

STUDY OF SOME PHARMACOLOGICAL ACTIONS OF BERBERINE*

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Summary : Berberine produced reversible and dose-dependant hypotension in the anaesthetized dog, cat, rat and frog. The hypotension was studied in details in the dog. It was not due to the release of tissue histamine and was not blocked by atropine, mepyramine, phenoxybenzamine, propranolol, pentolinium, bilateral vagotomy and ablation of brain. Propranolol-pentolinium combination, however, blocked this effect in some animals and, for a short period, reversed it to hypertension in the others. Berberine did not alter the actions of carbachol, histamine, bradykinin, isoprenaline, adrenaline and nor-adrenaline on the blood pressure. It increased the volume of spleen and hind limb. Berberine appears to induce hypotension by directly acting on the blood vessels.

Berberine stimulated the *in situ* dog heart and produced tachycardia which outlasted hypotension. In smaller doses, it stimulated the isolated heart of the rabbit and the frog. It temporarily reversed the depression of the frog heart perfused with low calcium Ringer. In the dog and frog, myocardial depression is not likely to contribute to the berberine-induced hypotension.

The respiratory stimulant action was parallel to, and could be a reflex phenomenon provoked by hypotension.

In mice, berberine lowered the rectal temperature, reduced the spontaneous motor activity and prolonged the hexobarbitone sleeping time. It increased the incidence of death but not the severity of tremors in the tremorine-treated mice.

The LD_{50} of berberine sulphate in mice (intraperitoneal route) was 24.3 mg/kg.

In the frog, berberine produced peripheral vasodilatation, hypotension and aggregation of the melanin granules in the melanophores.

Its anticholinesterase action may explain the potentiation of acetylcholine-induced hypotension and angiotensin-induced hypertension in the dog, increase in the intestinal motility in the dog, antitubocurarine action and potentiation of acetylcholine on the isolated frog rectus muscle, slowing of the inactivation rate of acetylcholine by the dog and rabbit sera, stimulation of the frog ciliary movements and potentiation of the action of angiotensin on the guineapig ileum.

Aqueous solution of berberine kept at room temperature for about 11 months was as active as the fresh one.

Berberine had local anaesthetic action.

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It inhibited the contractions induced by acetylcholine, carbachol, histamine, bradykinin, $BaCl_2$ and KCl on the guineapig ileum and by 5-HT on the rat uterus. The inhibition was dose-dependant and reversible. It blocked the Schultz-Dale phenomenon on the ileum of the sensitized guineapig.

A possibility is suggested that berberine interferes with the process of depolarization and repolarization of the excitable tissues.

INTRODUCTION

Berberine is an alkaloid (Fig. 1) which occurs in *Berberis aristata* and certain other plants (8, 9, 10, 14). In India some of these plants have been used, for many centuries,

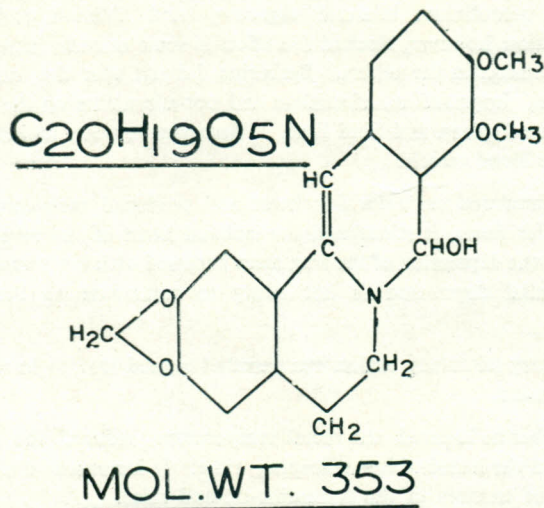


Fig. 1 : Chemical structure of berberine.

for the treatment of jaundice, chronic dysentery, infantile diarrhoea, leucorrhoea, fever and certain diseases of the skin and the eye (9, 10, 12, 16, 40, 41, 42). Varma (40) discovered and Das Gupta and Dikshit (11) established the utility of berberine in the treatment of oriental sore (dermal leishmaniasis). The drug failed to reduce mortality though prolonged the survival time of rats inoculated with *Trypanosoma equiperdi* (34) and *Trypanosoma evansi* (32). It had no chemotherapeutic action against human malaria (8). In recent years, there has been an intense reawakening of interest in berberine particularly on its usefulness in various diarrhoeas (1, 13, 15, 19, 22, 24, 25, 26, 30, 31, 36, 37). Experiments were planned to study the detailed pharmacological properties of berberine and the first part of the data is presented here.

MATERIALS AND METHODS

For convenience, berberine sulphate which was used in most of the experiments is mentioned hereafter as "berberine". The stock solution strength was 5 mg/ml in distilled water and on electric pH meter its pH was 4.05.

Blood pressure of the dog: Dogs (8-15 kg) of either sex were anaesthetized with sodium pentobarbitone (35 mg/kg ip). In one dog, only ether was used to induce and maintain anaesthesia. Spinal dog preparations were made by the technique described for the cat by Burn (6). Carotid artery blood pressure was recorded with mercury manometer and drugs were injected through the cannulated femoral vein.

For recording the *movements of the auricle and the ventricle* the method described for the cat (38) was followed. The Starling heart lever which was used for recording gave a magnification of 20 fold. The *respiration* was recorded by connecting one limb of the tracheal cannula to a Marey's tambour. The *intestinal motility* was recorded with Jackson's enterograph. The *limb volume* was recorded in 3 experiments, by the method described by Jog (18). The right hind limb of the anaesthetized dog was placed in a glass plethysmograph (18 cm length, 4 cm diameter, open at both the ends). A sleeve of skin obtained by a circular incision on the skin half an inch above the proximal end of the plethysmograph was everted down and tied over its proximal edge. The volume displacements of air in the plethysmograph were recorded by connecting its distal end to a volumetric pressure transducer PT-5-A (Grass Instrument Co., U.S.A.) which was in turn connected to a polygraph (Model 5C). The calibration of the transducer was done before and after the experiment by injecting a known volume of air into the plethysmograph. The *spleen volume* was recorded by using the spleen plethysmograph (6) which was connected to a Marey's tambour (4 experiments) or a pressure transducer (1 experiment).

Histamine level and PCV of the blood samples of the anaesthetized dogs: One, 3, 10 and 15 min after the intravenous injection of berberine, histamine or compound 48/80, blood samples were drawn from the saphenous or radial vein of the dogs; these samples were immediately assayed for histamine content on the isolated guineapig ileum preparation. The acid phosphate of histamine was used throughout and the doses refer, unless specified, to the histamine base. For PCV, 1 ml heparinized blood was centrifuged in the Wintrobe's tube at 3000 rpm for 20 min.

Blood pressure of the cat: Female cats (3-4 kg) were anaesthetized with pentobarbitone sodium (35 mg/kg ip) and carotid artery blood pressure was recorded with mercury manometer. One spinal cat preparation was made. Drugs were injected through the cannulated femoral vein.

Blood pressure of the rat: Rats (200-300 g) of either sex were anaesthetized with urethane (1.25 g/kg sc) and the carotid artery was cannulated with a polyethylene tube which was filled with heparinized normal saline and was connected to Condon's manometer. Berberine was dissolved in 0.2 ml of distilled water and injected through the cannulated external jugular vein of the opposite side.

Blood pressure of the frog (17): Frogs (300-500 g) were anaesthetized with sodium pentobarbitone (40 mg/kg intraabdominally) and ventral portion of the pectoral girdle

was removed without injuring the ventral abdominal vein. After opening the pericardium, the left branch of the aorta was dissected clean and a ligature was applied about 1.5 cm away from its commencement. On either side of the ligature 2 polyethylene cannulae (external diameter 2 mm) were introduced into the aorta and tied. The cannula inserted proximal to the ligature and directed towards the heart was filled with heparinized frog saline (0.6% NaCl). It was connected to a Statham transducer (P 23AA) which was in connection with the pressure meter (Atlas Druck-Messgerat); the pressure meter, in turn, was connected to a preamplifier channel of a polygraph (Sanborn model 150). The second cannula inserted into the aorta distal to the ligature, was directed away from the heart and was used for injecting drugs into the general circulation. Heparin (300 i.u.) was injected into the general circulation to prevent blood clotting. Berberine dissolved in distilled water was injected in 0.1-0.5 ml volume.

Rectal temperature was studied in 4 groups each of 10 adult albino mice (14-42 g) of either sex by the method described by Bhide (4). All the drugs were injected intraperitoneally in such concentrations that the volume injected per kg animal weight was 10 ml. The room temperature was about 80°F. The control group received 10 ml/kg distilled water; the remaining 3 groups received 1.0, 3.0 and 10.0 mg/kg berberine respectively. The temperature was recorded by keeping the lubricated mercury bulb of a clinical thermometer into the rectum for 2 min.

Hexobarbitone sleep was studied in the adult albino mice (14-44 g) of either sex by the method described by Bhide (4). All the drugs were injected intraperitoneally in such concentrations that the volume injected per kg animal weight was 10.0 ml. The room temperature was about 80°F. Berberine or chlorpromazine was injected 30 min before the injection of hexobarbitone sodium.

Effect on the tremorine-induced tremors was studied in 3 groups each of 10 adult albino mice (14-35 g) of either sex. The control group received distilled water (10 ml/kg); the remaining 2 groups received 3.0 and 10.0 mg/kg (ip) of berberine respectively. The concentrations of berberine and tremorine were so adjusted that the volume injected per kg animal weight was 10 ml. Tremorine 25.0 mg/kg (sc) was injected 30 min after injecting distilled water or berberine. Atropine 100 mg/kg (ip) was administered in 2 mice as positive control. The room temperature was about 70°F. The degree of tremors was assessed by gross observation.

Local anaesthetic action: (a) *Guineapig corneal technique* (7) was followed in 5 groups each of 6 animals. The first group served as control and received only distilled water; the second one received 2% cocaine. The remaining 3 groups were used for 0.5, 1.0 and 2.0% berberine respectively. (b) *Skin infiltration technique* (5) was followed in 4 groups each of 10 guineapigs. The skin of the control group was infiltrated with distilled water; the remaining 3 groups were for procaine (0.5 and 2.0%) and berberine (0.5%).

Acute toxicity: Seven groups each of 20 albino mice (16-45 g) of either sex received 0.3, 1.0, 3.0, 10.0, 20.0, 25.0, and 30.0 mg/kg (ip) berberine respectively and mortality during the first 24 hr was recorded. The room temperature was 80-82°F.

The isolated rabbit heart (6), the frog heart and the rat (6) and the frog (17) hind limb perfusion experiments were also made for studying the effects of berberine.

The isolated tissues: Pieces of guineapig ileum, rabbit jejunum, frog rectus abdominis muscle (6) or oestrogenized rat uterus were suspended in 10.0 ml capacity isolated tissue bath containing Tyrode (37°C), Ringer (37°C), amphibian Ringer (room temperature 21°-37°C) or de Jalon (31° C) solutions respectively; the solutions were bubbled with oxygen. The contractions were recorded on a smoked drum with a simple or frontal writing lever. For oestrogenization, adult female non-pregnant rats received 0.1 mg (ip) oestradiol benzoate in 1.0 ml groundnut oil, daily, on 2 successive days. The animals were killed 24 hr after the last injection and the uterine horns were used.

Angiotensin solution in distilled water (50 µg/ml) lost about 95% of its potency when stored at -4°C for about a month. Therefore, fresh solution was prepared every week.

Schultz-Dale phenomenon (28): Guinea pigs were sensitized by an ip injection of 3 ml normal horse serum or 5 ml of 25% V/V fresh egg-white in water per animal. The animals were killed 3-18 weeks after sensitization. The entire small intestine was removed and cut into 3 cm long pieces. Individual pieces were discarded after one exposure to the antigen. Berberine was added to the bath 1 min before the antigen.

Ciliary movements: The dissection described by Burn (6) was followed; in some experiments, the chamber for keeping the preparation moist and warm was not used because the results obtained with and without the chamber were almost identical. Instead of white poppy seeds (average weight 0.29 mg/seed) the black ones of *Amaranthus gangeticus* (known as Maath in Marathi and Marsa in Hindi; average weight 0.95 mg/seed) were used. The solutions of berberine or carbachol were applied in 0.2 ml volume on the ventral mucous surface of the oesophagus and were gently washed off, after 3-5 min, with about 8 ml of frog Ringer. The room temperature ranged between 86° and 100°F.

Skin melanophores of the frog (17): Frogs were anaesthetized (details described above) and the abdomen was dissected open. Berberine was injected through the liver lobe. The effect of berberine on the colour of the dorsal skin was noted and the distribution of melanin granules in the melanophores in the web skin of the hind limb toes was observed under the microscope (x60).

RESULTS

Blood pressure: In the anaesthetized dog, cat, rat and frog, berberine produced fall in blood pressure which was proportional to its dose (0.1-6.0 mg/kg). On weight basis, rat appeared more sensitive to its hypotensive action than the dog, cat and frog. In the frog the

initial blood pressure was about 25-50 mm Hg and 1 mg/kg dose of berberine reduced it by 15-20 mm Hg. Recovery occurred in about 15-20 min (Fig. 2; 4 experiments). In the dog, the initial blood pressure was 100-140 mm Hg and 3 mg/kg dose of berberine sharply reduced it by about 60 mm Hg (Fig. 3; 31 experiments); the recovery was gradual and complete in about 3-5 min. In the dog anaesthetized with ether (1 experiment) as also in the spinal dog (2 experiments) and cat (1 experiment) preparations the degree and duration of hypotension induced by berberine were comparable to those in the pentobarbitone-anaesthetized animals. Repeated administration of berberine did not induce tachyphylaxis (7 experiments). Atropine sulphate (1 mg/kg), mepyramine maleate (10 mg/kg), pentolinium tartrate (1 mg/kg), propranolol (Inderal, 1 mg/kg) or phenoxybenzamine (3 mg/kg) administered intravenously 10-45 min before the injection of berberine blocked the effects of acetylcholine (1-3 μ g/kg; 4 experiments), histamine (0.3-1.0 μ g/kg; 2 experiments), nicotine (20 μ g/kg; 2 experiments), isoprenaline (1 μ g/kg; 7 experiments) and adrenaline (3 μ g/kg; 3 experiments) respectively. However, these antagonists or bilateral vagotomy (4 experiments) neither blocked nor reduced the action of berberine; indeed, in some experiments, after injecting atropine or propranolol or after bilateral vagotomy berberine-induced hypotension took longer time (about 20 min) to recover.

In 12 dogs, the effect of injecting both propranolol and pentolinium on berberine-induced hypotension was studied. This treatment produced blockade of berberine-induced hypotension in 2 dogs. In 4 dogs (one of whom had also received atropine), this blockade was preceded by a short phase (about 30 min) during which berberine produced rise (instead of fall) in the blood pressure by about 80 mm Hg. In the remaining 6 animals propranolol-pentolinium combination lowered the blood pressure to 20-40 mm Hg and then not only berberine (3.0-6.0 mg/kg) but even large dose of histamine acid phosphate (100 μ g/kg) could not elicit further fall in the blood pressure.

The latent period of hypotension in the dog was about 18 sec (range 14-22 sec). The latent period was not altered significantly when berberine (3 mg/kg) was injected before and after vagotomy and into femoral vein (4 experiments) or femoral artery (3 experiments) or jugular vein (2 experiments), or common carotid artery (2 experiments), or left ventricle (1 experiment).

After receiving berberine, dogs became more sensitive to the hypotensive action of acetylcholine (Fig. 3; 6 experiments); however, berberine had no effect on the hypotension induced by carbachol (1 μ g/kg, Fig. 3; 7 experiments), histamine (1 μ g/kg; 7 experiments) or isoprenaline (1 μ g/kg; 10 experiments) or bradykinin (3 μ g/kg; 4 experiments).

Hypertension induced by the carotid artery occlusion (30-45 sec; 5 experiments) or by adrenaline (3-5 μ g/kg; 10 experiments) and noradrenaline (3-5 μ g/kg; 4 experiments) was not influenced by the prior injection of berberine (3 mg/kg).

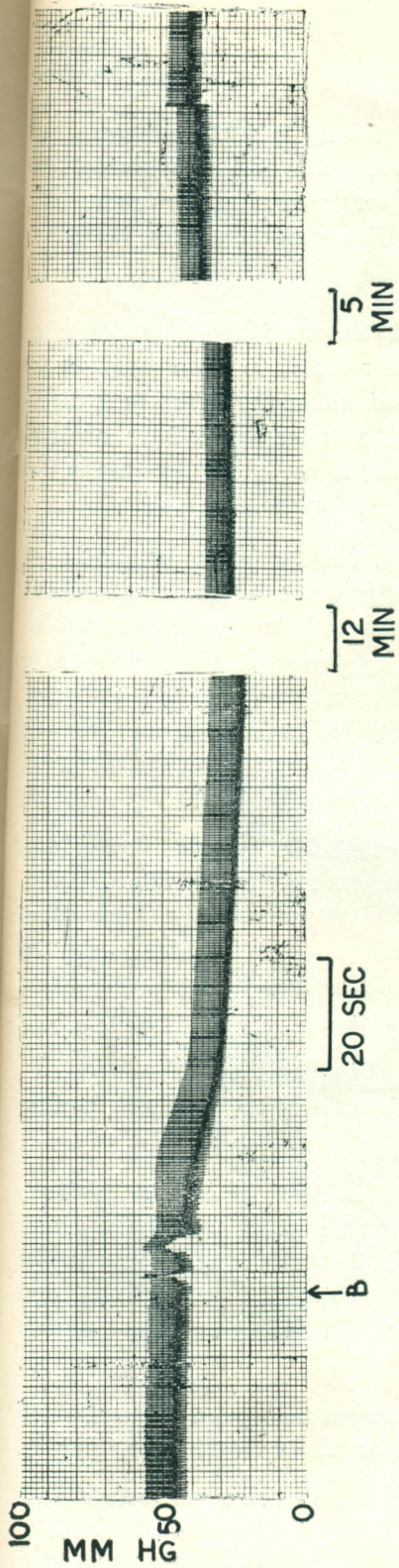


Fig. 2: Blood pressure of the anaesthetized frog (490 g). B, berberine sulphate (1 mg/kg).

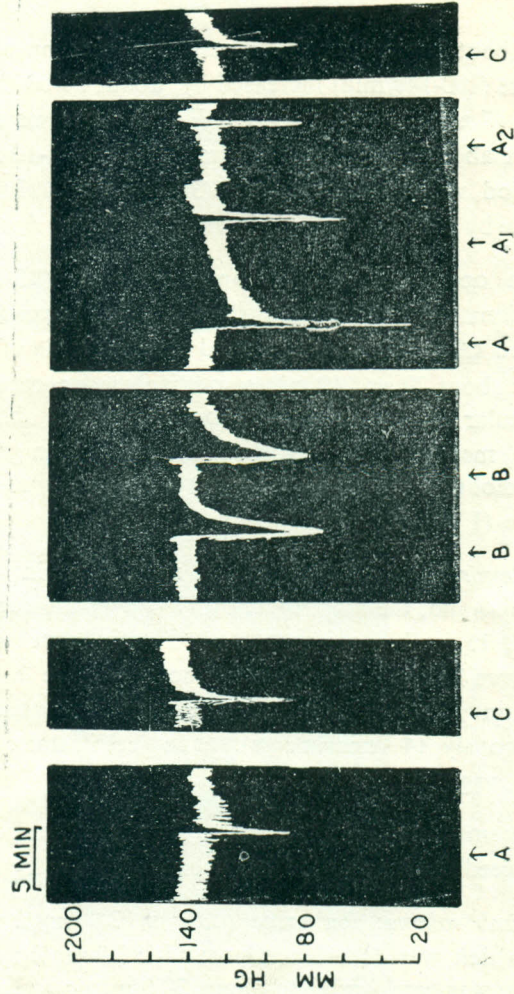


Fig. 3: Blood pressure of the anaesthetized dog (10 kg). A, acetylcholine (1 μ g/kg); C, carbachol (1 μ g/kg); B, berberine sulphate (3 mg/kg); A₁, acetylcholine (0.5 μ g/kg); A₂, acetylcholine (0.1 μ g/kg).

Neostigmine (0.2 mg/kg; 2 experiments), physostigmine (0.1 mg/kg; 1 experiment) and berberine (3 mg/kg; 6 experiments) potentiated the hypotensive action of acetylcholine (3.0 µg/kg) on the dog blood pressure by about 10 fold. Further, they potentiated by about 25% the hypertensive action of angiotensin (1 µg/kg); however, this effect was inconsistent in that it could not be elicited in some dogs.

About 2 min after the intravenous injection of 1-45 mg/kg doses of berberine, the initial heart rate (100-140/min) increased by about 20-60 beats/min as revealed by the lead II of E.C.G. (7 experiments). Other E.C.G. parameters remained unaltered. The tachycardia lasted for about 30 min with the smaller doses (1-10 mg/kg) and, over the entire 2 hr observation period, with the higher doses (30-45 mg/kg). In only one dog there was a decrease in the heart rate by about 40 beats/min after 30 mg/kg dose and it lasted for about 30 min.

In open-chest preparations berberine (0.3-10.0 mg/kg) increased the amplitude of contractions of the auricle and the ventricle (4 experiments). In one such experiment, berberine in 0.3 and 1.0 mg/kg dose increased the amplitude of the auricular and the ventricular contractions for about 20 and 40 min respectively. Isoprenaline (2 µg/kg) increased the amplitude of the auricular and the ventricular contractions for about 4 and 1 min respectively; carbachol (2 µg/kg) markedly inhibited the heart which then gradually recovered in about 5 min (Fig. 4). Propranolol (1 mg/kg; 1 experiment) blocked the stimulant action of isoprenaline (2 µg/kg) and berberine (1 mg/kg).

Respiration: Berberine in 10 mg/kg dose increased the rate by about 96% (from 26 to 51 per min) and, the amplitude, by 77% over the control; when the effect of the first injection subsided, 15 mg/kg dose was repeated in the same animal and the rate increased by about 204% (from 26 to 79 per min) and the amplitude by 54% over the control. The respiratory stimulation and hypotension induced by berberine occurred and disappeared almost simultaneously (4 experiments). The respiratory stimulant action could be elicited after bilateral vagotomy.

Intestinal motility: Berberine (3 mg/kg; 5 experiments) increased the intestinal motility which exceeded that induced by carbachol (2 µg/kg).

Limb volume: Berberine (0.06 to 3.0 mg/kg) produced marked increase in the limb volume which was dose-dependant; with 3.0 mg/kg dose the increase which lasted for about 10-15 min was comparable to about 5-10 ml air volume (Fig. 5; 3 experiments). The increase in limb volume by histamine (1 µg/kg) and bradykinin (1 µg/kg) was of the same degree and duration as was obtained with berberine (3 mg/kg); compound 48/80 (0.3 mg/kg) had the same degree of effect lasting for about 25 min.

Spleen volume: Berberine (3.0 mg/kg; 5 experiments) increased the spleen volume which was comparable to about 10 ml air volume. The effect lasted for about 10-15 min (Fig. 5).

Estimation of blood histamine in the dog: Blood samples (1.0 ml) removed before and

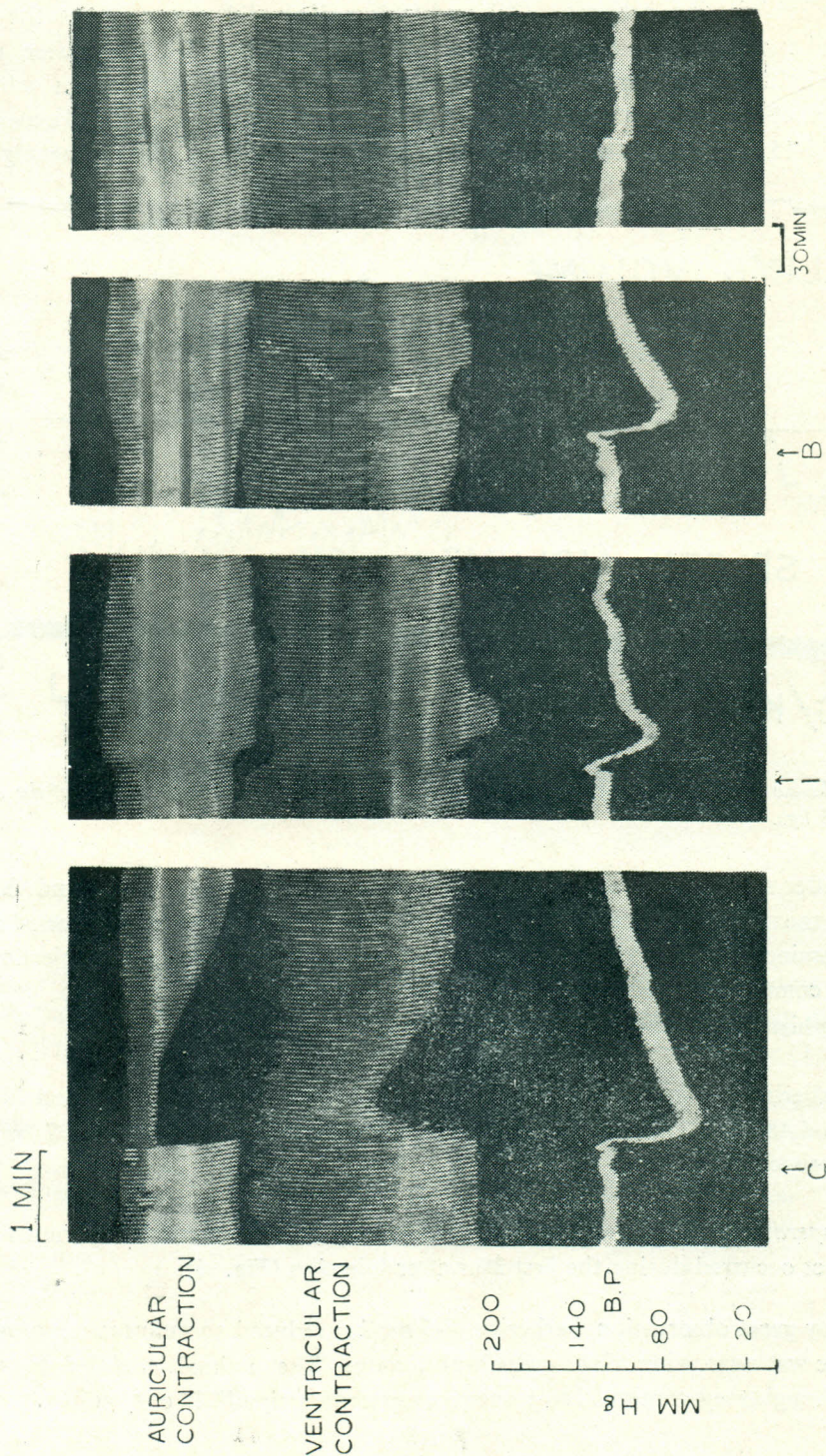


Fig. 4: From above downwards tracings of auricular and ventricular contractions and blood pressure of the anaesthetized dog (11 kg).
C, carbachol (2 μ g/kg); I, isoprenaline sulphate (2 μ g/kg); B, berberine sulphate (1 mg/kg).

1, 3 and 10 min after injecting berberine (3.0 and 10.0 mg/kg) did not contract the isolated guineapig ileum (4 experiments). Blood samples (1.0 ml) collected 1-2 min after injecting histamine (0.1, 0.3, 1.2 and 2.0 $\mu\text{g}/\text{kg}$) induced contraction of the guineapig ileum which was respectively comparable to that induced by 0.02, 0.2, 0.3 and 1.2 μg of histamine (4 experiments). Blood collected 12-15 min after histamine injection failed to contract the guineapig ileum.

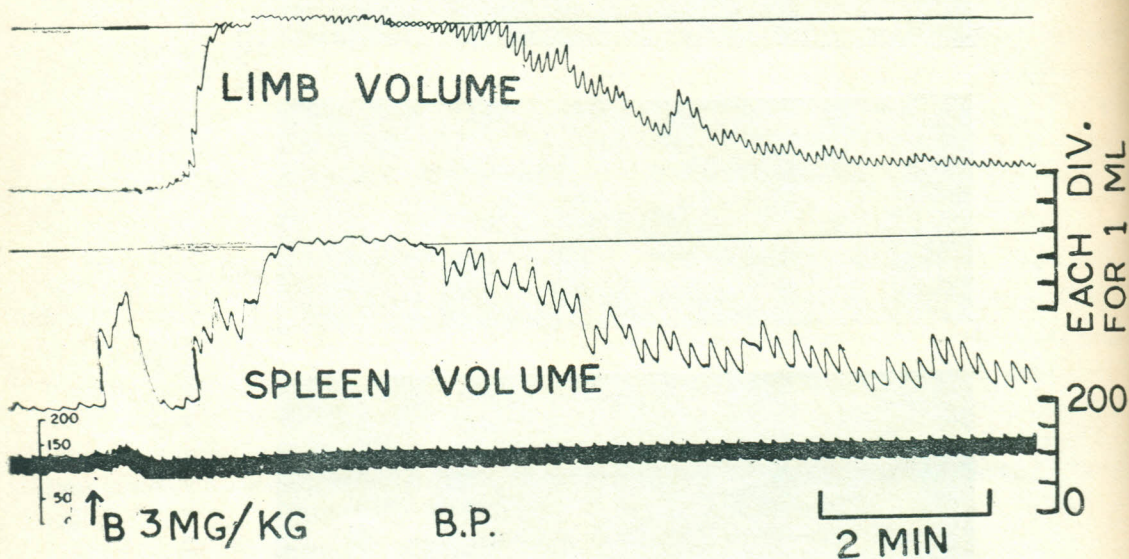


Fig. 5: Effect of berberine sulphate (B) 3 mg/kg on the limb and spleen volumes and blood pressure of the anaesthetized dog (13.5 kg).

Blood collected after simultaneous injection of berberine (3.0 or 10.0 mg/kg) and histamine (1 $\mu\text{g}/\text{kg}$) induced the same degree of contraction as was obtained by the blood collected after the injection of histamine alone (2 experiments). Blood collected 3-8 min after the injection of 0.1 and 0.3 mg/kg of compound 48/80 contracted the guineapig ileum and the effect was comparable to 0.1 and 0.2 μg histamine respectively (2 experiments).

PCV: Berberine (3.0 and 10.0 mg/kg) had no effect on the PCV of the blood (4 experiments). Compound 48/80 (0.3 mg/kg) increased the PCV from 32 to 68% within 7 min after injection (2 experiments).

Rectal temperature: Berberine (1.0-10.0 mg/kg) lowered the rectal temperature in mice. The maximal effect occurred during the first 3 hr after injection (Fig. 6).

As assessed by gross observation berberine (3-5 mg/kg) reduced spontaneous motor activity in mice and there was narrowing of the palpebral fissure. The animals huddled together and remained quiet though they continued to respond to external stimuli like handling.

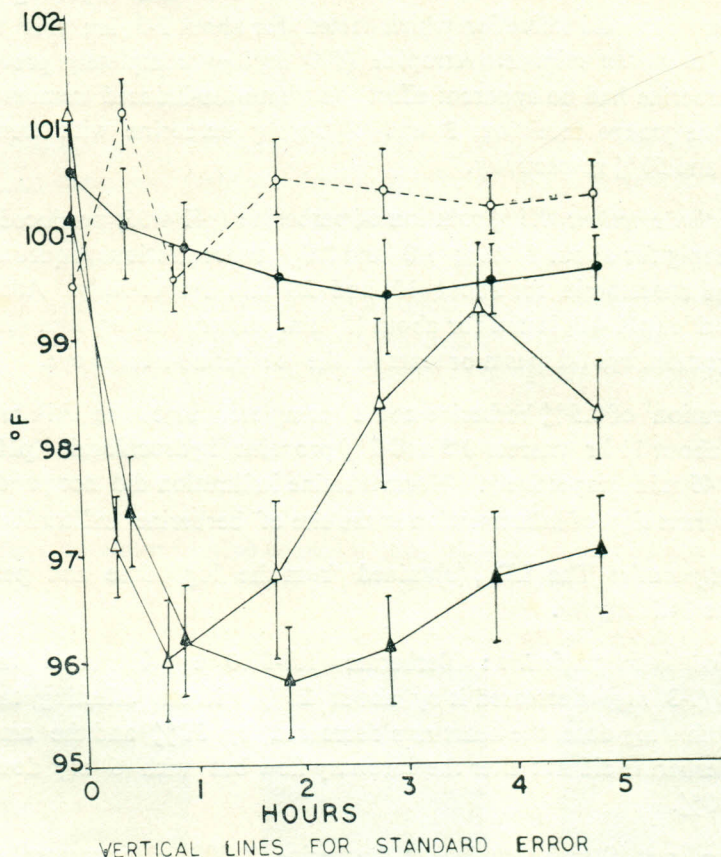


Fig. 6: Effect of berberine sulphate on the rectal temperature of mice. Control (○—○); berberine sulphate 1 mg/kg (●—●), 3 mg/kg (▲—▲) and 10 mg/kg (△—△) respectively.

Hexobarbitone sleep: Berberine prolonged the hexobarbitone sleeping time in mice. The sleeping time as also the per centage of sleeping animals were generally proportional to the dose. Chlorpromazine markedly potentiated the effect of hexobarbitone (Table I).

TABLE I: Effect of berberine on sleeping time in mice induced by 50 mg/kg hexobarbitone sodium.
Sleep index = Average sleeping time of the group X per cent sleeping animals in the group

Drug (dose ml or mg/kg)	Number of mice	Per cent animals that slept	Average sleeping time min (range)	Sleep index
Distilled water 10.0	15	33	1.3 (1-7)	43
Chlorpromazine 5.0	15	82	22.0 (8-49)	1804
Berberine 0.3	15	53	4.1 (3-7)	217
Berberine 1.0	15	60	5.7 (3-37)	342
Berberine 3.0	15	93	7.6 (1-28)	709
Berberine 5.0	13	61	15.7 (8-37)	966

Effect on the tremorine-induced tremors: About 20 min after injecting tremorine, mice developed severe tremors and salivation which lasted for about 2-3 hr; this was followed by complete recovery in all the animals. Atropine (100 mg/kg) completely prevented the effects of tremorine. Berberine had no apparent effect on tremorine-induced tremors and salivation; however, in both the groups receiving 3 and 10 mg/kg berberine with tremorine, the 24 hr mortality was 40 and 50% respectively.

Local anaesthetic action: 0.5% solution of berberine (pH 4.05) produced loss of corneal reflex in the guineapig for about 5 min; 1.0 and 2.0% concentrations (aqueous suspension) of berberine produced anaesthesia for about 10 and 20 min respectively. Anaesthesia by 2% cocaine hydrochloride (pH 4.0) lasted for about 15 min. There was no sign of local irritation like vascular congestion, eye lid spasm or tears in the berberine-treated eye.

Local infiltration of 0.5% berberine could completely block the skin reflex response of the guineapig for about 11 hr whereas 0.5 and 2% procaine hydrochloride (pH 4.0) blocked it for about 10 and 45 min respectively. Normal saline infiltration did not produce anaesthesia. There was no apparent sign of inflammation at the site of berberine infiltration.

Acute toxicity study: The LD₅₀ (obtained from the log dose and per cent mortality graph) in mice was 24.3 mg/kg.

Isolated rabbit heart perfusion: Berberine (1.0-30.0 µg) increased the heart rate by 12%; larger doses (1-3 mg) decreased it by about 25% without affecting the amplitude (12 experiments). With 6 mg dose, the heart rate decreased by 38% and the amplitude by 43%. Upto 3 mg dose berberine did not alter the coronary flow but with 6 mg dose, the flow was reduced by 10-60%.

Isolated frog heart (Straub technique): Berberine (1-300 µg) increased the amplitude of contraction by 8-46% without significantly changing the heart rate (5 experiments). With 1 mg dose, the heart stopped in diastole.

Frog heart perfusion: Three mg berberine increased the amplitude of contraction by 125% without significantly altering the heart rate; 6.0 mg dose stopped the heart completely in diastole (16 experiments). After berberine-induced arrest, adrenaline (10.0 µg), theophylline ethylenediamine complex (Aminophylline, 10 mg), digoxin (20.0 µg) and atropine (5.0 mg) could resuscitate the arrested heart only for about 2 to 8 min; moreover, these drugs could not restore the rate and contractility of the heart to the pre-injection level.

Berberine (0.2 mg/ml) reversed the cardiac arrest induced by perfusion with the amphibian Ringer solution containing 1/4 CaCl₂ for about 30-45 min in all the 7 experiments.

Hind limb perfusion: In the frog, berberine (20 ng/ml of perfusion fluid) decreased both the inflow and the outflow by about 13%. Perfusion with 0.2 µg berberine per ml reduced the inflow and the outflow by 19 and 55% respectively; these were further reduced on increasing the dose of berberine and, with 300 µg/ml dose, the inflow stopped completely (21

experiments). The hind limb preparation became oedematous after perfusion with berberine solutions and its weight increased by 50-60%. Perfusing the preparation only with the amphibian Ringer for a comparable period did not change the perfusion rate or the weight of the preparation.

In rat, berberine (60 $\mu\text{g/ml}$ of perfusion fluid) reduced both the input and the output by about 50%; and these were further reduced by about 66% on perfusing with 100 $\mu\text{g/ml}$ (10 experiments). The berberine-perfused preparations became oedematous and increased in weight by about 30-35%.

Isolated guineapig ileum : Berberine had no effect of its own on the tissue upto a dose of 0.5 mg/ml . The contractions induced by histamine (7.0 ng/ml , Fig. 7; 13 experiments), carbachol (5.0 ng/ml ; 6 experiments), acetylcholine (1.0 ng/ml ; 9 experiments), bradykinin 6.0 ng/ml ; 4 experiments), BaCl_2 (5.0 $\mu\text{g/ml}$; 4 experiments) and KCl (1.0 mg/ml ; 5 experiments) were reduced by about 50% by 50.0, 5.0, 3.0, 6.0, 1.0 and 50.0 $\mu\text{g/ml}$ doses of berberine respectively. The antagonism was proportional to the dose of berberine. Antagonism by the smaller doses of berberine was quickly reversible. The inhibition induced by a certain dose of berberine could be reversed by increasing the dose of the agonist. The hydrochloride of berberine blocked the action of BaCl_2 as effectively as did its sulphate.

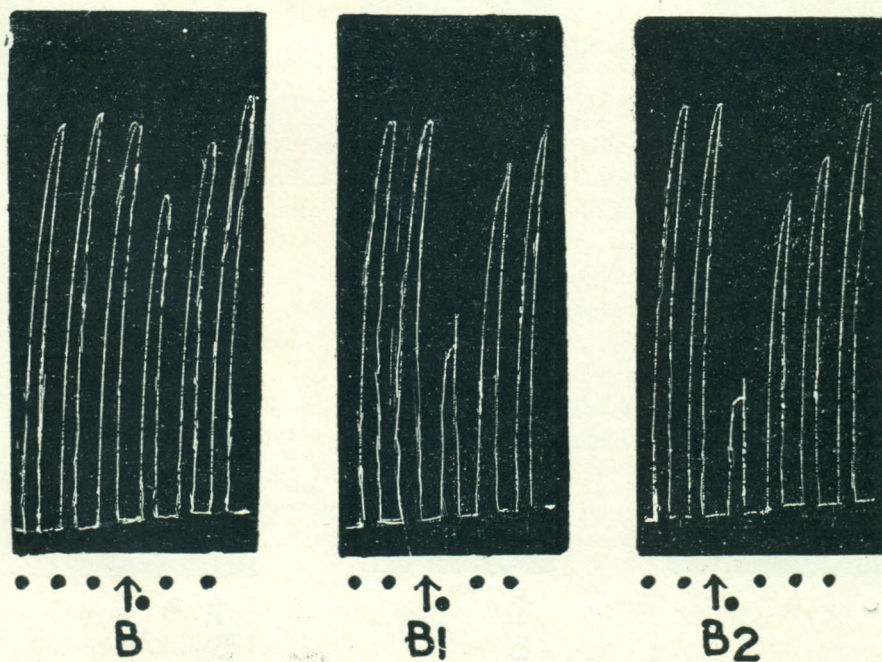


Fig. 7: *Isolated guineapig ileum*. Time interval 3 min. Doses per 10 ml bath volume. Berberine was added 1 min before histamine. Histamine (70 ng) was given at dots; berberine sulphate (B —0.1 mg ; B_1 —0.5 mg ; B_2 —1.0 mg) was added at arrows.

Smaller doses (5 ng-3.0 $\mu\text{g/ml}$) of berberine increased the contraction induced by angiotensin (0.05-30 ng/ml; 13 experiments). This potentiation was proportional, within limits, to the concentration of berberine; thus 0.01, 0.03, 0.3, 1.0 and 3.0 $\mu\text{g/ml}$ berberine increased the contraction induced by 3.0 ng/ml of angiotensin by 25, 44, 53, 72 and 97% respectively (Fig. 8).

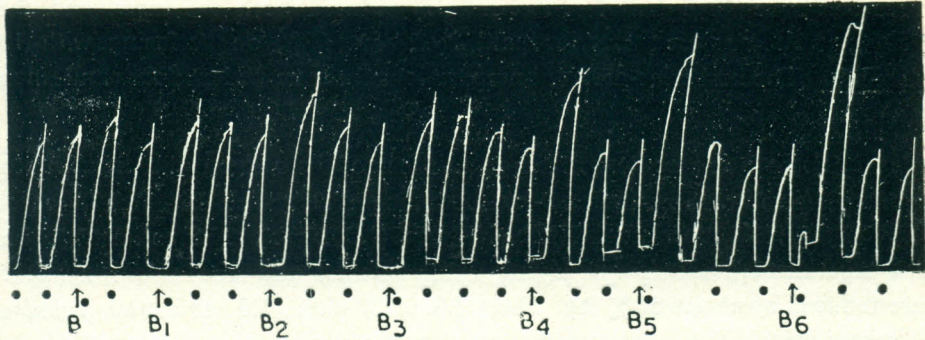


Fig. 8: Isolated guinea pig ileum. Time interval 3 min. Doses per 10 ml bath volume. Berberine sulphate (B —0.03 μg , B_1 —0.1 μg , B_2 —0.3 μg , B_3 —1.0 μg , B_4 —3.0 μg , B_5 —10.0 μg , B_6 —30.0 μg) was added at arrows 1 min before angiotensin (30 ng) which was added at dots.

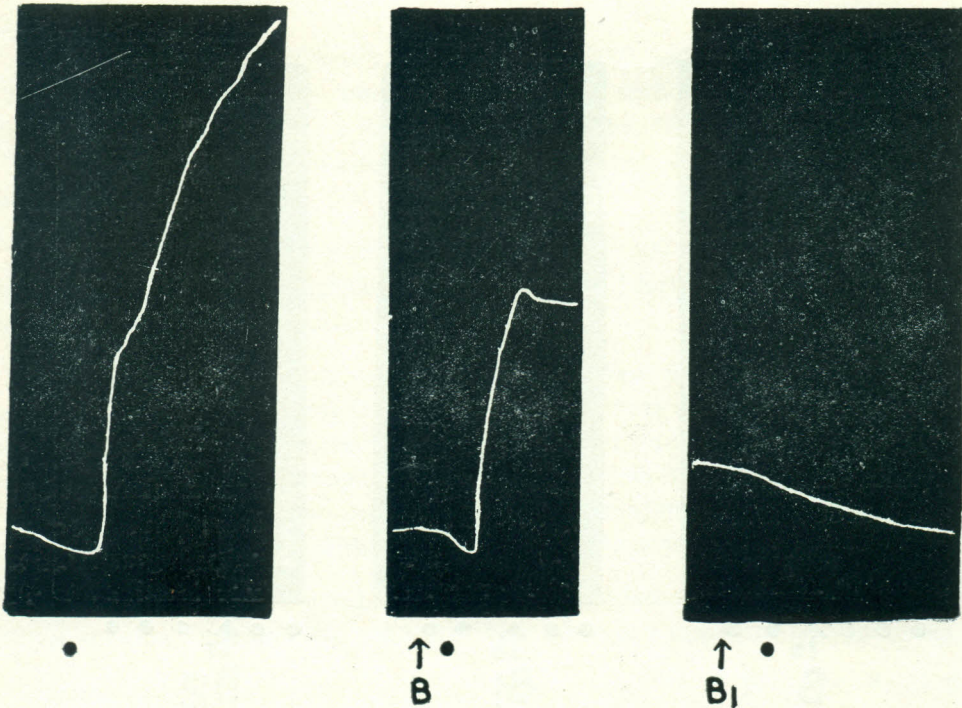


Fig. 9: Schultz—Date phenomenon. Three tracings of 3 pieces of ileum of a sensitized guinea pig. Doses per 10 ml bath volume. Horse serum antigen (0.2 ml) was added at dots. Berberine sulphate (B , 0.3 mg and B_1 , 1 mg) was added 1 min earlier at arrows.

Larger doses (10.0 $\mu\text{g/ml}$ and more) of berberine inhibited (about 80%) the angiotensin response. Berberine solution kept at room temperature for about 11 months potentiated the angiotensin-induced contraction as effectively as did its fresh solution.

Neostigmine (0.1 $\mu\text{g/ml}$; 2 experiments) and physostigmine (0.1 $\mu\text{g/ml}$; 3 experiments) increased the contraction induced by angiotensin (0.08 ng/ml) by about 4) and 70% respectively. Atropine sulphate (10.0 ng/ml ; 2 experiments) completely blocked the contractions induced by angiotensin (0.015 ng/ml); berberine (2.0 $\mu\text{g/ml}$; 2 experiments) and physostigmine (0.5 $\mu\text{g/ml}$; 2 experiments) partly (about 30-50%) reversed this blockade.

As tested on the guineapig ileum, angiotensin (5-300 ng/ml) lost all activity when incubated with 0.3 ml of freshly drawn heparinized dog blood at 37°C for 60 sec (6 experiments); prior addition of berberine (0.3 μg —1.0 mg/ml) to the blood samples did not alter the rate of inactivation of angiotensin.

Three weeks after sensitization, pieces of the small intestine of the guineapig responded by contraction when challenged with 0.2 ml (per 10 ml bath volume) of the antigen (horse serum or egg-white). This was followed by a slow relaxation after which the tissue did not respond to the subsequent addition of the antigen though it remained sensitive to histamine (3.0 ng/ml). Pieces of the intestine kept at 8°C in about 50 ml Tyrode solution for upto 24 hr did not lose sensitivity to the antigen. At 0.03 mg/ml dose, berberine partially suppressed the *Schultz-Dale phenomenon*; 0.1 mg/ml dose produced complete blockade (Fig. 9: 5 experiments).

Isolated rabbit jejunum: Berberine (0.1 mg/ml) blocked the pendular movements of the rabbit jejunum. The contractions induced by carbachol (50 ng/ml ; 3 experiments) and acetylcholine (50 ng/ml ; 5 experiments) were reduced by about 50% by 2.0 and 50.0 $\mu\text{g/ml}$ of berberine; the antagonism was proportional to the dose of berberine and was quickly reversible.

Oestrogenized rat uterus: Berberine (30 $\mu\text{g/ml}$) reduced the contractions induced by 5-HT (4.0 ng/ml as creatinine sulphate) by 50% (3 experiments). The antagonism was proportional to the dose of berberine and was quickly reversible.

Frog rectus abdominis muscle: Berberine markedly potentiated the magnitude of contractions induced by acetylcholine (0.1 $\mu\text{g/ml}$). The potentiation (23, 53, 70 and 76%) was proportional to the dose of berberine (respectively 0.5, 1.0, 3.0 and 6.0 $\mu\text{g/ml}$; 11 experiments). Berberine solution kept at room temperature for about 11 months was as effective as the fresh solution. Larger doses (0.05-0.1 mg/ml) reduced the effect of acetylcholine by 88% but, when berberine was washed out the response to the subsequent addition of acetylcholine was again exaggerated (93-135%) for about 20 min. The anti-acetylcholine action of tubocurarine (0.5 $\mu\text{g/ml}$) was antagonized by berberine (0.5 $\mu\text{g/ml}$; 3 experiments).

Berberine (5.0 μg) delayed the inactivation of acetylcholine (1.0 μg) incubated with 0.2 ml dog or rabbit serum (Fig. 10; 3 experiments).

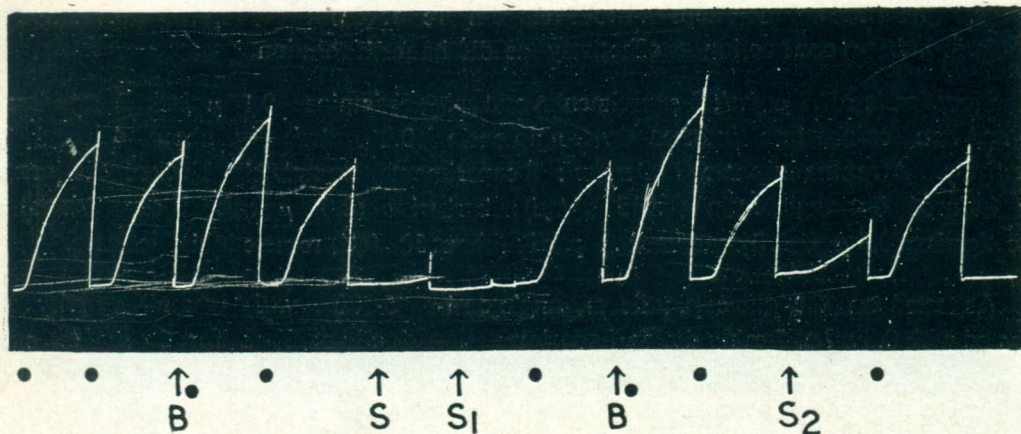


Fig. 10: Isolated frog rectus abdominis muscle. Time interval 5 min. Dose per 10 ml bath volume. Acetylcholine (1 μg) was added at dots. B, 5 μg berberine sulphate + 1 μg acetylcholine; S, 0.2 ml dog serum; S₁, 0.2 ml dog serum + 1 μg acetylcholine (incubated); S₂, 0.2 ml dog serum + 5 μg berberine sulphate + 1 μg acetylcholine (incubated).

The contractions induced by carbachol (0.2 $\mu\text{g}/\text{ml}$; 6 experiments) and suxethonium bromide ('Brevdil' E, 0.3 $\mu\text{g}/\text{ml}$; 2 experiments) were not potentiated by small doses of berberine and reduced by about 50% by 10.0 and 3.0 $\mu\text{g}/\text{ml}$ of berberine respectively. The contraction induced by KCl (1.0 mg/ml ; 4 experiments) was not altered by berberine (upto 0.3 mg/ml).

Ciliary movements: Seventyeight observations were carried out in 16 experiments with 0.1 μg -3 mg of berberine. In 65 observations berberine stimulated the ciliary movements and the time required for the seed to travel the fixed distance decreased by 8-72% of the control values; in the 12 observations berberine had an opposite effect in that the time for the seed travel increased by 1 to 50% of the control values. The quality and intensity of the effect on cilia were unrelated to the dose of berberine. Atropine in doses about 3 times those of berberine could block the stimulation by the latter. In all the 16 experiments, carbachol (0.3-1.0 $\mu\text{g}/\text{ml}$) consistently stimulated the ciliary movement and the time for the seed travel decreased by 23-83% of the control values. The black seeds of *Amaranthus gangeticus* could be more conveniently watched than the white poppy seeds. Burn (6) has used a transparent chamber to maintain the preparation warm. In this laboratory (room temperature 88-100°F) it worked equally well without the chamber.

Skin melanophores of the frog: Berberine (10-30 mg/kg) induced marked vasodilatation of the cutaneous blood vessels. It also brought about aggregation of melanin granules in the melanophores which changed the colour of the frog skin from brown to yellow (12 experiments).

DISCUSSION

The sulphate of berberine was used in most of the experiments because it is about five times more soluble in water than the hydrochloride.

As reported by the previous workers (8, 39a) berberine was found to produce hypotension in anaesthetized dogs and cats. The present work shows that in anaesthetized rats and frogs also berberine produced hypotension which was not influenced by the type of anaesthetic agent used.

An attempt was made to determine the mechanism of hypotension. The hypotension induced by histamine liberators has the following characteristics : (i) The latent period is longer than that taken by the directly acting drugs like histamine, bradykinin; it is about 20-25 sec in the anaesthetized cat (23) and about 30 sec in the anaesthetized dog (31). (ii) Histamine can be detected in the blood circulation in quantities which can be assayed on the isolated guineapig ileum (27). (iii) There is a definite (if not always complete) blockade by antihistamines. (iv) Tachyphylaxis occurs when safe doses are repeatedly injected. (v) The PCV value increases due to haemoconcentration (27). The present work shows that berberine-induced hypotension did not manifest any of these characteristics.

Atropine could not counteract berberine-induced hypotension ; therefore, it cannot be attributed to the direct stimulation of the muscarinic receptors nor to the anticholinesterase action of berberine.

Veratrum-induced hypotension is partly due to the stimulation of the afferent vagal fibres and is reduced by vagotomy (2) and thus differs from that induced by berberine.

In the doses used here, pentolinium could block the action of nicotine and, propranolol, of isoprenaline though neither of them individually affected berberine-induced hypotension. Therefore, the role of the autonomic ganglia or beta-adrenergic receptors can be excluded. However, when both propranolol and pentolinium were injected in the individual animals, berberine failed to produce hypotension in 2 dogs and produced marked rise in the blood pressure in 4 dogs. It is difficult to interpret this observation.

The carotid sinus occlusion reflex (which has centre in the midbrain) remained unaltered in the dogs after repeated injections of berberine. Also, hypotension could be seen in the spinal preparations. These findings suggest that berberine does not produce hypotension by depression of centres in the medulla and above.

The hypertension caused by adrenaline and noradrenaline was not altered by berberine. Therefore, berberine-induced hypotension is probably not due to blockade of alpha-adrenergic receptors.

Though a cholinesterase inhibitor, berberine usually produced only tachycardia in the dogs. This effect which lasted much longer than its hypotensive action could be due to direct stimulation (Fig. 4) of the heart.

Smaller doses of berberine were found to stimulate the isolated frog and rabbit heart. Frog heart arrested during perfusion with low calcium Ringer, could be promptly revived by berberine. Further, in the open-chest experiment in the dog, berberine was found to induce hypotension at doses which clearly stimulated the auricle and the ventricle (Fig. 4). Therefore, hypotension observed in the dog and the frog in this work cannot be attributed to myocardial depression. However, earlier work (8) shows that in the cat myocardial depression might be a contributory factor to berberine-induced hypotension.

Berberine is found in this study and in an earlier report (8) to increase the spleen volume. This work clearly shows increase in the volume of the intact limb in the dog by berberine. Therefore, the site of berberine-induced hypotension in the dog appears to be the blood vessels in the visceral and somatic organs. Further, from the results discussed above the action of berberine on the blood vessels seems to be a direct one.

Vasodilatation was obtained with berberine in the perfusion of isolated frog and cat limb preparations (8). Also, when the frog hind limb was perfused with 0.6% sodium chloride solution, berberine was found to produce vasodilatation (31). However, in the present work where Ringer solution was used for perfusion, berberine was found to consistently induce vasoconstriction in the frog and rat hind limb experiments.

The respiratory stimulation in the dog could be due to an activation of the baroreceptor reflexes by the berberine-induced hypotension.

Berberine in smaller doses potentiated the action of angiotensin on the isolated guineapig ileum and, occasionally, on the dog blood pressure. However, berberine did not delay the inactivation of angiotensin by the blood angiotensinase. Therefore, anti-angiotensinase mechanism is probably not involved in this action.

On the isolated guineapig ileum angiotensin is considered to exert its stimulant action by bringing about the release of intrinsic acetylcholine (21) and this action is potentiated by cholinesterase inhibitors and blocked by atropine (20, 29). In smaller doses (5 ng-3 $\mu\text{g/ml}$) berberine, a cholinesterase inhibitor, potentiated the action of angiotensin and also partially reversed the anti-angiotensin action of atropine. However, in larger doses (10.0 $\mu\text{g/ml}$ and above) it blocked the action of angiotensin. Further, berberine (1-30 μg) blocked the effect of extrinsic acetylcholine. Therefore, it is possible that in the isolated guineapig ileum preparation berberine might be having different actions on extrinsic and intrinsic acetylcholine. Also, the direct, non-specific depressant action of berberine on the excitable tissues and the anticholinesterase action would seem to exert opposite effects in this preparation. Apparently, this complex action needs further study.

Although berberine showed anticholinesterase action in smaller doses, when several times larger doses were injected into the intact animals there were no signs of overt cholinesterase inhibition. In this respect berberine would seem to differ from physostigmine and neostigmine. An explanation for this must await further work.

Tetrahydroberberine has depressant effect on the central nervous system (CNS) of mice (33). In the present study, berberine was also found to induce signs of CNS depression in mice besides increasing the hexobarbitone sleeping incidence and time. In a recent report, Shanbhag *et al* (35) have independently observed that berberine produces sedation in conscious cats and mice and potentiation of pentobarbitone sleep in mice. It is not known if such an action occurs in the clinical use of berberine.

Tremorine induces tremors by central cholinergic action which is effectively blocked by atropine. The high mortality in mice receiving both berberine and tremorine could be due to the potentiation of the latter by the anticholinesterase action of the former.

In the traditional medical practice berberine-containing plants have been used as antipyretics (12). The experiments conducted here show that berberine does produce hypothermia in mice.

The LD_{50} of berberine sulphate in mice was 24.3 mg/kg; this is in close agreement with that of berberine bisulphate (27.5 mg/kg) found by Uchizumi (39 b). In dogs intravenous doses upto 45 mg/kg did not produce any lethal or gross toxic effect and the animals appeared normal for several days after the injection. The mouse, therefore, appears to be more sensitive than the dog to berberine. This is unusual because, to a large number of drugs and poisons, the mouse is definitely more resistant than the dog.

The prolonged local anaesthesia of the berberine-infiltrated guineapig skin observed in this work agrees with the earlier finding (34) on the human skin; it is interesting to point out that in the earlier work (34) permanent motor paralysis was observed when berberine was injected in the rabbits as a spinal anaesthetic.

Berberine is known to be an inhibitor of true and pseudo cholinesterases (39 a). This property can explain the increase in the intestinal motility in the dog, potentiation of acetylcholine on the frog rectus muscle, anti-tubocurarine action on the frog rectus muscle, potentiation of acetylcholine-induced hypotension in the dog, inhibition of acetylcholine inactivation by the dog and rabbit sera, potentiation of angiotensin (by smaller doses of berberine) on the isolated guineapig ileum, high mortality in tremorine-treated mice and stimulation of the ciliary movement in the frog observed in this study. It may also explain the prolongation, by berberine, of the local anaesthetic action of procaine (34) which is known to be hydrolyzed by cholinesterase. The effects of carbachol on the dog blood pressure and on the frog rectus muscle were not potentiated by berberine; this appears appropriate because carbachol is not hydrolyzed by cholinesterases.

Berberine inhibited the pendular movements of the isolated rabbit intestine. It blocked the stimulant action of histamine, acetylcholine, carbachol, bradykinin, $BaCl_2$ and, in large doses, of angiotensin, on the isolated guineapig ileum and of 5-HT on the oestrogenized rat uterus. Some of these findings have been reported (3) while this work was in progress. Further, the contraction of the isolated iluem of the sensitized guineapig induced by the

antigen (Schultz-Dale phenomenon) could be completely inhibited by berberine. That berberine reversibly inhibited KCl-induced contraction of the guineapig ileum is interesting because KCl acts directly by causing surface depolarization of the excitable tissues. All these findings may also explain the clinically conspicuous (13, 19) antispasmodic action of berberine.

The local anaesthetic and the smooth muscle inhibitory actions of berberine could be due to interference with those processes of depolarization and repolarization which are essential for the function of excitable organs.

Thus, many pharmacological actions of berberine could be categorized under two broad headings (1) the stimulant actions, some of which are due to its anticholinesterase mechanism and (2) the inhibitory actions, many of which could be due to inhibition of depolarization and repolarization of the excitable tissues. On some individual organs the net effect would then depend upon which of the two actions dominates at a given dose of berberine.

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