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# THE EFFECT OF ADRENALINE, NORADRENALINE AND ISOPRENALINE ON THE GUINEA-PIG UTERUS

BY

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The actions of adrenaline, noradrenaline and isoprenaline were compared on the isolated guinea-pig uterus. In uteri from immature animals (200 to 350 g) adrenaline caused relaxation which changed to a biphasic effect and finally to a contraction in the course of an experiment (6 to 8 hr). Noradrenaline always caused contraction and isoprenaline relaxation. In uteri from oestrogen-treated animals adrenaline and noradrenaline caused contraction and isoprenaline caused relaxation. Isoprenaline potentiated the contraction produced by adrenaline and reversed the adrenaline relaxation to a contraction. The change of the pharmacological action of adrenaline was not related to the $Na^+$ and $K^+$ content of the uterus, which remained constant throughout an experiment involving repeated application of the amines. Nor could it be related to a change in the glycogenolytic effect of adrenaline estimated by determinations of total glycogen of the muscle which, however, may not reflect a momentary change in rate of breakdown.

The effect of adrenaline and noradrenaline on the isolated uterus from the guinea-pig has been investigated by several workers, particularly by Holtz & Wölpert (1937) and later by Greeff & Holtz (1951). They found that adrenaline caused a relaxation in uteri from immature guinea-pigs or from adult guinea-pigs in anoestrus. This relaxation was changed to a biphasic response—a contraction followed by a relaxation—or a pure contraction, when the guinea-pigs had been treated with gonadotrophic hormones. They did not consider the possibility that the change in the pharmacological action of adrenaline, which they produced by hormonal treatment, might occur by itself during a single experiment in the course of 8 hr.

Lundholm & Mohme-Lundholm (1956) tried to relate the mechanical response of adrenaline with its metabolic action. They found no significant decrease in the glycogen content when adrenaline was applied to the guinea-pig uterus. However, when the uteri were divided in two groups, one in which adrenaline caused a relaxation and another in which it caused a contraction or had an indefinite effect, then there was a significant decrease in muscle glycogen in the first group (from an average of 183 mg % the glycogen fell by 27.4 mg %). They were unable to show any glycogenolytic effect of adrenaline when glucose was present in the nutrient solution and they concluded that the relaxing effect of adrenaline was due to formation of lactic acid in the muscular tissue.

The investigation to be described in this paper was carried out in order to compare the effect of adrenaline and noradrenaline with that of isoprenaline. Another aim was to find if factors other than hormonal treatment might produce

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the same changes in the action of adrenaline as those described by Holtz and his co-workers.

## METHODS

### In vitro *experiments on the guinea-pig uterus*

Virgin female guinea-pigs weighing 200–350 g were used. Immediately after killing and bleeding the animals the right horn of the uterus was removed and set up in a 20 ml. isolated organ bath at 35° C. The composition of the bathing solution was (mM): $Na^+$ 137, $K^+$ 5.9, $Ca^{++}$ 2.5, $Mg^{++}$ 1.2, $Cl^-$ 134, $HCO_3^-$ 15.5, $H_2PO_4^-$ 1.2 and glucose 11.5. It was aerated with a gas mixture of 97% $O_2$ and 3% $CO_2$; the *p*H was 7.3.

Mechanical records were obtained on a smoked drum by means of an isotonic lever with frontal-writing point and a load of 1 g/cm muscle.

Most of the experiments were carried out by using uteri from untreated immature guinea-pigs. A few animals were treated with oestradiol (Dimenformon, Organon) 1 $\mu$g/g intramuscularly 48 hr before starting the experiment. The stage of the oestrus cycle was determined by vaginal smears.

### *Sodium and potassium determinations*

To determine the $Na^+$ and the $K^+$ content of the tissue before and after an experiment, one horn was taken for $Na^+$ and $K^+$ determination immediately after killing the guinea-pig, while the other horn was used for the determination at the end of the experiment. The fresh weight of the first horn was determined, but only the wet weight of the second horn was obtained at the end of the experiment. Both horns were transferred to small containers which were kept overnight in a drying oven at 105° C in order to determine the dry weight. In this way the ratio $\frac{\text{fresh wt.}}{\text{dry wt.}}$ of one horn and $\frac{\text{wet wt.}}{\text{dry wt.}}$ of the other horn could be calculated.

The pots containing the dried uteri were placed in small recesses in the top of a heavy steel block and to each pot was added 1 ml. concentrated Analar nitric acid and 1 ml. concentrated Analar perchloric acid. The temperature was then raised slowly to 150° C and in this way the uteri were ashed until no organic material was left. The residue was dissolved in distilled water. The $Na^+$ and $K^+$ content was determined by flame photometry with a Beckman DU flame photometer at a wavelength of 589 m$\mu$ and 769 m$\mu$ respectively and compared with standard solutions of NaCl and KCl.

### *Glycogen determinations*

Many workers have found that in some species, for instance, rat (Walaas, 1952), a considerable part of the uterine glycogen is situated in the endometrium. As the present work was concerned with the glycogen content of the myometrium a preliminary experiment was carried out to see whether the glycogen distribution was the same in the guinea-pig and in the rat. A technique similar to that described by Walaas (1952) was used. Uteri were quickly dissected and transferred to a watch-glass placed on solid $CO_2$. The uteri from which the endometrium was to be removed were split longitudinally and the peritoneal side was frozen to the watch-glass. The endometrium was then scraped off with a scalpel. The remaining myometrium was quickly weighed and transferred to boiling potassium hydroxide for glycogen estimation which was carried out as follows. The uterine horns, either freshly removed from the body, or at the end of an experiment from the isolated organ bath, were weighed and placed in 10 ml. graduated centrifuge tubes containing 1 ml. 30% potassium hydroxide in a boiling water bath. After boiling for 15 min the tissue was usually dissolved completely and the cotton knots were then removed. The volume was made up to 5 ml. with 95% ethanol and the centrifuge tubes were kept overnight in a deep freeze at −20° C for glycogen precipitation. The next morning the glycogen was spun down for 5 min at 3,000 rev/min and the supernatant was discarded. The solid glycogen was hydrolysed to glucose by boiling it in 1 ml. 3N sulphuric acid for 2 to 3 hr. The glucose was estimated by the method of Huggett & Nixon (1957). This very sensitive method was necessary because the total amount of glycogen was only about 50 $\mu$g. Furthermore the method is claimed to be very specific, and this might account for the glucose values in this paper being rather small compared with those of other authors. The glycogen content was expressed in mg glucose per 100 g fresh wt. throughout.

Lundholm & Mohme-Lundholm (1956) found that there was a big animal variation in the glycogen content. In the present experiments the scatter was not so great, possibly because only immature guinea-pigs were used. Lundholm & Mohme-Lundholm (1956) used adult animals, and the scatter which they found might be connected with different stages of the oestrus cycle.

To avoid the effect of variation between animals the changes in the glycogen content caused by adrenaline were expressed as percentages by comparing the test horn with a control horn from the same animal. The right and the left horn were taken alternately as control and test object.

The experiments in which the glycogenolytic effect of adrenaline was determined were carried out as follows. Three isolated organ baths were set up and kept at the same temperature. The first bath contained the usual bathing solution. Both horns from each guinea-pig uterus were set up in this solution and stretched by 20% of their *in situ* length. After a predetermined period one horn of each pair was removed to the second bath, the other to the third bath, both containing glucose-free solution. To the third bath adrenaline was added 20 sec before the uterus was immersed to give a concentration of $1 \times 10^{-6}$. After 5 min both horns were removed at the same time and the glycogen was determined.

## RESULTS

### *Mechanical effect of adrenaline, noradrenaline and isoprenaline*

*Effect of hormonal treatment.* When a horn from the guinea-pig uterus was set up in the isolated organ bath it usually exhibited a spontaneous tone at first. This gradually declined, but, after several hours, most preparations showed a rhythm of activity, contracting and relaxing at regular intervals.

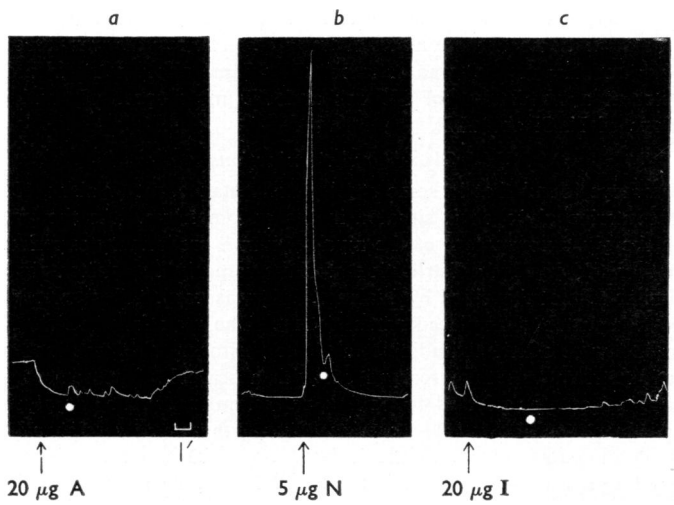


Fig. 1. The effect of adrenaline (A), noradrenaline (N) and isoprenaline (I) on the isolated uterus from an immature guinea-pig, recorded at the beginning of an experiment. In this and all subsequent tracings a dot indicates the time at which the amines were washed out. Time : 1 min.

The effects of adrenaline, noradrenaline and isoprenaline on an isolated uterus from an immature guinea-pig are illustrated in Fig. 1. The responses were obtained at the beginning of an experiment, when a slight spontaneous tone was present. Adrenaline caused a relaxation and noradrenaline caused a contraction. Isoprenaline caused a relaxation and it stopped the spontaneous activity.

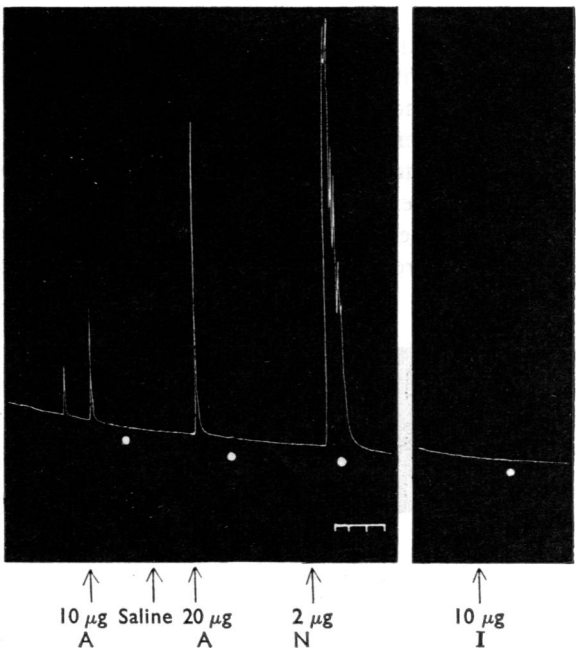


Fig. 2. The effect of adrenaline (A), noradrenaline (N) and isoprenaline (I) on an isolated uterus from a guinea-pig in oestrus. Time: 1 min.

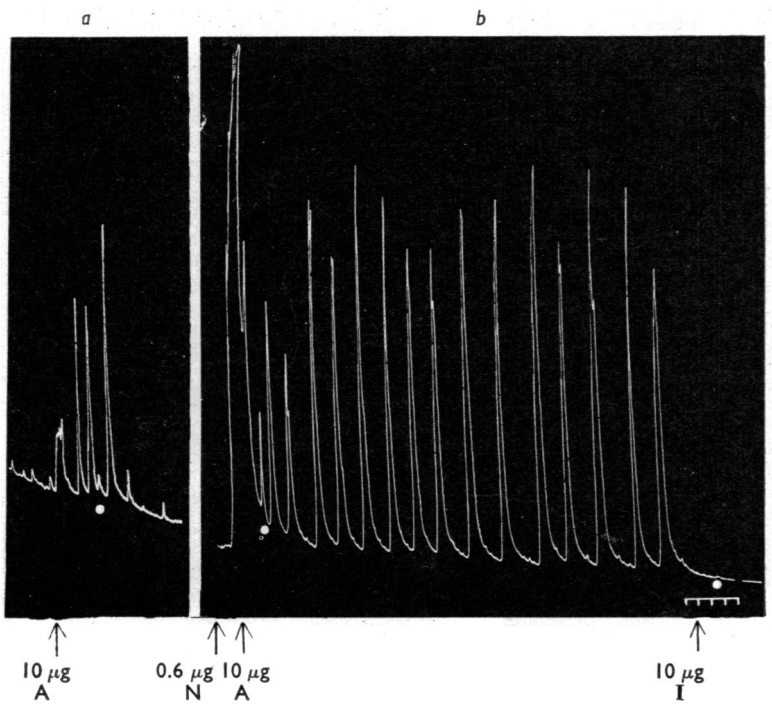


Fig. 3. Uterus from an oestrogen-treated guinea-pig. Rhythmic contraction initiated by adrenaline (A) and effects of noradrenaline (N) and isoprenaline (I). For description, see text. Time: 1 min.

Fig. 2 shows the responses to the same three amines of a uterus from a guinea-pig which had been treated with oestradiol. Both adrenaline and noradrenaline now caused a contraction, and isoprenaline had no visible effect because the preparation was at that time completely relaxed.

Adrenaline frequently initiated a rhythm of spontaneous activity in a uterus taken from a guinea-pig in oestrus. Fig. 3*a* shows a sequence of contractions produced by 10 $\mu$g adrenaline and an additional contraction on washing out. Fig. 3*b* shows a contraction produced by noradrenaline; and while this was still

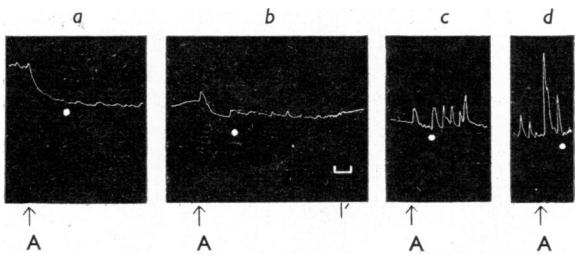


Fig. 4. Responses to 20 $\mu$g adrenaline (A) by the uterus from an immature guinea-pig. (*a*) 30 min, (*b*) 2 hr, (*c*) 3 hr and (*d*) 4 hr after the preparation was set up. Time: 1 min.

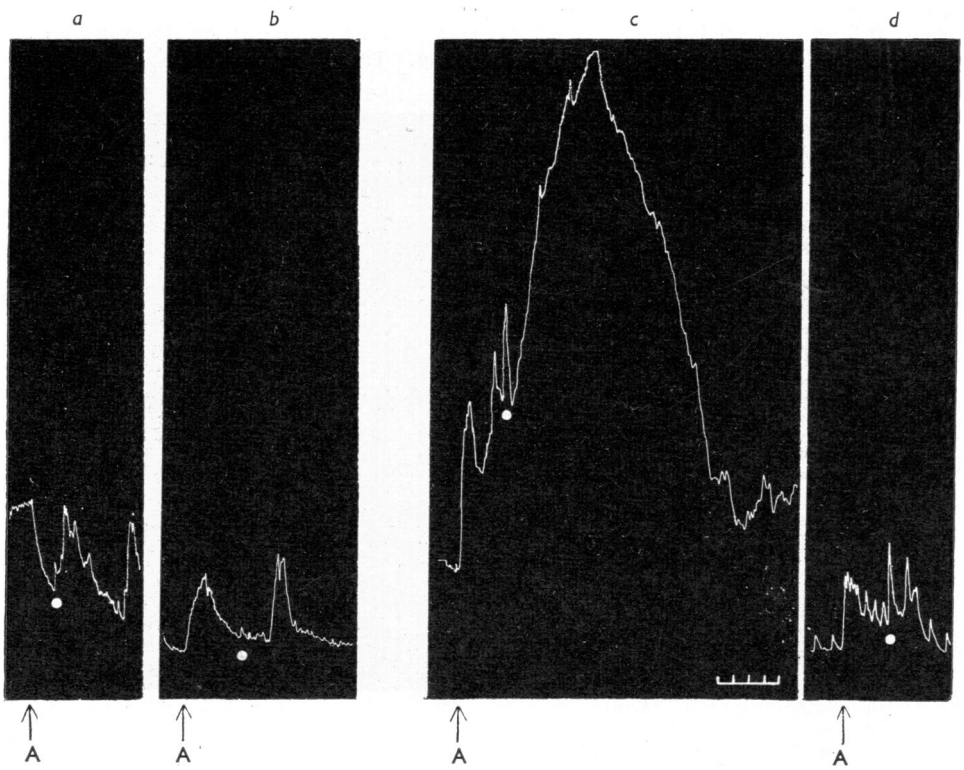


Fig. 5. Immature guinea-pig uterus. Response to 4 $\mu$g adrenaline (A) (*a*) 1 hr, (*b*) 4 hr after setting up the preparation on the first day; (*c*) 30 min and (*d*) 2½ hr after setting up the same preparation on the second day, having been kept at 4° C overnight. Time: 1 min.

present the muscle relaxed. Adrenaline interrupted the relaxation causing a small contraction after which the relaxation continued. When both amines were washed out a regular rhythm of contractions started and continued for several min until isoprenaline was applied which stopped all activity.

*Gradual change of the response to adrenaline in the course of an experiment.* In six out of eight experiments on uteri from immature, untreated guinea-pigs a gradual change in the action of adrenaline was observed. At the beginning the response to adrenaline was a relaxation as shown in Fig. 4*a*. After some time this changed to a biphasic effect, usually a contraction followed by a relaxation (Fig. 4*b*). The biphasic effect was not observed in every experiment and occasionally the sequence was reversed, that is, a relaxation was followed by contraction. Often, while the muscle still relaxed in the presence of adrenaline, a big contraction was seen as an after-effect on washing out the adrenaline. During the later stages of an experiment the preparation responded to adrenaline by a contraction (Fig. 4*c* and *d*) or a series of contractions, unless a high spontaneous tone had developed.

A more marked change in the effect of adrenaline was noticed when it was applied to a preparation which had been kept overnight at 4° C. The responses shown in Fig. 5*a* and *b* were obtained from an immature guinea-pig uterus at the beginning and at the end of the first day. In Fig. 5*a* the preparation had a spontaneous rhythm which was interrupted by a relaxation when adrenaline was applied. In Fig. 5*b*, which was recorded several hr later on the same day,

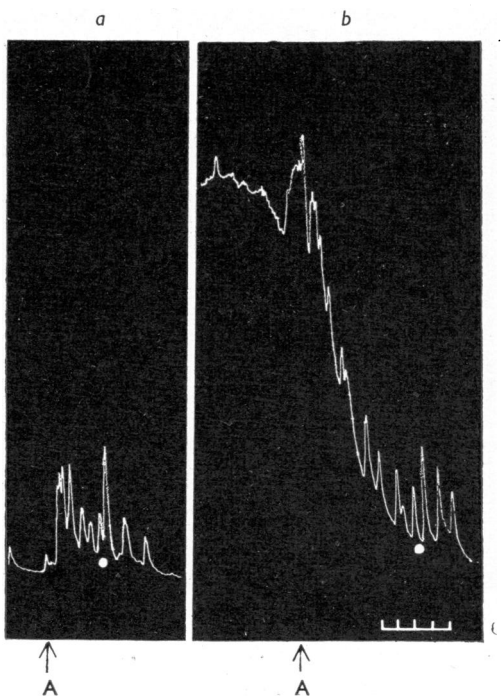


Fig. 6. Immature untreated guinea-pig uterus. 4 $\mu$g adrenaline (A) was applied when the tone was low in (*a*), when the tone was high in (*b*). Time: 1 min.

adrenaline caused a contraction. The records in Fig. 5*c* and *d* were obtained on the following day, after the preparation had been kept overnight at 4° C. Shortly after rewarming the uterus to 35° C it responded to adrenaline by a big contraction which was further increased when the adrenaline was washed out. Later in the same experiment adrenaline caused also a contraction, but this was smaller.

*Dependence of the response to adrenaline on the tone.* It was found that the effect of adrenaline depended on the tone which was present when it was applied. In Fig. 6 adrenaline was applied at first when the tone was low and it caused a contraction (*a*). Some time later when a high spontaneous tone had developed it caused a relaxation (*b*). Applied on this high tone adrenaline never caused a contraction, not even at the end of an experiment, unless isoprenaline had been given beforehand.

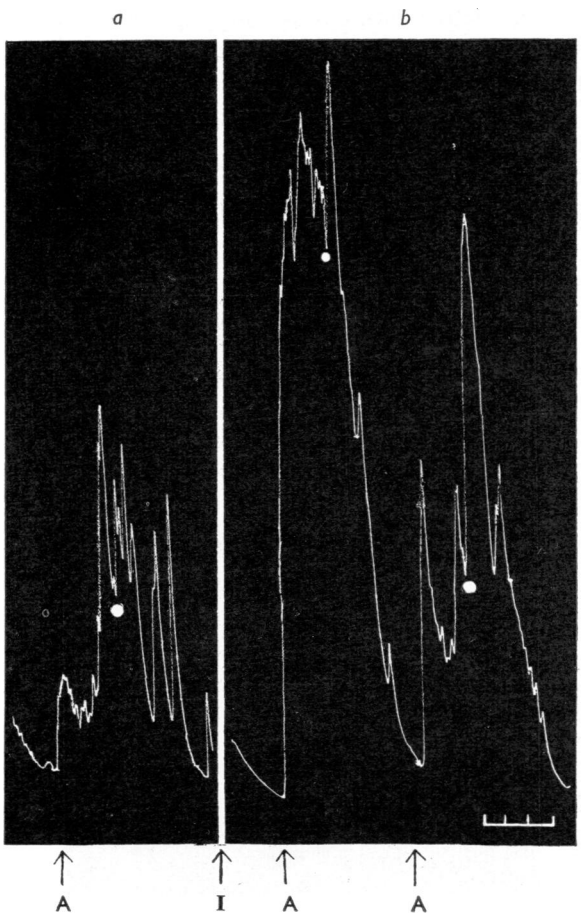


**Fig. 7. The potentiation of the adrenaline contraction by isoprenaline. Contraction produced by 20 μg adrenaline (A) at (*a*) before and at (*b*) 5 min after 4 μg isoprenaline (I) had been added to the bath, and 4 min after both had been removed (see text). Time: 1 min.**

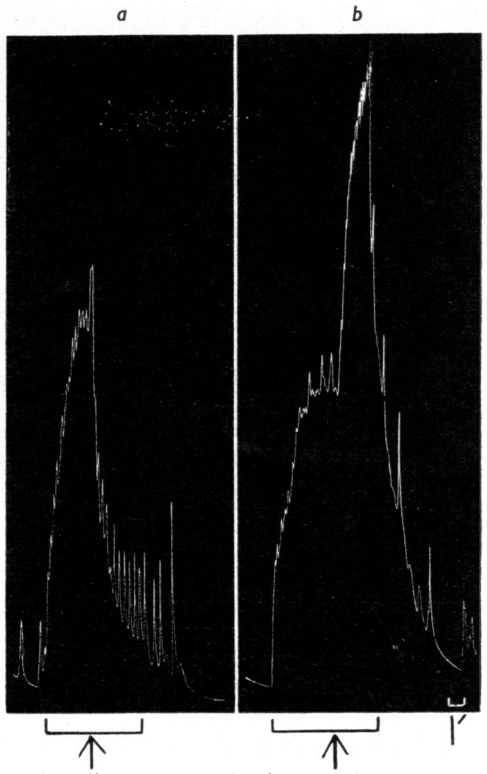


Fig. 8. Reversal of the adrenaline response by isoprenaline. Response of guinea-pig uterus to acetylcholine 5 $\mu$g, shown by the bars, and effect of adrenaline 20 $\mu$g (↑) on this contraction (*a*) and in the presence of isoprenaline (*b*). Time: 1 min.

*Changes of the adrenaline effect caused by isoprenaline.* Isoprenaline relaxed the uterus (Fig. 1). It also potentiated the contraction produced by adrenaline, and if adrenaline caused a relaxation this effect was reversed to a contraction when isoprenaline was present in the bath.

Fig. 7*a* shows a contraction caused by 20 $\mu$g adrenaline. Between 7*a* and *b* 4 $\mu$g isoprenaline was given. When adrenaline was applied 5 min later with isoprenaline still present the response to adrenaline was much greater. After washing out, the contraction initiated by adrenaline became smaller again.

The relaxing effect of adrenaline could be observed not only when the tone was spontaneously high, but also when it was produced by noradrenaline or by acetylcholine. In Fig. 8*a* adrenaline was given at the peak of a contraction caused by acetylcholine ; it caused relaxation. Between *a* and *b* isoprenaline was added to the bath. To obtain the same size of contraction as before it was necessary to increase the dose of acetylcholine 30 times. When adrenaline was now applied, on the same level of contraction as before, it caused a further contraction instead of a relaxation.

Adrenaline also caused relaxation when it was given at the peak of a contraction produced by noradrenaline. In Fig. 9 noradrenaline was twice allowed to act for 3 min. The first contraction was cut short by giving adrenaline, while the second

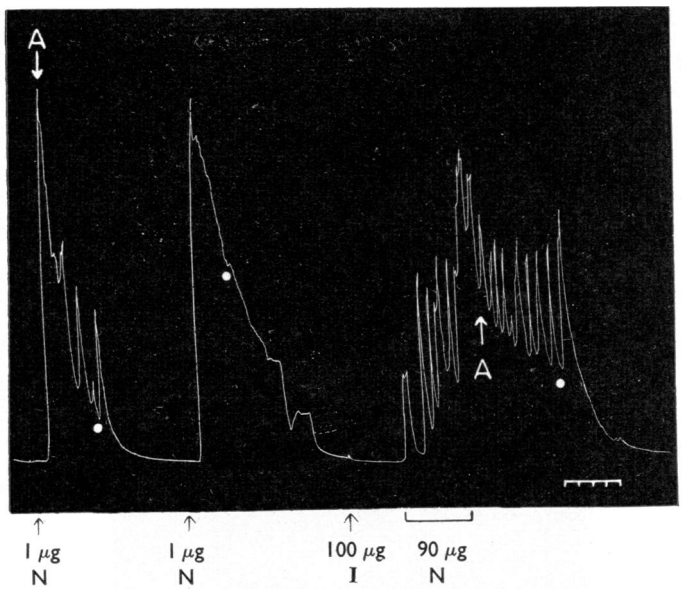


Fig. 9. The effect of isoprenaline on the relaxation caused by 10 μg adrenaline (A) when a tone was produced by noradrenaline (N). The first contraction was cut short by giving adrenaline at the peak. The second contraction was more maintained. In the presence of isoprenaline (I) adrenaline had no effect. For further description, see text. Time: 1 min.

contraction was more maintained. After the addition of isoprenaline the dose of adrenaline had to be increased 90 times and even then the contraction obtained was slower and smaller than before. When adrenaline was applied now it caused neither a contraction nor a relaxation.

One experiment was carried out using dichloroisoprenaline (1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol) in a concentration of $1 \times 10^{-6}$. It reversed a relaxation produced by 10 μg adrenaline to a large contraction.

### *$Na^+$ and $K^+$ content of guinea-pig uterus*

For the $Na^+$ and $K^+$ estimations the fresh weight was determined in one horn which served as a control and the wet weight in the other horn which was used for the experiment. In Table 1, therefore, the $Na^+$ and $K^+$ content is expressed in mM/kg fresh wt. for the control muscle, while the corresponding value after 8 hr application of the amines is expressed in mM/kg wet wt. The figures are

TABLE 1

$Na^+$ AND $K^+$ IN GUINEA-PIG UTERUS (mM PER KG FRESH WT. AND WET WT. RESPECTIVELY±S.D.)

| Condition | Time after killing | dry wt. / fresh wt. | dry wt. / wet wt. | $Na^+$ | $K^+$ |
|---|---|---|---|---|---|
| In anoestrus | 0 | 0·18±0·02 | | 71·5±12·0 | 80·2±6·6 |
| | 8 hr | | 0·18±0·01 | 70·8±7·0 | 77·8±12·4 |
| In oestrus | 0 | 0·16±0·02 | | 68·1±3·7 | 82·9±11·8 |
| | 8 hr | | 0·12±0·02 | 101·5±10·0 | 70·2±8·4 |

the mean of 8 determinations. The averages of the ratios $\frac{\text{dry wt.}}{\text{fresh wt.}}$ and $\frac{\text{dry wt.}}{\text{wet wt.}}$ were both 0.18 in the immature guinea-pigs in anoestrus. The table shows that after repeated applications of adrenaline, noradrenaline and isoprenaline in the course of an 8 hr experiment the $Na^+$ and $K^+$ content were the same as those in the initial control.

The same analysis was applied to the uteri from animals in oestrus and the results were entirely different. As seen from Table 1, the ratio $\frac{\text{dry wt.}}{\text{fresh wt.}}$ was 0.16 compared with 0.18 in the untreated animals. These results indicate that the water content was increased by the treatment with oestrogen although the difference was not significant. Futhermore the ratio $\frac{\text{dry wt.}}{\text{fresh wt.}}$ was reduced from 0.16 to 0.12 during the experiment indicating that the uteri had become oedematous. This difference was significant at the 5% level. Finally, although the $K^+$ content remained unchanged, the $Na^+$ content in oestrous uteri increased significantly ($P<0.001$) after repeated application of the amines. However, the uteri from the oestrogen-treated guinea-pigs were considerably heavier than those from untreated animals. Therefore the accumulation of $Na^+$ might be due to a larger degree of anoxia or possibly to $Na^+$-fixation in the connective tissue. On the other hand Goodford & Hermansen (1960) have shown that there was no accumulation of $Na^+$ in pieces of guinea-pig taenia coli kept in a poorly oxygenated organ bath compared with pieces in a well-oxygenated bath.

### *Glycogen determinations*

*Total glycogen content of uterine horns compared with that of the endometrium.* In these experiments all the animals were treated with oestradiol 1 $\mu$g/g body weight given intramuscularly. The glycogen content in whole horns was $110.3 \pm 9.5$ mg %, while in the endometrium it was only about 15 mg %. This is less than 15% of the amount in the whole horn and was considered to be insignifi-

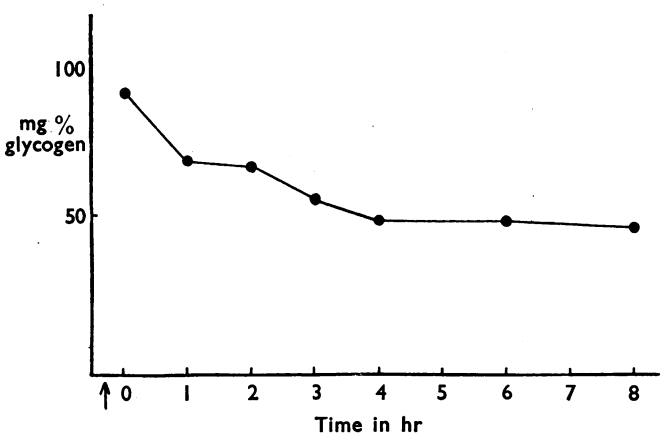


Fig. 10. Glycogen content (ordinates) of isolated guinea-pig uteri. The guinea-pigs were killed at a time marked by (↑) and the uteri set up in the organ bath at 0.

cant. Furthermore the loss of glycogen during the removal of the endometrium was an additional source of experimental error. Therefore all the estimations described were carried out on whole horns.

No comparison of the normal glycogen contents of the right and left horns was made. In order to avoid errors, if there existed an uneven distribution between the right and the left horn, they were taken alternately as controls. In order to avoid the effects of variation between animals, the experimental results were expressed relatively by comparing them with those of a control horn from the same guinea-pig.

*Changes in the glycogen content during an experiment.* In a few control experiments the amount of glycogen in uteri from seven untreated immature guinea-pigs kept for 1 to 8 hr in the usual bathing solution without any application of drugs was determined (see Fig. 10). The initial content immediately after killing the animal was about 90 mg %. During the first 4 hr in the isolated organ bath the glycogen content of the uteri gradually decreased to about 50 mg %. Between 4 and 8 hr *in vitro* there was apparently no further decline. The remarkable decrease of glycogen content occurred in the course of 8 hr in spite of the fact that no treatment was carried out and that external glucose was available in the normal concentration.

In a single experiment in which a preparation was exposed to glucose-free solution for 6 hr, the uterus was not completely depleted of glycogen, but 10% of the initial content was still present.

*Glycogenolytic effect of adrenaline at the beginning and the end of an experiment.* The finding that the glycogen content changed with the time in isolated conditions led to the investigation whether the glycogenolytic effect of adrenaline might also change during the course of an experiment. We therefore determined the change of the glycogen content produced by exposure to adrenaline $1 \times 10^{-6}$ for 5 min in pieces kept for 30 min and for 8 hr in the isolated organ bath (Table 2). The mechanical effect was not recorded. The average effect of

TABLE 2

THE INFLUENCE OF ADRENALINE ON THE GLYCOGEN CONTENT (EXPRESSED IN MG % GLUCOSE) IN GUINEA-PIG'S UTERUS

| 30 min in organ bath | | | 8 hr in organ bath | | |
|---|---|---|---|---|---|
| Control | Adrenaline | ±% of control | Control | Adrenaline | ±% of control |
| 69·2 | 60·6 | −12·5 | 71·4 | 76·8 | +7·6 |
| 56·5 | 60·2 | +6·5 | 63·4 | 67·5 | +6·5 |
| 55·9 | 63·2 | +13·1 | 74·4 | 73·8 | −0·8 |
| 77·5 | 74·0 | −4·5 | 80·1 | 61·2 | −23·6 |
| 60·7 | 53·8 | −11·4 | 73·2 | 71·1 | −2·9 |
| 75·3 | 72·7 | −3·5 | 67·9 | 58·5 | −13·9 |
| 75·4 | 71·7 | −4·9 | 72·2 | 69·4 | −3·9 |
| 66·3 | 59·5 | −10·2 | | | |
| Average 67·1 | 64·4 | −4·1 | 71·8 | 68·3 | −4·9 |

adrenaline was the same in both groups, that is, −4.1 and −4.9% respectively, but the action of adrenaline was not consistent. In both groups it caused an increase of the glycogen content in some and a decrease in other uteri. This confirmed the findings of Lundholm & Mohme-Lundholm (1956) that it was

impossible to show a significant change of the glycogen content after adrenaline, unless the uteri were separated into groups according to the mechanical reponse which adrenaline produced.

It must be pointed out that in Table 2 the controls removed after 8 hr had the same glycogen content as those removed after 30 min. This finding seems to disagree with the decline in glycogen content during an 8 hr experiment which has been described in the preceding section. However, the animals of the two groups in Table 2 were killed at different times of the day ; the 8 hr group at noon, the 30 min group between 3 and 7 p.m. Fluctuations in glycogen content may well occur during the day and the controls of the 2 groups are therefore not directly comparable.

## DISCUSSION

Recently Axelsson, Bueding & Bülbring (1959) showed that in intestinal smooth muscle the relaxing effect of adrenaline coincided with an increased phosphorylase activity and they interpreted the adrenaline relaxation as a result of an accelerated supply of metabolic energy for processes stabilizing the membrane. If the same mode of action is assumed in uterine smooth muscle it might be possible to explain some of the observations described in this paper.

Ellis, Davis & Anderson (1955) found that isoprenaline in the rat diaphragm had a 10 times stronger glycogenolytic effect than adrenaline. If the same ratio existed in the guinea-pig uterus the phosphorylase activity might be very high in the presence of isoprenaline and adrenaline might then fail to cause a further increase. Hence it would fail to produce its inhibitory effect and the motor effect would become predominant. This is what was observed.

It is interesting that the di-chloro analogue of isoprenaline dichloroisoprenaline which has been investigated by Powell & Slater (1958) did not reverse the adrenaline-induced relaxation in the rat uterus. It is probably due to a species difference because they also found in a single experiment that dichloroisoprenaline could reverse the response of the guinea-pig uterus to adrenaline.

The reversal of the adrenaline effect by isoprenaline is complicated by the fact that isoprenaline itself relaxes the uterus, and that the stimulant action of adrenaline is more likely to appear when the tone is low. It was possible, however, with acetylcholine to bring the tone of the organ to the same level whether isoprenaline was present or not; the reversal of the action of adrenaline by isoprenaline was then unequivocal. If noradrenaline was used to control the tone of the uterus, a different result was obtained ; in the presence of isoprenaline and sufficient noradrenaline to bring the tone to a standard level, adrenaline was now inactive. Under these conditions, the motor receptors were presumably saturated with noradrenaline, and the metabolic receptors with isoprenaline, so that adrenaline would have no scope for action.

On the basis of the hypothesis that relaxation is correlated with an accelerated glycogen breakdown and the finding that the amount of glycogen declined during the course of an experiment, it was tempting to speculate that there might be a causal connexion between the glycogen content and the change in the pharmacological action of adrenaline. However, no difference was found in the glycogenolytic effect of adrenaline between the beginning and the end of an experiment. From

these results it is not possible to deduce that the phosphorylase activity was also unchanged because glycogen estimations give only a very rough indication of this activity and further work is required to elucidate the change in the adrenaline effect.

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