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THE CHANGE IN PHARMACOLOGICAL ACTION PRODUCED BY THE INTRODUCTION OF A METHYL GROUP INTO PRISCOL

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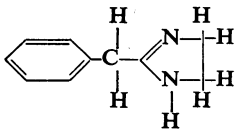
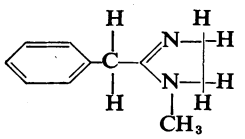
Within the last decade a new series of chemical compounds, the 2-substituted imidazolines, have claimed wide attention because of their vascular actions. Several of them, phedracin, priscol, privine and otrivin, have been used clinically in peripheral vascular disorders.

Hartmann and Isler (1939) made a preliminary pharmacological investigation of a large number of these imidazolines. They found that small changes in the imidazoline molecule sometimes caused great quantitative or even qualitative changes in the effect upon the blood pressure. Similar abrupt transitions have been described among sympathomimetic amines (Beyer, 1946). Since the substitution of an N-methyl group in the noradrenaline molecule causes significant differences in its properties (Barger and Dale, 1910) it was decided to investigate what changes the introduction of an N-methyl group would effect in priscol. The structural formulae of these compounds are as follows:



2-benzylimidazoline (priscol)



2-benzyl-1-methyl-imidazoline (N-methylpriscol)

2-Benzyl-1-methyl-imidazoline was one of the compounds investigated by Hartmann and Isler. They stated that it had approximately the same toxicity and effect on the rabbit's intestine as priscol, but caused a rise of blood pressure, whereas priscol caused a fall.

The general pharmacology of priscol and its N-methyl derivative is here described as part of an attempt to elucidate the mechanism of action of the imidazolines.

Experimental Results

Cardiovascular action of priscol.—The action of priscol on the blood pressure varies in different species. Meier and Müller (1939) observed a fall of blood pressure in rabbits; Hermann, Jourdan and Bonnet (1941) found that in dogs priscol usually caused a fall but occasionally a rise of blood pressure. In cats Chess and Yonkman (1945) observed either no effect or a fall with larger doses.

Since priscol is structurally related to histamine it seemed possible that the fall of blood pressure might be antagonized by neoantergan. However, in a cat under ether anaesthesia, a dose of neoantergan which virtually abolishes the depressor effect of histamine did not prevent the fall of blood pressure caused by a large dose of priscol. All the workers quoted above observed that priscol reduced or reversed the pressor action of adrenaline. An example of this in the spinal cat is shown in Fig. 1. After the injection of 10 mg. priscol, which was itself without action on the blood pressure or spleen volume, 10 μg. adrenaline caused a small fall of blood pressure accompanied by dilatation of the spleen; the pressor effect and the contraction of the spleen caused by 5 μg. adrenaline before the injection of priscol were absent. When a larger dose of adrenaline was injected, however, the original effect was seen

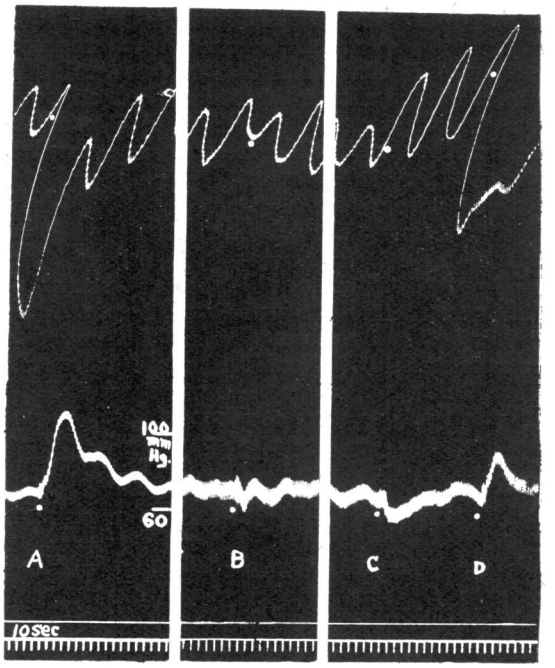


FIG. 1.—Upper record; spleen volume ; lower record: blood pressure of spinal cat. At A, 5 μg. adrenaline caused contraction of spleen and rise of pressure. At B, 10 mg. priscol. At C, 10 μg. adrenaline caused slight dilatation of spleen and fall of blood pressure. At D, 0.1 mg. adrenaline caused contraction of spleen and a small rise of pressure.

once more ; 0.1 mg. adrenaline caused a small rise of blood pressure and contraction of the spleen.

This observation suggested that the effect of priscol on the pressor response of adrenaline might be attributed to competition between priscol and adrenaline molecules for the same " receptor." The effect of different amounts of priscol on a range of doses of adrenaline was therefore determined in a series of spinal cats. The results are shown in Fig. 2 in which curve A shows the mean height of the pressor response to a given dose of adrenaline in the absence of priscol. Curve B shows the effect on the adrenaline response of administering priscol in a dose of 2.5 mg./kg., and curve C that of 5 mg./kg. It can be seen from Fig. 2 that the reversal of the pressor action of adrenaline by priscol is only possible when small doses of adrenaline are injected, and that the reversal is easily overcome by larger doses.

Priscol on blood vessels.—The action of priscol on the blood vessels was determined by perfusing the rabbit ear, using the method of Gaddum and Kwiatkowski (1938). In Fig. 3 the vasodilatation produced by 0.5 mg. priscol is shown. The action on the adrenaline response was also examined in this preparation and the record in Fig. 4 shows the reversal of the constrictor action when priscol was added to the Ringer's solution perfusing the ear: (*a*) shows two vasoconstrictor effects due to the injection of 0.1 μg. adrenaline ; at (*b*), in the presence of priscol, 0.8 μg. adrenaline caused

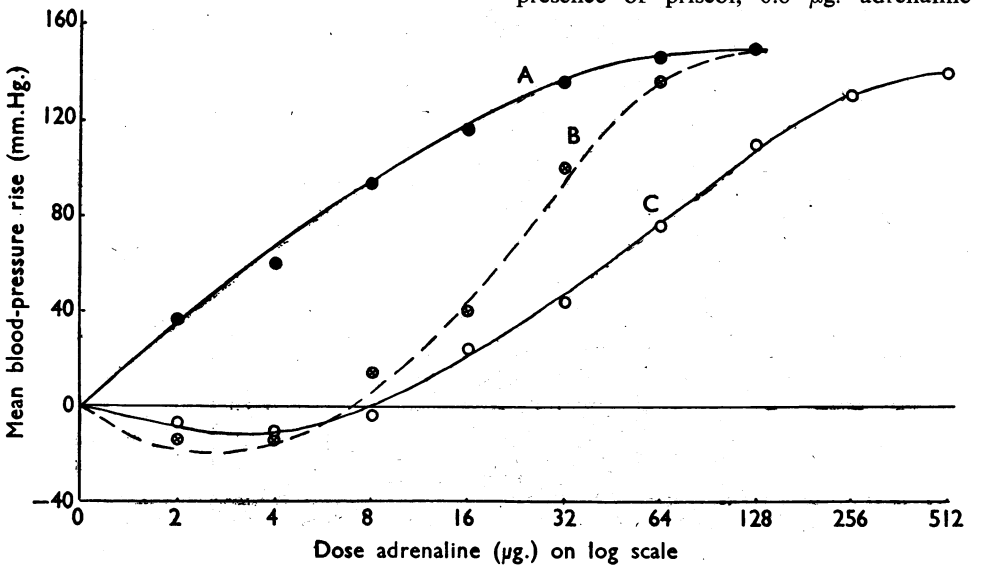


FIG. 2.—Priscol-adrenaline antagonism on blood pressure of spinal cat. Mean blood-pressure rise (ordinate mm. Hg.) plotted against dose (μg) adrenaline as abscissa on logarithmic scale. Curve A shows blood-pressure rise before priscol (mean of 3 exp.); curve B after 2.5 mg./kg. priscol (mean of 2 exp.); curve C after 5.0 mg./kg. priscol (mean of 3 exp.).

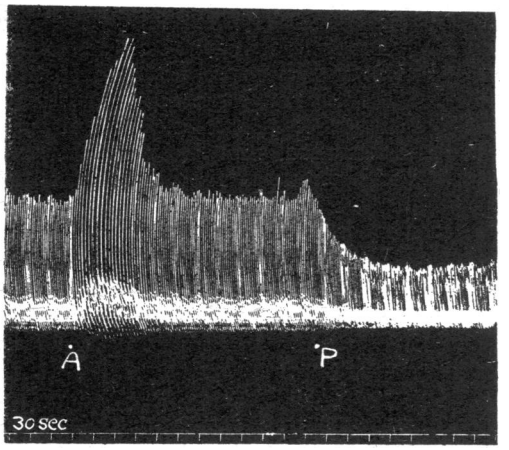


FIG. 3.—Record of outflow from vessels of rabbit ear obtained by Gaddum's drop timer. At A, 0.025 μg. adrenaline caused vasoconstriction; at P, 0.5 mg. priscol caused vasodilatation.

vasodilatation, and 1.6 μg. was without effect; at (*c*) 3.2 μg. adrenaline caused a slight vasoconstriction, and at (*d*) 4 μg. a constriction similar to the initial effects. Thus not only does priscol cause vasodilatation and reverse the vasoconstrictor action of adrenaline in isolated perfused vessels, but as in the spinal cat the relationship between priscol and adrenaline is quantitative and not qualitative. Histamine also causes vasoconstriction in the rabbit's ear; larger doses of priscol than those necessary to abolish the action of adrenaline abolished the vasoconstriction caused by histamine, but no reversal was seen.

Similar observations were made when the hind-leg of a dog was perfused with blood (containing heparin) by a Dale-Schuster (1928) pump. The injection of 10 mg. priscol caused vasoconstriction (in contrast to the vasodilatation seen in the rabbit's ear) and converted the previous constrictor action of 4 μg. adrenaline to a dilator action. When 20 mg. priscol was injected the dilator action of 4 μg. adrenaline became greater and resembled that of 20 μg. histamine. The effect of histamine itself was unchanged by priscol.

Priscol on the heart.—The action of priscol on the heart was examined by perfusing the isolated heart with Locke's solution by Langendorff's method. In two rabbit hearts, the injection of 10–20 μg. priscol decreased the amplitude and reduced the coronary flow. A similar action was observed in three experiments on cat hearts, but in other experiments on cat hearts an increase in the heart's action was observed especially with larger doses (1–3 mg.). An example of this is

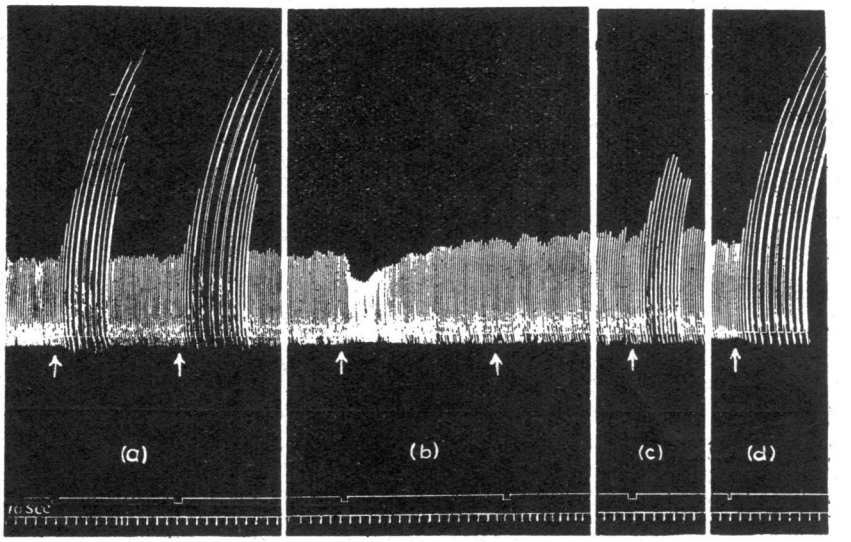


FIG. 4.—Record as in Fig. 3. In (*a*) two injections of 0.1 μg. adrenaline causing vasoconstriction. Between (*a*) and (*b*) perfusion with Ringer containing priscol (0.2 mg. per ml.) was begun. In (*b*) at first arrow 0.8 μg. adrenaline caused vasodilatation, and at the second, 1.6 μg. was without effect. In (*c*) 3.2 μg. caused vasoconstriction, and in (*d*) 4.0 μg. caused an effect similar to that in (*a*).

given in Fig. 5 which shows the effect of 2 mg.; this dose not only increased the amplitude but also both the rate (from 102–200 per min.) and the coronary flow (from 6.0–10.4 ml. per min.). Although the increase in amplitude did not last more than 8 min. the increase in rate and in coronary flow continued for 15 min.

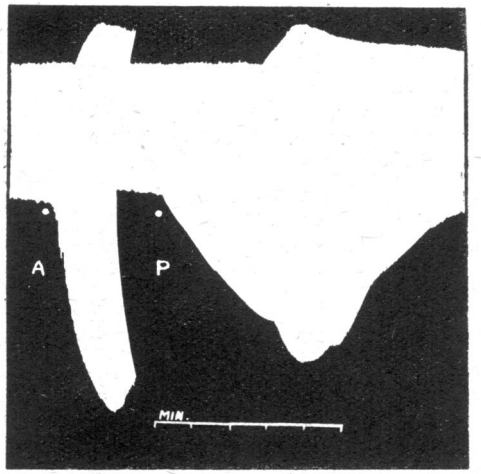


FIG. 5.—Record of contractions of isolated cat heart perfused by method of Langendorff. At A, 0.3 μg. adrenaline increased force of contraction; heart rate from 104/min. to 140/min., and coronary flow from 5.6 ml./min. to 7.8 ml./min. At P, 2 mg. priscol increased force of contraction; heart rate from 102/min. to 200/min., and coronary flow from 6.0 ml./min. to 10.4 ml./min. There is prolonged stimulation due to priscol: 8 min. after injection, heart rate still 173/min. and coronary flow 9.6 ml./min.

On isolated rabbit auricles at 29° C. priscol in concentrations of 2 μg.–4 μg. per ml. augmented the amplitude though it slowed the rate. Priscol lengthened the refractory period when examined by Dawes's method (1946a), though the concentrations required were large; 20 μg. per ml. increased it by 6 per cent., and 0.1 mg. per ml increased it by 22 per cent. Priscol did not affect the action of either adrenaline or acetylcholine on the spontaneous beats of the isolated auricles.

Cardiovascular action of N-methylpriscol.—The N-methyl derivative of priscol, unlike priscol, always caused a rise of blood pressure, as Hartmann and Isler (1939) had observed. An example is given in Fig. 6 in which the effect of injecting N-methylpriscol (20 mg.) is shown at A_1; the prolonged rise of blood pressure was accompanied by a great increase in heart rate. At B_1 10 μg. adrenaline was injected. A series of doses of nicotine was then given, until no further pressor action was observed; a total of 31.5 mg. nicotine acid tartrate was required. The injection of 10 μg. adrenaline at B_2 then caused about the same rise of blood pressure as at B_1, but the injection of 20 mg. N-methylpriscol at A_2 was almost without effect. This suggested that the pressor action of N-methylpriscol was due to liberation of adrenaline and it was found that the pressor action of N-methylpriscol was greatly reduced by adrenalectomy.

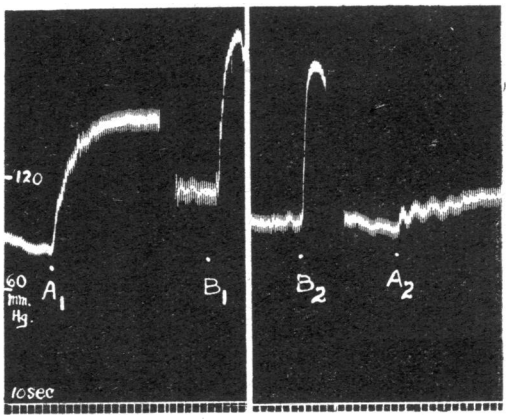


FIG. 6.—Record of blood pressure of spinal cat. At A_1, 20 mg. N-methylpriscol caused rise of blood pressure; at B_1, 10 μg. adrenaline had pressor action. Between B_1 and B_2 nicotine was injected until it no longer caused a rise (total injected 31.5 mg.). At B_2, 10 μg. adrenaline had a similar effect to that at B_1, but 20 mg. N-methylpriscol at A_2 had almost no action on blood pressure.

Since the pressor action of N-methylpriscol is largely due to the release of adrenaline, and since priscol abolishes the pressor action of adrenaline, it would be expected that priscol would modify the pressor action of N-methylpriscol. This proved to be so. In Fig. 7 a record of the nictitating membrane and of the blood pressure of a spinal cat is shown. At A, 20 μg. adrenaline was injected and, at B, 5 mg. N-methylpriscol. The latter caused a small prolonged contraction of the nictitating membrane. At C, 5 mg./kg. priscol was injected, and the nictitating membrane contracted strongly and persistently. At D, the same dose of adrenaline as was previously given at A caused a fall of blood pressure. The injection of 5 mg. N-methylpriscol at E now had a much smaller pressor action.

N-methylpriscol on the blood vessels.—When examined on the vessels of the rabbit's ear, doses of N-methylpriscol up to 1 mg. were without action.

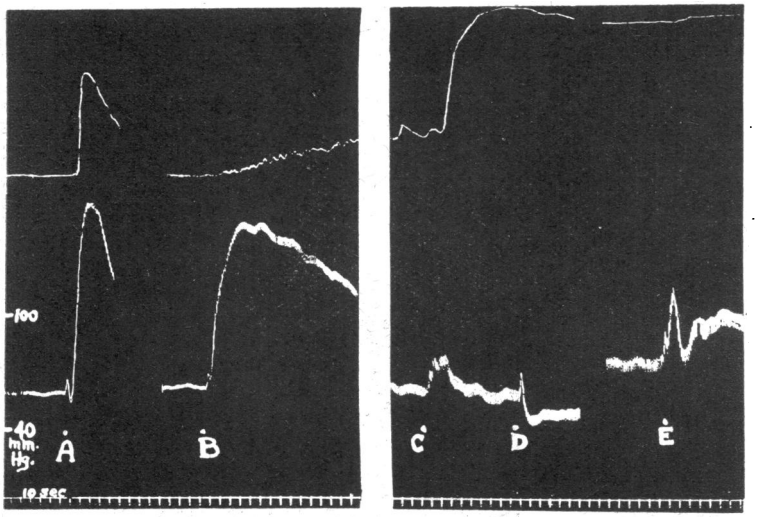


FIG. 7.—Upper record: contractions of nictitating membrane; lower record: blood pressure of spinal cat. At A, 20 μg. adrenaline caused contraction of nictitating membrane and rise of blood pressure; at B, 5 mg. N-methylpriscol caused slow contraction of nictitating membrane and rise of pressure; at C, 5 mg./kg. priscol caused powerful prolonged contraction of nictitating membrane; now at D, 20 μg. adrenaline was depressor, and 5 mg. N-methylpriscol at E had little pressor action.

N-methylpriscol on the heart.—On the cat heart perfused with Locke's solution through the coronary vessels, N-methylpriscol was found to have a stimulant action as shown in Fig. 8. At A, 0.5 mg. N-methylpriscol increased the rate from 120 to 206 per min., the coronary flow from 9.0 to 15.0 ml. per min. and the amplitude as shown in the figure. At B, 50 μg. nicotine produced a similar effect but of much shorter duration. Since N-methylpriscol caused a rise of blood pressure and increase of heart rate by a "nicotine-like" liberation of adrenaline from the adrenals, it was expected that its action upon the isolated heart would be another manifestation of the same "nicotine-like" property. But it was found that perfusion of the cat's heart with nicotine (2 mg. per ml.) abolished the stimulant action of nicotine, but not that of N-methylpriscol. In this respect N-methylpriscol resembles adrenaline and histamine, rather than nicotine, in its action upon the isolated heart.

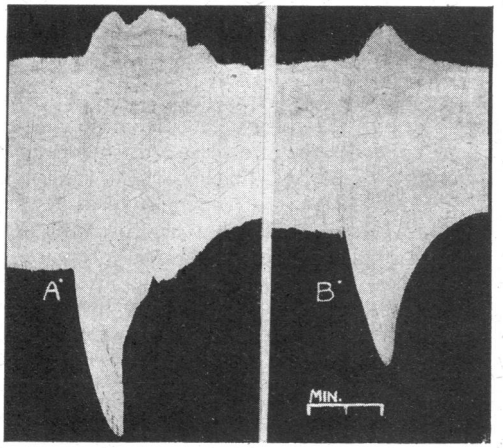


FIG. 8.—Record of contractions of isolated cat heart perfused by method of Langendorff. At A, 0.5 mg. N-methylpriscol increased force of contraction; heart rate from 120/min. to 206/min., and coronary flow from 9.0 ml./min. to 15.0 ml./min. At B, 50 μg. nicotine acid tartrate increased amplitude of contraction; heart rate from 128/min. to 160/min., and coronary flow from 8.6 ml./min. to 11.0 ml./min.

N-methylpriscol increased the rate and the amplitude of contraction of the isolated rabbit auricles; again it differed from nicotine, since tetraethylammonium iodide abolished the action of nicotine, but had no effect on that of N-methylpriscol; nor was the action of N-methylpriscol reduced by the antihistamine substance neoantergan.

N-methylpriscol did not affect the refractory period of the electrically-driven auricles even in a concentration as high as 10^{-4}, though this was enough to increase the spontaneous rate and the amplitude.

Relation to amidines.—The chemical structure of the imidazoline ring is related to that of the amidines. Dawes (1946b) showed that the latter potentiated the pressor action of adrenaline when injected into the splenic vein so as to pass through the portal system before entering the general circulation. Similar experiments were therefore performed with N-methylpriscol. Fig. 9 shows the blood pressure responses of a spinal cat to six injections of 20 μg. adrenaline; 5 mg. N-methylpriscol was mixed with the adrenaline in the

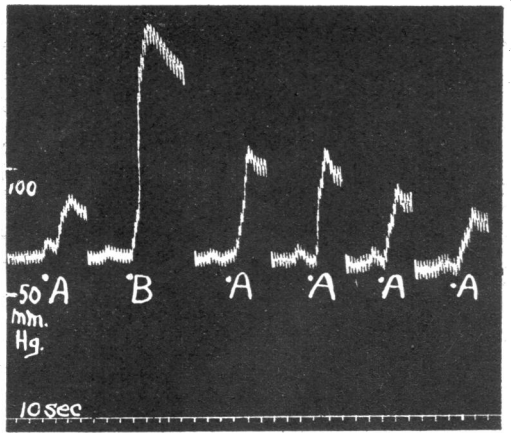


FIG. 9.—Record of blood pressure of spinal cat, all injections made into portal circulation through splenic vein. At each A, 20 μg. adrenaline produced rise of blood pressure; at B, 5 mg. N-methylpriscol was mixed with 20 μg. adrenaline and caused much greater rise of blood pressure.

second injection. The effect of the second injection was much greater than that of the first, and the succeeding injections progressively declined. Since the injection of 5 mg. N-methylpriscol alone had no effect on the blood pressure when given by this route, it clearly potentiated the action of adrenaline.

Smooth muscle.—The action of priscol was studied in the anaesthetised cat by inserting balloons in the oesophagus, duodenum and colon. In some experiments the vagi were stimulated below the heart, and in others the spontaneous intestinal movements were increased by the injection of eserine. Fig. 10 shows the record of the duodenal contractions; they were inhibited by adrenaline; a large contraction was induced by priscol (5 mg./kg.); 13 min. later another injection of adrenaline increased the contractions of the intestine, at the same time causing a fall of blood pressure. This reversal of the effect of adrenaline on the duodenum was not seen in other parts of the gastro-intestinal tract.

In the isolated duodenum of the rabbit priscol reduced the inhibitory action of both adrenaline and privine, but did not reverse their action.

Both priscol and N-methylpriscol were found to potentiate the action of acetylcholine on the guinea-pig ileum, while N-methylpriscol also potentiated the action of histamine in the guinea-pig ileum as shown in Fig. 11. It should, however, be observed that the spontaneous movements were also increased.

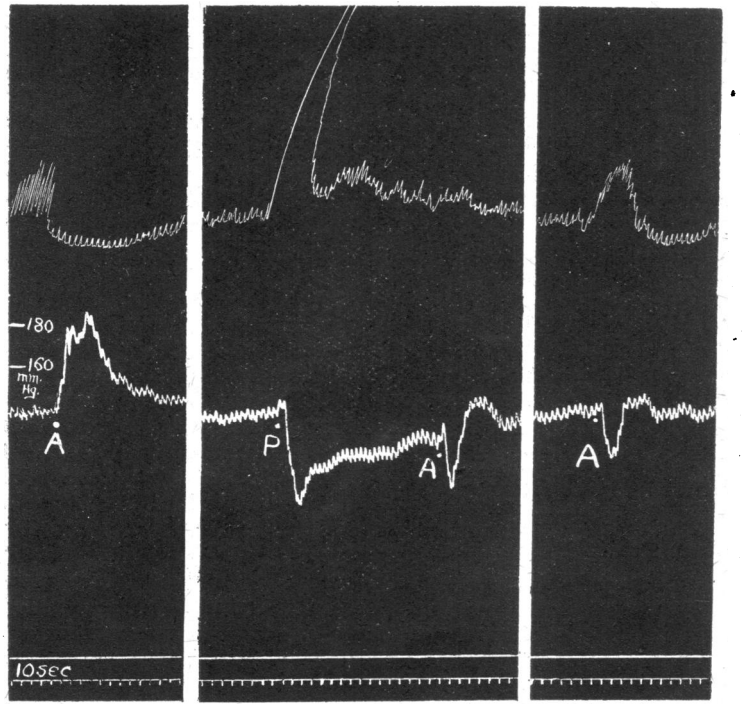


FIG. 10.—Upper record: contractions of duodenum recorded by intestinal balloon, water manometer and piston recorder; lower record blood pressure of cat under chloralose anaesthesia. At the first A, 10 μg. adrenaline inhibited duodenum and caused rise of blood pressure; at P, 5 mg./kg. priscol caused fall of blood pressure and large contraction of duodenum; 3 min. after priscol 10 μg. adrenaline, at second A, had depressor action but no effect on duodenum; 13 min. after priscol 10 μg. adrenaline, at last A, caused fall of blood pressure and *contraction* of duodenum.

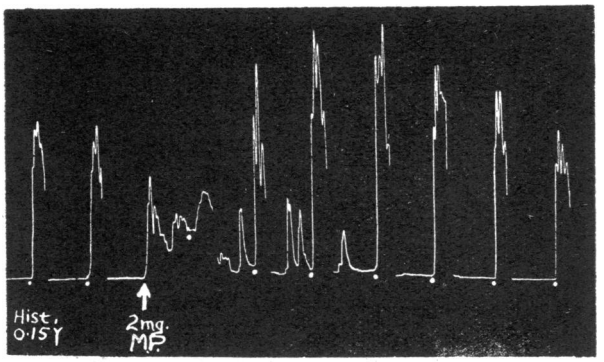


FIG. 11.—Record of contractions of isolated guinea-pig ileum in Tyrode solution. All contractions caused by 0.15 μg. histamine except at arrow where 2 mg. N-methylpriscol was added to bath and left in for 30 sec. before the next 0.15 μg. histamine was added (at dot). Note the increased spontaneous activity after N-methylpriscol.

Skeletal muscle.—Priscol and N-methylpriscol potentiate the contractions of the frog's rectus abdominis caused by acetylcholine. When the cat's sciatic nerve is stimulated by maximal single shocks (16 per min.) the injection of either priscol or N-methylpriscol into the central end of the external iliac artery near the bifurcation caused a curariform depression of the contractions of the gastrocnemius muscle as shown in Fig. 12. The same effect was observed in the phrenic nerve diaphragm preparation of the rat (Bülbring, 1946), and in the frog gastrocnemius.

Superior cervical ganglion.—When the superior cervical ganglion of the cat was perfused with Locke's solution, and the contractions of the nictitating membrane were recorded, it was found that

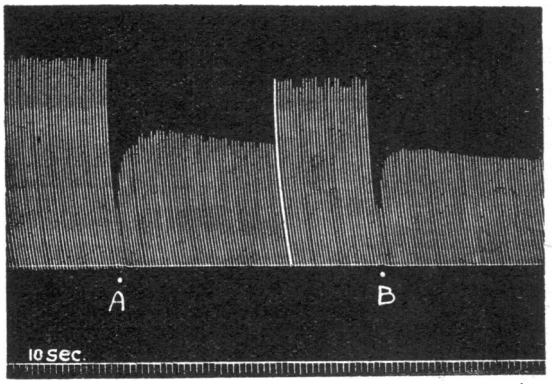


FIG. 12.—Record of contractions of gastrocnemius muscle of spinal cat caused by supra-maximal electrical stimulation (rate 16/min.) of sciatic nerve. At A, 8 mg. N-methylpriscol injected into external iliac artery had curariform action, and at B, 8 mg. priscol had same action.

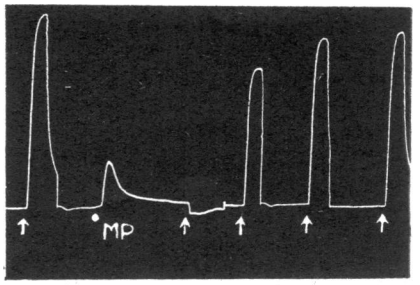


FIG. 13.—Record of contractions of nictitating membrane caused by supra-maximal electrical stimulation of pre-ganglionic fibres of cat's superior cervical ganglion perfused with warm Locke solution. At arrows stimulations lasting 15 sec. (given every 3 min.). At MP, 0.5 mg. N-methylpriscol caused contraction; it abolished the contraction due to the next stimulation, and reduced the second succeeding contraction.

both priscol and N-methylpriscol when injected into the perfusing fluid caused a contraction of the membrane. The contraction due to N-methylpriscol was more rapid in onset and in relaxation than that due to priscol, in contrast to the effect produced in the whole animal (Fig. 7). The injection of N-methylpriscol also abolished the effect of the next stimulation of the preganglionic fibres as shown in Fig. 13.

Gastric secretion.—When priscol was infused at a uniform rate into a vein it was found to cause a large output of gastric juice (collected by a wide cannula introduced through the pylorus in the stomach); this was observed in six cats anaesthetised with pentobarbitone. When N-methylpriscol was infused in four times the concentration, it failed to produce any secretion in two cats.

DISCUSSION

The observations described in this paper show that the reversal of the constrictor action of adrenaline by priscol, which is seen in the whole animal, can also be observed in the perfused vessels of the rabbit's ear. It is interesting to record that Rothlin (1925) found that ergotamine also would reverse the action of adrenaline in isolated vessels. The reversal of the pressor action of adrenaline is usually demonstrated in the whole animal; the fact that it also occurs in isolated vessels makes it more probable that it is due to a direct action of adrenaline itself, rather than to liberation of, for instance, histamine, as has been suggested by Staub (1946).

The observations here described show further that the introduction of a methyl group into the imidazoline ring of priscol changes the vascular action so that the peripheral vasodilatation is no longer seen ; instead a pressor action appears which can be attributed to a release of adrenaline by stimulation of the sympathetic ganglia. The pressor effect of N-methylpriscol is greatly reduced by the administration of either full doses of nicotine or of tetraethylammonium iodide, and also by the removal of the suprarenal glands. It is therefore very curious that though N-methylpriscol stimulates the isolated cat heart and the rabbit auricles, this does not appear to be a nicotine-like action, but may more properly be compared to the effect of adrenaline. In cardiac tissue the action of N-methylpriscol was in some respects similar to that of priscol.

While priscol converts the motor effects of adrenaline on the vessels to inhibitor effects, it reduces the inhibitory effects of adrenaline on the intestine, and in at least one instance converts them to motor. Thus after the injection of priscol which itself caused a contraction of the cat's duodenum (*in vivo*), the injection of adrenaline caused a contraction. The observations are very similar to those which have been made with ergotoxine and ergotamine, for these substances also convert the motor action of adrenaline on the vessels to an inhibitor action and as Planelles (1925) found, convert the inhibitor action on the intestine to a motor action. Priscol potentiated the action of acetylcholine on the intestine.

On skeletal muscle priscol and N-methylpriscol were alike in action. On the one hand, the stimulation of the frog rectus by acetylcholine was augmented, and on the other the contractions of the cat's gastrocnemius, when evoked by stimulation of the sciatic nerve, were diminished as by an injection of tubocurarine. This curariform action was also observed in the phrenic-diaphragm preparation of the rat. In the perfused superior cervical ganglion there was again evidence of a double action. Both priscol and N-methylpriscol, on injection into the perfusion fluid, stimulated the ganglion ; after the injection of N-methylpriscol, stimulation of the preganglionic fibres was temporarily ineffective. We therefore have in priscol and in N-methylpriscol substances which augment or imitate the action of acetylcholine in the sympathetic ganglion and in skeletal muscle ; in certain circumstances they have, as nicotine has, the opposite, curariform, action as well.

Perhaps the most striking conclusion to which we are driven by a study of the pharmacology of these substances is that they will not fit into any of the provisional classifications of drugs which in the past have proved so useful. This point can be more effectively illustrated by the following table: —

	Priscol	*N-Methylpriscol*
Blood pressure	Fall	Nicotine-like rise
Adrenaline action on blood pressure	Reversed	Unaffected
Adrenaline pressor effect after portal injection	?Unaffected	Augmented
Blood vessels	Vasodilatation	No direct effect
Adrenaline effect on vessels	Reversed	Unaffected
Smooth muscle	Abolishes adrenaline *in vitro* Reverses adrenaline *in vivo* (cat)	No effect on response to adrenaline
Spleen *in vivo*	Slight dilatation	Contraction
Cardiac muscle	Stimulated	Stimulated
Refractory period	Prolonged	Unaffected
Nictitating membrane	Strongly stimulated (ganglion removed)	Slight stimulation
Gastric secretion	Stimulated	Unaffected
Skeletal muscle	Like nicotine	Like nicotine
Superior cervical ganglion	Like nicotine	Like nicotine

It will be impossible to explain their mode of action until we have achieved more knowledge of the physiology of these tissues, but two points may provide a lead for subsequent investigations. The first is the structural relationship between the imidazolines, adrenaline and histamine, and the second is the observation that the reversal of the pressor action of adrenaline by priscol is dependent upon the relative concentrations of the two substances.

SUMMARY

1. Reversal of the constrictor action of adrenaline by priscol, demonstrated in the whole animal, can also be observed in the perfused vessels of the rabbit's ear. The dilator action of adrenaline in these circumstances is more likely to be due to the action of adrenaline itself, rather than to the liberation of a substance such as histamine.

2. The introduction of an N-methyl group into priscol changes the vascular action so that, instead of peripheral vasodilatation, a pressor action is observed, which can be attributed to a release of adrenaline by stimulation of the sympathetic ganglia. The stimulating effect of N-methylpriscol on the isolated heart, however, does not appear to

be a nicotine-like action, but may be compared to the effect of adrenaline. In cardiac tissue the action of priscol is, in some respects, similar to that of N-methyl derivative.

3. Priscol reduces the inhibitory effects of adrenaline on the intestine, and in at least one instance converts them to motor. Both priscol and N-methylpriscol potentiate the action of acetylcholine on the intestine.

4. In the sympathetic ganglion and in skeletal muscle priscol and N-methylpriscol augment or imitate the actions of acetylcholine, and in certain circumstances they have the opposite, curariform, action as well.

5. A study of the pharmacology of these substances indicates that they cannot be fitted into the provisional classifications of drugs which have hitherto been so valuable.

The N-methylpriscol was very kindly supplied by Dr. W. F. Short, Director of the Chemical Research Laboratories of Messrs. Boots Pure Drug Co., Ltd. I wish to thank Prof. J. H. Burn and Dr. G. S. Dawes for the guidance and advice which they have given throughout this study.

References

Barger, G., and Dale, H. H. (1910). *J. Physiol.*, **41**, 19.
Beyer, K. H. (1946). *Physiol. Rev.*, **26**, 169.
Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.
Chess, D., and Yonkman, F. F. (1945). *Fed. Proc.*, **4**, 114.
Dale, H. H., and Schuster, E. H. J. (1928). *J. Physiol.*, **64**, 356.
Dawes, G. S. (1946a). *Brit. med. J.*, **1**, 43.
Dawes, G. S. (1946b). *Brit. J. Pharmacol.*, **1**, 21.
Gaddum, J. H., and Kwiatkowski, H. (1938). *J. Physiol.*, **94**, 87.
Hartmann, M., and Isler, H. (1939). *Arch. exp. Path. Pharmak.*, **192**, 141.
Hermann, H., Jourdan, F., and Bonnet, V. (1941). *C.R. Soc. Biol.*, **135**, 1653.
Meier, R., and Müller, R. (1939). *Schweiz. med. Wschr.*, **69**, 1271.
Planelles, J. (1925). *Arch. exp. Path. Pharmak.*, **105**, 38.
Rothlin, E. (1925). *Klin. Wschr.*, **4**, 1437.
Staub, H. (1946). *Helv. Physiol. Acta*, **4**, 539.