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# THE ACTION OF SUBSTANCES WHICH ANTAGONIZE ACETYLCHOLINE ON THE BODY TEMPERATURE OF MICE, BEFORE AND AFTER ADRENALECTOMY

BY

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In 1931 Glaubach and Pick described the fall of temperature produced in guinea-pigs by the injection of procaine. Recently Peczenik (1947) has extended this observation to castrated mice, and has shown in addition that the fall is greater after adrenalectomy. By administration of desoxycorticosterone acetate, progesterone, and other steroids before adrenalectomy, the mice were protected to a varying extent, so that the fall of temperature due to procaine was smaller.

No light has hitherto been shed on the reason for this action of procaine. Peczenik says that the fall is probably the result of a shock to the autonomic nervous system. Recently, however, attention has been drawn to other properties possessed by procaine in addition to its local anaesthetic action: Dawes's work (1946) on quinidine substitutes led him to point out that quinine, quinidine, and procaine reduce the action of acetylcholine on the intestine, on the heart, and, as was shown by Harvey (1939a and b), on skeletal muscle. Dawes showed that procaine, atropine, and pethidine act like quinidine on the electrically driven auricle, and having pointed out the local anaesthetic action of atropine, which has long been known, he demonstrated that pethidine possesses a local anaesthetic action too. Thus atropine, pethidine, procaine, and quinidine are four substances having several properties in common, and de Elío (1948) has determined their relative potency in reducing the action of acetylcholine on the frog rectus, the rabbit intestine, and on the isolated rabbit auricle. The question then arose whether atropine, pethidine, and quinidine would also share with procaine the ability to cause a fall of body temperature in mice. The experiments here described supply the answer. The antihistamine substance benadryl was also included in the investigation because of the observations of Dews and Graham (1946); they tested the antihistaminé substance neoantergan, which is known to have some atropine-like action, and found it to possess a quinidine-like action on the auricle, and a local anaesthetic action. I have found that neoantergan also depresses the action of acetylcholine on the frog rectus and the rabbit auricle, though the doses required are rather large. Benadryl also has been shown to have a local anaesthetic action (Leavitt and Code, 1947) and to reduce the action of acetylcholine on the intestine (Loew, MacMillan, and Kaiser, 1946).

## METHODS

Since Peczenik has described the conditions for observing the fall of temperature produced by procaine in mice, his procedure was followed in several respects. Male mice weighing not less than 26 g. were used, and all were castrated. Peczenik states that non-castrated mice "did not behave uniformly." They had access to food and water except during the observation of body temperature. They were fed on a diet of constant composition consisting of ground grain; its percentage composition was: wheat 19, bran 19, oats 14, maize 9, barley 9, fish-meal 5, bone-meal 9, skimmed milk 14, yeast 1, salt 0.5, and cod-liver oil 0.5 per cent. The mice were kept at about 25° C. and experiments were carried out at 25° ± 1° C. in a small room with a thermostat.

The rectal temperature of each mouse was recorded by inserting a thermocouple into the rectum for 2.5 cm.; this thermocouple was connected to a moving coil galvanometer, and the other thermocouple was placed in melted sodium sulphate crystals in a thermos flask. The thermocouple in the rectum of the mouse was kept in position by adhesive tape fixed to the tail, and the tail was fixed along its length to a copper wire which continued forwards over the back of the mouse as far as the forelegs, where it turned around the body of the mouse in a ring behind

them ; the mouse was thus held comfortably throughout the period of observation and passed faeces as usual.

The temperatures of 8 mice were followed in each experiment during the same period of time ; 4 mice were used as controls and were injected with saline, while 4 were injected with the substance being tested. The injections of atropine, pethidine, and procaine were subcutaneous, while those of benadryl and quinidine were intraperitoneal. Before each experiment all mice were kept in the room until their temperatures remained steady ; this usually required 30–45 min.

## RESULTS

*Pethidine (demerol).*—In order to illustrate the performance of the tests, the results with one dose of pethidine will be given in detail so that the remaining results can be given in summarized form.

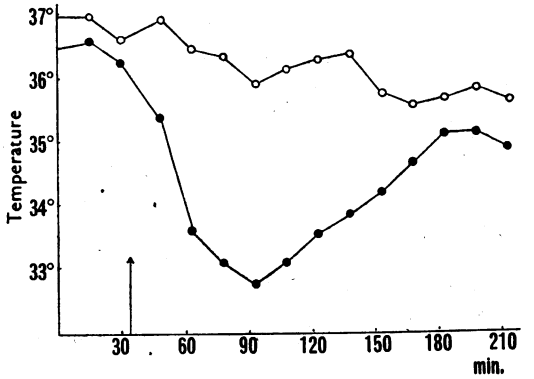


FIG. 1.—Abscissae: time in min.; ordinates: body temperature in °C. White circles, mean body temperature of 4 control mice injected at arrow with saline. Black circles, mean body temperature of 4 mice injected at arrow with pethidine hydrochloride, 30 mg./kg.

Observations were made on three groups of 8 castrated mice, half of which received 30 mg./kg. pethidine hydrochloride, and half received the same volume of saline. The results in one of these groups are given in Fig. 1. The body temperature of the control mice fell during the 3 hours after the injection from a mean value of 36.6° C. to a mean value of 35.6° C. The temperature of the mice injected with pethidine reached a minimum of 32.7° C. one hour after injection and slowly recovered to 35.2° during the next 1½ hours. The results in the other two groups were similar to those shown in Fig. 1 ; they are given in Table I. It will be noted that the fall of temperature in the control mice was greater in both groups than that in Fig. 1. In Group 2 the mean temperature fell from 35.5 to 33.8°, while in Group 3 it fell from 34.6 to 32.9° in the course of the experiment. In

TABLE I

TEMPERATURES OF MICE RECEIVING 30 MG./KG. PETHIDINE HYDROCHLORIDE

| Time min. | Group 2 | | | Group 3 | | |
|---|---|---|---|---|---|---|
| | Control | Injected | Difference | Control | Injected | Difference |
| 0 | 35.5 | 35.5 | 0 | 34.6 | 35.8 | −1.2 |
| 15 | 35.9 | 35.1 | 0.8 | 34.6 | 35.0 | −0.4 |
| 30 | 35.1 | 33.7 | 1.4 | 34.3 | 33.8 | 0.5 |
| 45 | 34.7 | 32.6 | 2.1 | 34.2 | 33.2 | 1.0 |
| 60 | 34.6 | 32.3 | 2.3 | 34.3 | 32.9 | 1.4 |
| 75 | 34.5 | 32.6 | 1.9 | 34.1 | 32.8 | 1.3 |
| 90 | 34.4 | 33.1 | 1.3 | 33.7 | 32.8 | 0.9 |
| 105 | 34.4 | 33.1 | 1.3 | 33.7 | 33.0 | 0.7 |
| 120 | 34.2 | 33.5 | 0.7 | 33.3 | 32.5 | 0.8 |
| 135 | 34.2 | 33.6 | 0.6 | 33.0 | 32.5 | 0.5 |
| 150 | 34.2 | 34.5 | −0.3 | 33.1 | 32.6 | 0.5 |
| 165 | 33.8 | 34.1 | −0.3 | 32.9 | 32.6 | 0.3 |

Table I the differences between the control mice and those injected with pethidine are also shown. The mean differences for the three groups were calculated and the resulting figures are plotted as ordinates against time as abscissa in Fig. 2. In this and succeeding Figures, the mean difference of temperature between the two groups of mice before

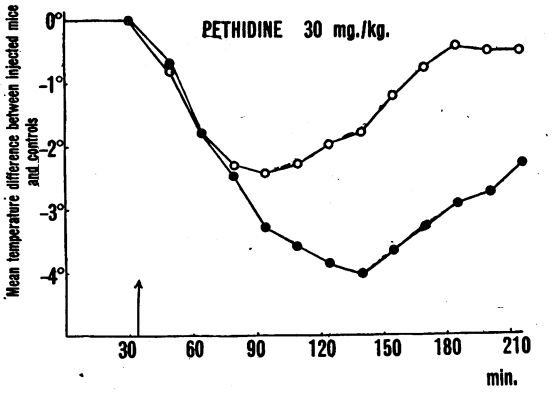


FIG. 2.—Ordinates: difference between mean body temperature of mice injected with 30 mg./kg. pethidine hydrochloride and of control mice, taking difference before injection as zero and correcting throughout for this difference. White circles show observations on 12 injected and 12 control mice before adrenalectomy. Black circles show observations on 9 injected and 8 control mice after adrenalectomy.

injection is shown as 0° ; this was often not actually so (see Fig. 1), but the difference of temperature between the two groups throughout the period of observation was corrected by the difference which existed before injection.

The mice used in obtaining these results were kept for one week after the observations were

made ; adrenalectomy was then carried out under ether. Two days after the operation the effect of the same dose of pethidine was redetermined. Of the 24 mice tested before the operation, 7 died during the next two days, so that only 17 were available ; of these 8 were injected with saline and 9 were injected with pethidine. The differences of temperature between the mice receiving pethidine and the control mice are recorded in the lower curve of Fig. 2. Fig. 2 shows that after adrenalectomy the fall of temperature caused by pethidine was much greater than before.

Observations were also made with a higher dose of pethidine, namely 100 mg./kg. In the mice before adrenalectomy this dose produced a rise of body temperature, the mean maximum rise in 12

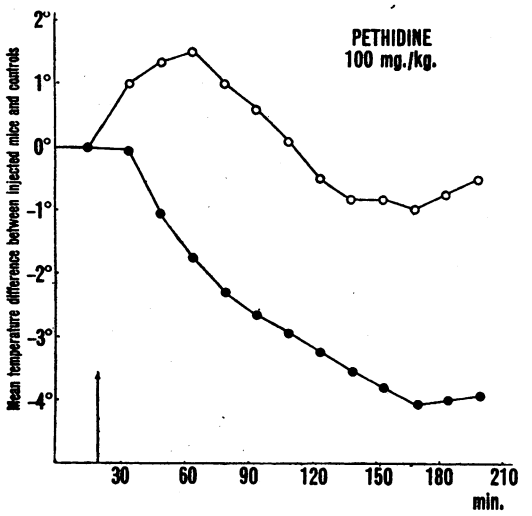


FIG. 3.—Similar to Fig. 2. Dose of pethidine 100 mg./kg. Note the rise of temperature before adrenalectomy when 12 mice were compared with 12 controls. Black circles show results after adrenalectomy; (10 mice injected and 9 controls).

animals being to a point 1.5° above the mean temperature of the 12 control mice. The rise, however, subsided after 1.5 hours and was followed by a fall. The change is shown in Fig. 3. This high dose of pethidine not only produced a rise of temperature but also caused increased muscular movements in all the mice.

The rise disappeared after adrenalectomy: 10 mice were injected with pethidine and 9 were injected with saline. The pethidine caused a large fall of temperature relative to the temperature of the control mice ; this is shown in the lower curve of Fig. 3.

*Atropine.*—Similar experiments were then made with atropine, using first of all 150 mg./kg. of atropine sulphate. This dose was too high ; in two of the three groups used, the dose was fatal to the mice after adrenalectomy. In one of the groups, however, the tests were carried out successfully, and, though the maximum fall of temperature before adrenalectomy was only 1.3° more than in the controls, after adrenalectomy the maximum fall was 3.2° more than in the controls. A further

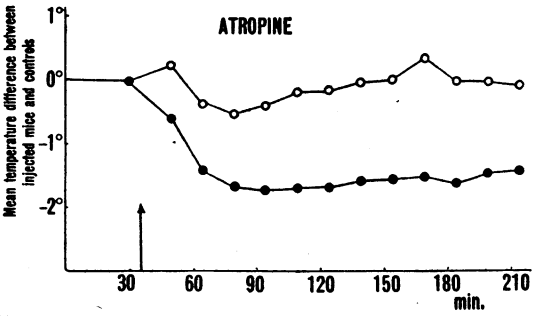


FIG. 4.—Similar to Fig. 2. Mice injected with atropine sulphate 30 mg./kg. White circles, 12 injected and 12 control mice before adrenalectomy. Black circles, 10 injected and 10 control mice after adrenalectomy.

test was then made using 30 mg./kg. atropine sulphate, and the results which were obtained in 24 mice before adrenalectomy and in 20 of these after adrenalectomy are given in Fig. 4. Before the operation the fall of temperature was slight, the maximum being 0.5° more than the controls. This is an effect too small to be discovered except by careful investigation. After adrenalectomy it was increased to about 1.5°, and this was maintained.

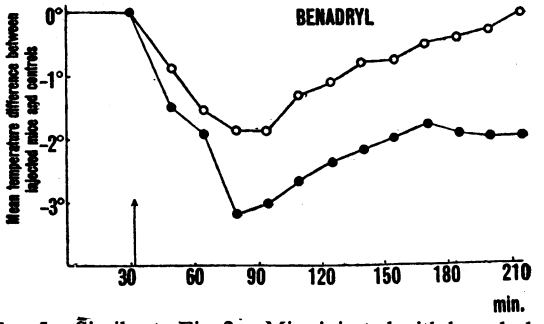


FIG. 5.—Similar to Fig. 2. Mice injected with benadryl 20 mg./kg. White circles, 8 injected and 8 control mice before adrenalectomy. Black circles, 7 injected and 7 control mice after adrenalectomy.

*Benadryl.*—Results with the hydrochloride of β-dimethylaminoethylbenzhydrylether, which is called benadryl, are shown in Fig. 5 ; they were obtained with a dose of 20 mg./kg. Before adren-

alectomy 16 mice were used, and after adrenalectomy 14 of the same mice were used. The results were similar to those obtained with the lower dose of pethidine, though the fall of temperature was less.

*Procaine.*—Peczenik injected his mice with procaine hydrochloride in a dose of 100 mg./kg. When this amount was used, the fall of temperature was very small, and was not much greater after adrenalectomy. The maximum fall in 8 mice compared with 8 controls was 0.5° before and

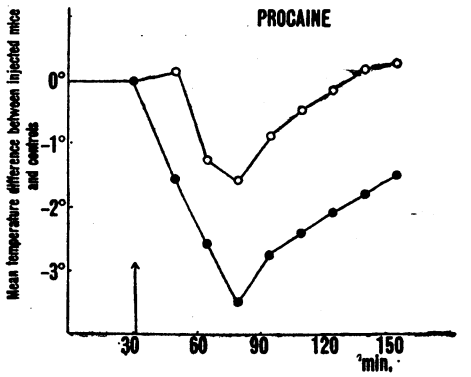


FIG. 6.—Similar to Fig. 2. Mice injected with procaine hydrochloride 200 mg./kg. White circles, 8 injected and 8 control mice before adrenalectomy. Black circles, 8 injected and 6 control mice after adrenalectomy.

1.5° after the operation. Further observations were then made with 200 mg./kg., and the results in 8 mice, compared with 8 controls, are shown in Fig. 6. The difference in sensitiveness between mice in this laboratory and those in Peczenik's laboratory is not surprising, for similar differences are often seen.

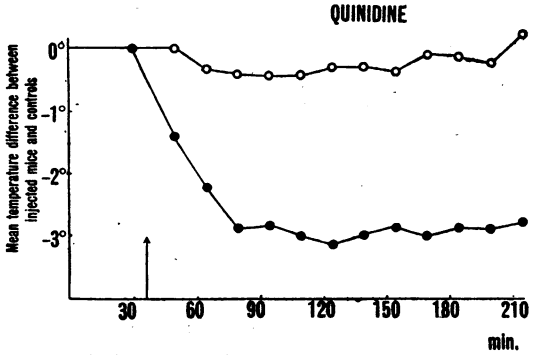


FIG. 7.—Similar to Fig. 2. Mice injected with quinidine hydrochloride 30 mg./kg. White circles, 12 injected and 11 control mice before adrenalectomy. Black circles, 9 injected and 7 control mice after adrenalectomy.

*Quinidine.*—The final observations were made when quinidine hydrochloride was given in a dose of 30 mg./kg. As after atropine, there was a very small fall of temperature before adrenalectomy, not exceeding 0.5°. After the operation there was, however, a fall of 3°, which was maintained throughout the period of observation. The results are shown in Fig. 7.

## DISCUSSION

So far as the action of procaine is concerned, the results described confirm the finding of Peczenik that procaine causes a fall of body temperature in mice and that this fall is greater after adrenalectomy. However, the " collapse " of temperature which Peczenik observed after adrenalectomy was not seen ; the temperature fell, but so did that of many control mice, and the effect of procaine was not great. Peczenik found the mean fall in 20 mice after adrenalectomy to be 4.9° when procaine was given in the dose 100 mg./kg. ; he does not record the temperature changes in control mice kept alongside. In the work here described, the maximum difference between injected mice and control mice was found to be only 1.5° after 100 mg./kg., and only 3.5° after twice this amount. The difficulty which a mouse has in maintaining a constant temperature has been discussed by Fuhrman (1946) ; on account of its large surface per unit weight, the body temperature falls more easily than in larger animals, and control observations are therefore essential.

The main result of this research is to demonstrate that the property which procaine possesses of reducing temperature is shared by a number of other substances—atropine, quinidine, pethidine, and benadryl. The experiments were undertaken in order to see if this would be so, and the hypothesis concerning their action has thus gained additional support. It was pointed out that all these drugs reduce or abolish the action of acetylcholine on the frog rectus, on the spontaneous contractions of the rabbit auricle, and on the rabbit intestine. Further, atropine, procaine, and pethidine act like quinidine on the refractory period of the electrically driven auricles of the rabbit (Dawes, 1946). I myself have observed that benadryl reduces the effect of acetylcholine on the frog rectus and the rabbit auricle, and I have found that it lengthens the refractory period. Since de Elío (1947) has shown that acetylcholine shortens the refractory period, the quinidine-like action of this group, if not one which can be described as a depression of the action of acetycholine, is at least an action in the opposite direction to that of

acetylcholine. Thus there is some ground for the view that the common property of reducing body temperature may be connected with the power to reduce or antagonize the action of acetylcholine.

It is at least clear that the common property is not due to the possession of a local anaesthetic action, though all of these substances have one. Thus it was found by Glaubach and Pick (1931) that while cocaine caused a varying change of body temperature—namely, a rise in rabbits and a fall in guinea-pigs—nupercaine, one of the most potent local anaesthetics, had almost no effect on the temperature of either species. This is of interest because de Elío found that nupercaine did not reduce the action of acetylcholine on the rabbit auricles when spontaneously contracting, no matter in what dose nupercaine was applied. Thus nupercaine provides an example of a local anaesthetic which differs from procaine in reducing neither body temperature nor the action of acetylcholine in the cardiac tissue of the rabbit.

The conclusion that substances which reduce body temperature are also substances which reduce the action of acetylcholine must not be taken to imply that acetylcholine plays a role in the central nervous system to maintain body temperature. Feitelberg, Pick, and von Warsberg (1939) tested the action of acetylcholine on the temperature of the grey matter of the cortex of cats and found that sometimes it caused a fall and sometimes a rise. The fall which procaine, pethidine, etc., cause may be due to their inhibition of the action of acetylcholine in the skeletal muscles, on the activity of which the normal formation of heat depends. This suggestion is borne out by the fact that the large dose of pethidine which caused an initial rise of temperature also caused increased motor activity. The lower dose of pethidine, and also the doses of the other substances used, were too small to cause any visible effect in the mice.

The effect of adrenalectomy in augmenting the fall of temperature produced by these substances shows clearly that the adrenal glands are concerned in temperature control. Whether it is the cortex or the medulla which is the more important is not known for certain, though the power of cortical extracts to prevent the fall of temperature in adrenalectomized rats when exposed to low temperature indicates the greater importance of the cortex. Moreover, Peczenik claims to have shown that the administration of desoxycorticosterone acetate diminished the effect of removing the glands on the procaine fall of temperature.

A word is required in conclusion on the use of castrated mice. Observations on 16 normal male mice, 8 of which were injected with 30 mg./kg. pethidine and 8 of which were controls, gave a result almost the same as that obtained with castrated mice.

## Summary

1. The finding that procaine causes a fall of body temperature in mice, and that this fall is greater after adrenalectomy, has been confirmed.

2. It has been shown that this property of procaine is shared by the analgesic pethidine (demerol), by quinidine, by atropine, and by the antihistamine substance benadryl.

3. The fall of temperature produced by each of these substances is augmented by adrenalectomy.

4. All these substances reduce or abolish the action of acetylcholine on the frog rectus, the rabbit auricle, and the rabbit intestine. It is suggested that the fall of temperature may be due to lessened activity and lessened heat production in the skeletal muscles of the mice.

5. A large dose of pethidine causes a rise of temperature accompanied by increased motor activity.

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## References

Dawes, G. S. (1946). *Brit. J. Pharmacol.*, **1**, 90.
Dews, P. B., and Graham, J. D. P. (1946). *Brit. J. Pharmacol.*, **1**, 278.
Elío, F. J., de. (1947). *Brit. J. Pharmacol.*, **2**, 131.
Elío, F. J., de. (1948). *Brit. J. Pharmacol.*, **3**, 108.
Feitelberg, S., Pick, E. P., and von Warsberg, A. (1939). *Arch. intern. Pharmacodyn.*, **61**, 447.
Fuhrman, G. J. (1946). *Physiol. Rev.*, **26**, 247.
Glaubach, S., and Pick, E. P. (1931). *Arch. exp. Path. Pharmak.*, **162**, 551.
Harvey, A. M. (1939a). *J. Physiol.*, **95**, 45.
Harvey, A. M. (1939b). *Bull. Johns Hopk. Univ.*, **65**, 223.
Leavitt, M. D., and Code, C. F. (1947). *J. Lab. clin. Med.*, **32**, 334.
Loew, E. R., MacMillan, R., and Kaiser, M. E. (1946). *J. Pharmacol.*, **86**, 229.
Peczenik, O. (1947). *Proc. roy. Soc.*, **134B**, 218.