kg.), in addition to the fall in blood pressure, there is slowing of the heart rate with elevation and then inversion of the T wave. These latter changes, however, are not prevented by either vagotomy or atropinization.

3. The pressor responses to both adrenaline and ephedrine are reversed with single injections of doses of chlorpromazine (1 mg./kg.), while the pressor response to noradrenaline, though diminished, is not inverted. Chlorpromazine does not prevent the cardiac acceleration induced by either adrenaline or noradrenaline.

4. During the continuous infusion of adrenaline or following the injection of ephedrine, low doses of chlorpromazine (0.01 to 0.5 mg./kg.) induce a prompt, marked and sustained depressor response. This hypotension appears to be potentiated by morphine and diminished by vagotomy or atropinization.

5. On the other hand, during the continuous infusion of noradrenaline, injections of chlorpromazine (0.01 to 0.5 mg./kg.) produce a transient rather than a sustained depressor response. In the morphinized animal, the same procedure now produces a sustained rather than a transient depressor effect. This hypotension is diminished by vagotomy or atropinization.

6. In the pithed or spinal cat, injections of chlorpromazine (0.02 to 0.04 mg./kg.) during the continuous infusion of either adrenaline or noradrenaline produce identical depressor responses.

7. Prior injections of chlorpromazine (0.5 to 1 mg./kg.) effectively prevent the ventricular extrasystoles and fibrillations induced by the administration of adrenaline during chloroform inhalation although a marked tachycardia still occurs.

8. In the heart-lung preparation, high doses of chlorpromazine (20 to 40 mg.) produce a progressive depression of the cardiac rate and the output. This effect was reversible with such doses. Excessive doses (50 mg.) produce a very marked depression which ultimately led to the complete cessation of cardiac activity if no attempt was made to reverse the effect.

9. In the heart-lung preparation, chlorpromazine (20 to 40 mg.) could not prevent the tachycardia and increased cardiac output produced by injections of adrenaline or noradrenaline (100 µg.). Even with toxic doses of the drug (50 mg.), as stated above, the injection of adrenaline or noradrenaline (1 mg.) could reverse the depression.

10. In the heart-lung preparation, injections of chlorpromazine (40 mg.) diminish, but do not abolish, the depressant effects of acetylcholine (250 to 500 µg.).

11. In the isolated perfused rabbit heart, chlorpromazine (10 µg. to 2.5 mg.) produces a marked increase in coronary inflow, an increase in the heart rate, and a decrease in the amplitude of contraction. Low doses of

the drug (250 μg.) did not in any way alter the response to adrenaline or noradrenaline. However, in high doses (500 μg. to 2.5 mg.), the response to these amines was diminished, but not abolished. Chlorpromazine appeared to possess some slight "anti-acetylcholine" action in this preparation.

12. In the isolated frog heart, chlorpromazine (10 to 500 μg./ml.) produces a progressive depression of both the auricular and ventricular contractions associated with auriculo-ventricular dissociation. This effect is slowly reversible. Simultaneous continued perfusion with adrenaline or noradrenaline cannot prevent the development of these depressant effects. However, this action of the drug can be overcome by the simultaneous perfusion with ouabain.

13. The depressant effect of chlorpromazine resembles somewhat that of the structurally similar analogue, promethazine. However, recovery of the frog heart from the effects of the latter drug occurs much more rapidly than is the case with chlorpromazine. Again, the depressant action of promethazine cannot be antagonized by the simultaneous perfusion with adrenaline or noradrenaline.

14. The depressant effects of chlorpromazine in the isolated frog heart differ from those of the four (4) local anesthetics studied - procaine, cocaine, pontocaine and nupercaine - in the following respects: a) the local anesthetics, even in high concentrations, never produced complete cardiac arrest; b) recovery from these agents occurred very rapidly, and c) their depressant effects can be partially antagonized by the simultaneous perfusion with adrenaline or noradrenaline.

BIBLIOGRAPHY

1. Bartis, K., Morris, G., Rogers, S., and Knight, R.
"Clinical Results using Chlorpromazine as an Anti-emetic."
J. of Pharmacol. and Exper. Therap. 110. 29. 1954.

2. Birsohn, J., Namajuska, I., and Cochrane, L.S.G.
"Inhibitory Effect of Chlorpromazine on some Brain Enzyme Systems in Vitro."
Fed. Proceed. 14. 182. 1955.

3. Bertrand, I., Quivy, D., and Gayet-Hallion, T.
"Potentialisation par la Chlorpromazine de l'Effet Anticonvulsivant de la Diphenyl-hydantoine."
Compt. rend. Soc. Biol. 148. 1170. 1954.

4. Bovet, D., and Bovet-Nitti, F.
"Médicaments du Système Nerveux Végétatif."
Edition S. Karger, S.A. Bale, Paris, 1948.

5. Bovet, D., Fournel, J., and Charpentier, P.
Thérapie. 2. 115. 1947.

6. Boyd, E.M., Cassell, W.A., and Boyd, C.E.
"Prevention of Apomorphine-induced Vomiting by (dimethylamino-1-n-propyl-3)-N-(2-chloro)-phenothiazine hydrochloride."
Fed. Proceed. 12. 303. 1953.

7. Brand, E.D., Harris, T.D., Borison, H.L., and Goodman, L.S.
"The Anti-emetic Activity of 10-(γ-Dimethylaminopropyl)-2-chloro-phenothiazine (Chlorpromazine) in Dog and Cat."
J. of Pharmacol. and Exper. Therap. 110. 86. 1954.

8. Brunaud, M., Brunaud, S., and Decourt, P.
"Action de la Chlorpromazine sur l'Adrenaline-secrétion Surrénale."
Compt. rend. Soc. Biol. 147. 1764. 1953.

9. Burn, J.H.
"The Pharmacology of Chlorpromazine and Promethazine."
Proc. Royal Soc. of Med. 47. 617. 1954.

10. Cathala, H.P., and Pocidalo, J.J.
"Injection of 4560 R.P. into the Cerebral Ventricles of the Dog."
Compt. rend. Soc. Biol. 46. 1709. 1952.

11. Chauchard, B., and Chauchard, P.
"Comparison de l'Action Paralysante Ortho et Parasympathique de quelques Ganglioplégiques."
Compt. rend. Soc. Biol. 146. 528. 1952.

12. Cook, L., and Toner, J.J.
"The Anti-emetic Action of Chlorpromazine, SKF No. 2601-A (R.P. 4560)."
J. of Pharmacol. and Exper. Therap. 110. 12. 1954.

13. Coraboeuf, E., Distel, R., Lavigne, S., et Boistel, J.
"L'Action du Chlorpromazine sur l'Activité Electrique du Tissu Conducteur du Coeur de Chien."
Compt. rend. Acad. Sc. 239. 189. 1954.

14. Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., et Koetschet, R.
"Propriétés Pharmacodynamiques du Chlorhydrate de Chloro-3 (Diméthylamino-3-propyl)-10-phénothiazine (4560 R.P.)."
Arch. int. Pharmacodyn. 92. 305. 1953.

15. Das, N.N., Dasgupta, S.R., et Werner, G.
"Changes of Behaviour and EEG in Rhesus Monkeys caused by Chlorpromazine."
Arch. int. Pharmacodyn. 99. 451. 1954.

16. Dasgupta, S.R., and Werner, G.
"Inhibition of Hypothalamic, Medullary and Reflex Vasomotor Responses by Chlorpromazine."
Brit. J. of Pharmacol. and Chemotherapy. 9. 289. 1954a.

17. Dasgupta, S.R., Mukherjee, K.L., and Werner, G.
"The Activity of some Central Depressant Drugs in Acute Decorticate and Diencephalic Preparations."
Arch. int. Pharmacodyn. 97. 149. 1954.

18. Dasgupta, S.R.
"The Effect of Chlorpromazine on the Estrus Cycle in Rats."
Bull. Calcutta Sch. of Trop. Med. 3. 86. 1955.

19. Dawson, J.F., and Hiestand, W.H.
"Influence of Chlorpromazine on Body Temperature Control in small Mammals."
Fed. Proceed. 14. 36. 1955.

20. Decourt, P., Brunaud, M., et Brunaud, S.
"Etude Expérimentale de la Prétendue Action Ganglioplégique de la Chlorpromazine, (4560 R.P.)."
Compt. rend. Soc. Biol. 147. 1602. 1953.

21. Dilalme, J.R., and Catenacci, A.J.
"Chlorpromazine Protection against Hydrocarbon-Epinephrine Induced Ventricular Arrythmias."
Fed. Proceed. 14. 333. 1955.

22. Dobkin, H.B., Gilbert, R.G.G., and Lamoureux, L.
"Physiological Effects of Chlorpromazine."
Anesthesia. 9. 157. 1954.

23. Dundee, J.W., Scott, W.E.B., and Mesham, P.R.
"The Production of Hypothermia."
Brit. Med. J. 2. 1244. 1953.

24. Dundee, J.W., Mesham, P.R., and Scott, W.E.B.
"Chlorpromazine and the Production of Hypothermia."
Anesthesia. 9. 296. 1954.

25. Dundee, J.W.
"Chlorpromazine."
Brit. J. Anaesthesia. 26. 357. 1954.

26. Finkelstein, M., and Spencer, W.A.
"The Effect of Chlorpromazine on Heart Muscle, and its Influence on the Inotropic Action of Three Sympathomimetic Amines."
Fed. Proceed. 13. 354. 1954a.

27. Finkelstein, M., Spencer, W.A., and Ridgeway, E.R.
"Chlorpromazine and Tissue Metabolism."
Proc. Soc. Exper. Biol. and Med. 87. 343. 1954b.

28. Foltz, E.L.
"Cardiovascular Observations following Use of Dibenzylene and Chlorpromazine Singly and in Combination."
Fed. Proceed. 14. 339. 1955.

29. Foster, C.A., O'Mullane, E.J., Gaskell, P., and Churchill-Davidson, H.C.
"Chlorpromazine, A Study of its Action on the Circulation in Man."
Lancet. 267. 614. 1954.

30. Fournel, J.
"Action of 4560 R.P in Preventing Induced Shock."
Compt. rend. Soc. Biol. 146. 561. 1952.

31. Ganshirt, H., et Brilmayer, H.
"Effects of Largactil on Brain Slices and Homogenates."
Arch. int. Pharmacodyn. 98. 467. 1954.

32. Giequel, J., and Schmitt, H.
"Potentialisation par la Chlorpromazine (4560 R.P.) des Effets du Chloral et de l'Ethylurethane."
Compt. rend. Soc. Biol. 148. 899. 1954.

33. Giaja, J., and Giaja, L.M.
"La Chlorpromazine et la Thermorégulation."
Compt. rend. Soc. Biol. 148. 842. 1954.

34. Glaviano, V.V., and Wang, S.C.
"Dual Mechanism of Anti-emetic Action of Chlorpromazine."
Fed. Proceed. 13. 358. 1954.

35. Halpern, B.N.
"Recherches sur une Nouvelle Série Chimique de Corps Doués de Propriétés Anti-histaminiques et Anti-anaphylactiques: les Dérivés de la Thiodiphenylamine."
Arch. int. Pharmacodyn. 74. 314. 1947.

36. Halpern, B.N., and Liakopoulous, P.
"Action Comparée de la Prométhazine et de la Chlorpromazine sur la Température Chez le Rat et le Cobaye."
Compt. rend. Soc. Biol. 148. 955. 1954.

37. Hershey, S.G., and Guccione, I.
"Protective Action of Chlorpromazine in Hemorrhagic Shock in Rats."
Fed. Proceed. 14. 351. 1955.

38. Heymans, C., Estable, J.J., Castillo de Bonneveaux, S.
"Sur la Pharmacologie de la Phenothiazinyl-ethyldiethylamine (2987 R.P.)."
Arch. int. Pharmacodyn. 79. 123. 1949a.

39. Heymans, C., Estable, J.J., et Castillo de Bonneveaux, S.
"Nicotinolytic Action of Diethylaminoethylphenothiazine (2987 R.P. or Diparcol)."
Arch. int. Pharmacodyn. 79. 185. 1949b.

40. Hopkin, D.A.
"Brit. Med. J. 1. 1036. 1954.

41. Houde, R.H., and Wallenstein, S.L.
"Analgesic Power of Chlorpromazine alone and in combination with Morphine."
Fed. Proceed. 14. 353. 1955.

42. Huguenard, P.
"Hibernation Artificielle."
Anasth. et Analg. 9. 240. 1952.

43. Huidobro, F.
"Some Pharmacological Properties of Chloro-3(dimethylamino-3-propyl)-10- Phenothiazine or 4560 R.P."
Arch. int. Pharmacodyn. 98. 308. 1954.

44. Kelan, M., and Zweifach, B.W.
"Influence of Partial and Complete Evisceration on the Action of Drugs Protecting against Lethal Hemorrhage."
Surg. Gyn. and Obstetrics. 99. 707. 1954.

45. Knowlton, F.P., and Starling, E.H.
" The Influence of Variations in Temperature and Blood Pressure on the Performance of the Isolated Mammalian Heart."
J. Physiol. 44. 212. 1912.

46. Kopera, J., and Armitage, A.K.
"Comparison of Some Pharmacological Properties of Chlorpromazine, Promethazine and Pethidine."
Brit. J. Pharmacol. and Chemotherapy. 9. 392. 1954.

47. Laborit, H., and Huguenard, P.
"L'Hibernation Artificielle pas moyens Pharmacodynamiques et Physique."
La Press Méd. 59. 1329. 1951.

48. Laborit, H., Huguenard, P., et Alluaume, R.
"An Nouveau Stabilisateur Végétatif."
La Press Méd. 60. 206. 1952.

49. Laborit, H., et Huguenard, P.
"Pratique de L'Hibernothérapie en Chirurgie et en Médicine."
Masson et Cie., Paris, 1954.

50. Lamarche, M., et Arnould, P.
"Action de la Chlorpromazine sur l'Intestin Isolé du Lapin et du Rat."
Compt. rend. Soc. Biol. 148. 565. 1954.

51. Langendorff, O.
Arch. f. d. ges. Physiol. 61. 292. 1895.

52. Lu, F.C., and Melville, K.I.
"A New Apparatus and Procedure for Continuous Registration of Changes in Coronary Flow Concurrently with Changes in Heart Contractions."
J. of Pharmacol. and Exper. Therap. 99. 277. 1950.

53. Lu, F.C., and Melville, K.I.
"Effects of Noradrenaline on Coronary Flow and Heart Contraction, as Recorded Concurrently in the Isolated Rabbit Heart."
J. Physiol. 113. 365. 1951.

54. Marquardt, P.
"Etude des Propriétés Sympatholytiques et Adrénolytiques de la Chlorpromazine."
Thérapie. 8. 777. 1953.

55. Melville, K.I.
"The Protective Action of Atabrine against Chloroform-Adrenaline Ventricular Fibrillation."
J. of Pharmacol. and Exper. Therap. 87. 350. 1946.

56. Melville, K.I.
"Further Studies on the Anti-fibrillatory Action of Coronary Dilator Drugs."
J. of Pharmacol. and Exper. Therap. 94. 136. 1948.

57. Melville, K.I. and Lu, F.C.
"Effects of Epinephrine, Aminophylline, Nitroglycerine and Papaverine on Coronary Inflow and on Heart Contraction, as Recorded Concurrently."
J. of Pharmacol. and Exper. Therap. 99. 286. 1950.

58. Melville, K.I.
"Observations on the Adrenergic Blocking and Anti-fibrillatory Actions of Chlorpromazine."
Fed. Proceed. 13. 386. 1954.

59. Morris, G., Matthews, W., and Moyer, J.
"Clinical Experience with Chlorpromazine in Spinal Anesthesia."
Current Researches in Anesthesia and Analgesia. 33. 340. 1954.

60. Morris, G., Pontius, R., Herschberger, R., and Moyer, J.H.
"Cerebral Hemodynamics following Administration of Chlorpromazine."
Fed. Proceed. 14. 371. 1955.

61. Moyer, J.H., Kent, B., Knight, R., Morris, G., Huggins, R., and Handley, C.A.
"Laboratory and Clinical Observations on Chlorpromazine - Hemodynamic and Toxicological Studies."
Amer. J. of Med. Sc. 227. 283. 1954.

62. Nickerson, M.
"The Pharmacology of Adrenergic Blockade."
J. of Pharmacol. and Exper. Therap. 95. 27. 1948.

63. Pickering, R.W., and Ahlquist, R.R.
"Cardiovascular and Autonomic Effects of Chlorpromazine."
Fed. Proceed. 14. 378. 1955.

64. Pocidalo, J.J. et Tardieu, C.
"Prévention par la Chlorpromazine des Accidents Graves apres Lésion Expérimentale des Pédoncles Cérébraux."
Compt. rend. Soc. Biol. 148. 452. 1954.

65. Pocidalo, J.J., Cathala, H.P., Himbert, J., et Tardieu, M.
"Action sur l'Excitabilité des Nerfs Sympathiques du Chlorhydrate de Diméthylaminopropyl-N-chlorophénothiazine."
Compt. rend. Soc. Biol. 146. 386. 1952.

66. Reckless, D.
"Potentiation."
Anaesthesia. 9. 288. 1954.

67. Reilly, J., et Tournier, P.
"L'Action de la Chlorpromazine (4560 R.P.) sur l'Intoxication Typhique Expérimentale.
Bull. Acad. Nationale Méd. 137. 385. 1953.

68. Rutledge, L.T., and Doty, R.W.
"Differential Action of Chlorpromazine on Conditioned Responses to Peripheral versus Cortical Stimuli."
Fed. Proceed. 14. 126. 1955.

69. Salter, W.T.
Textbook of Pharmacology - Page 730.
W.B. Saunders Co. 1952.

70. Stehle, R.E., and Melville, K.I.
"The Influence of the Heart Beat upon the Flow of Blood into the Coronary Arteries."
J. of Pharmacol. and Exper. Therap. 46. 471. 1932.

71. Stevenson, T.D., and Sjoerdsma, A.
"Blood Pressure in Patients with Hypertension following Intramuscular Chlorpromazine."
Proc. Soc. Exp. Biol. and Med. 86. 726. 1954.

72. Terzian, H.
Rass. Neurol. Veg. 4. 211. 1952.

73. Vanlerenberche, J., Robelet, A., et Milliled, G.
"Action du 4560 R.P. sur le Système Sympathique."
Arch. int. Pharmacodyn. 98. 412. 1954.

74. Viaud, P.
Produits Pharmaceutiques. 2. 53. 1947.

75. Wallis, R.
"Potentiation of Hypnotics and Analgesics - Clinical Experience with Chlorpromazine."
N.Y. State Med. J. 55. 243. 1955.

76. Weese, H.
"Eine mechanische, automatisch registrierende Stromuhr Fur den geschlossenen Kreislauf."
Arch. Exper. Path. et Pharmakol. 166. 392. 1932.

77. Winkleman, N.W.
"Chlorpromazine in the Treatment of Neuropsychiatric Disorders."
J.A.M.A. 155. 18. 1954.

78. Winter, C.A.
"A Study of Comparative Anti-histamine Activity of Six Compounds."
J. of Pharmacol. and Exper. Therap. 90. 224. 1947.

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