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OXYTOCIC PROPERTIES OF BLOOD EXTRACTS AND THEIR PHYSIOLOGICAL SIGNIFICANCE.

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In a recent paper [Bell and Morris, 1934] experiments were described which suggested that the blood removed from the cow towards the end of pregnancy or during parturition contains considerable quantities of a substance capable of causing contraction of the isolated guinea-pig uterus. As data relating to the oxytocin content of body fluids are of great interest in a consideration of mechanisms concerned in the control of the uterine activity during gestation and parturition, it appeared desirable to obtain further information regarding the occurrence and nature of the substance described by Bell and Morris.

A series of experiments was therefore performed, repeating exactly the technique used by the previous authors, and the following additional experiments were carried out as a help in the elucidation of the problem:

(1) The blood of other species (the rabbit and the woman) was examined for its content of oxytocic substance; and

(2) The titration of all samples was performed not only on the uterus of the guinea-pig, but also on the uterus of the mouse. In the latter case uterine strips were removed usually from animals either during or shortly following parturition; though in several experiments when parturient mice were not available uteri from œstrous mice were used. The sensitivity of the uterus of the parturient mouse to oxytocin is high [Robson, 1934], while that of the œstrous uterus, though not so great, is also well marked. The uterus of the mouse has the advantage of being unaffected by comparatively large amounts of certain pharmacologically active substances (*e.g.* histamine, ergotoxine, choline derivatives), and is therefore particularly suitable for the qualitative investigation of oxytocic substances.

Technique.

Blood samples from cows and from women were in all cases obtained by venesection. We are greatly indebted to Mr A. D. Buchanan Smith, and to Dr J. B. Dewar of the Simpson Memorial Hospital, for their help in obtaining these samples. In the case of rabbits, samples of heart blood were removed under ether anæsthesia; in one case (see text) a sample from the ear vein was used.

The preparation of blood extracts for assay was consistently performed by the method described by Bell and Morris [1934]. This involved precipitation of the plasma proteins by hydrochloric acid and subsequent boiling.

Virgin dicestrous uteri of guinea-pigs were suspended in Burn-Dale solution through which a mixture of oxygen with 5 p.c. CO_2 was bubbled. The uterine strips from mice were suspended in Ringer-Locke solution through which oxygen was bubbled. Temperature and rate of bubbling were maintained constant. The capacity of the containers was 100 c.c. Preparations of the posterior pituitary lobe "Pituitrin" and "Pitocin", kindly supplied by Dr White of Parke, Davis and Co., were used.

Results.

Twenty-one samples were removed from pregnant or parturient cows and one sample from a non-pregnant cow, and the content of oxytocic substance of the extract determined on the uterus of guinea-pigs and mice. The results are given in Table I. An examination of these data shows:

(1) That the content of oxytocic substance of the blood from pregnant and parturient cows, as determined on the guinea-pig uterus, is in the great majority of cases low. For out of twelve samples taken in the last twelve days of pregnancy only three showed detectable amounts of oxytocic substance which assayed respectively at values corresponding to 4, 4, and 1 oxytocic units per litre of the original blood.

(2) That the assay performed on the mouse uterus gave a higher proportion of positive results and higher values for the content of oxytocic substance. Thus the samples which assayed at 4, 4, and 1 units per litre on the guinea-pig uterus gave values of 20, 8, and 10 units per litre respectively when tested on the mouse. Moreover, several samples in which no detectable amounts of oxytocic substance were present according to the guinea-pig test gave values up to 20 units per litre when assayed on the uterus of the mouse.

TABLE I. Oxytocic content of cow blood samples (expressed as oxytocic units per litre) measured on the mouse and guinea-pig uteri. The value obtained on the mouse uterus is in all cases given first.

Cow No.	Non-pregnant	Days before parturition: 54	47	12	11	10	8	7
1	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	10; —	—	10; 1
4	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
6	—	—	—	20; 4	<2; <2	—	—	<3; <1
7	—	—	—	—	—	—	—	—
8	<2; <2	—	—	—	—	—	—	—
9	—	—	—	—	—	—	1; <2	—
10	—	—	<6; <2	—	—	—	—	—
11	—	<6; <5	—	—	—	—	—	—

Cow No.	Days before parturition: 6	5	4	3	2	1	0	1
1	—	—	20; —	—	—	—	—	<3; —
2	<3; —	—	—	—	<5; —	—	<5; —	—
3	—	—	—	—	—	—	—	—
4	<5; <1·5	—	—	—	—	—	—	—
5	—	<1·5; <1	—	—	—	—	—	—
6	<6; <1·5	—	—	2; <2	—	—	—	—
7	—	5; <3	—	—	—	—	20; <3	—
8	—	—	—	—	—	—	—	—
9	8; 4	—	—	<10; <3	—	—	—	—
10	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—

(3) That three samples obtained, two from animals in the early stages of gestation and one from a non-pregnant cow, gave negative results both on the mouse and guinea-pig.

Six samples were obtained from women in the second stage of labour with strong pains. The results of assay of oxytocic substance on the guinea-pig and mouse are given in Table II. It will be seen that the

TABLE II. Oxytocic content of human blood samples taken during the second stage of labour (expressed as oxytocic units per litre) measured on the mouse and guinea-pig uteri.

Case	1	2	3	4	5	6
Guinea-pig	5	<2	<7	<2	<2	<2
Mouse	20	<6	2	<5	10	<5

guinea-pig tests gave negative results in all but one sample which assayed at 5 oxytocic units per litre. On the mouse uterus three of the samples were positive, giving values of 20, 10, and 2 oxytocic units per litre. It will again be noted that in the one case giving a positive value in the guinea-pig, a much higher value was obtained when the test was performed on the mouse.

Samples were obtained from eight rabbits and gave the following results:

(1) Three non-pregnant animals (RO 1, RO 5, C 25) showed no detectable amounts of oxytocic substance on the guinea-pig (<3, $<2{\cdot}5$, <5 units respectively). The extract from C 25 tested on the mouse gave a value equivalent to more than 10 O.U. per litre; the sample from C 25 was the only blood sample taken from the vein, all the other rabbit samples were taken from the heart. Another non-pregnant rabbit (C 19) gave less than 6 O.U. per litre when tested on the mouse.

(2) Four animals received injections of posterior lobe extracts, namely:

RO 2 received 0·5 c.c. of pituitrin intramuscularly, and one hour later the blood was taken and assayed at 3 O.U. per litre when tested on the guinea-pig.

RO 3 treated exactly in the same way assayed 8 O.U. per litre on the guinea-pig.

RO 6 received 1 c.c. of pitocin intramuscularly, and one hour later the blood assayed 40 O.U. per litre on the mouse and 10 O.U. per litre on the guinea-pig.

C 25 was injected intramuscularly with 1 c.c. of pitocin immediately after the taking of the sample already referred to, and one hour later a further sample showed no detectable amounts of oxytocic substance (<5 O.U. per litre) when tested both on the mouse and guinea-pig uterus.

(3) Rabbit RA 463 was hypophysectomized on the 25th day of pregnancy and aborted 33 hours later. The blood was taken immediately after abortion and assayed more than 20 O.U. on the mouse and less than 10 O.U. on the guinea-pig.

In their experiments Bell and Morris [1934] found that the oxytocic substance present in the blood during the last stages of pregnancy and parturition was unstable and that an active extract soon lost its potency when kept in the ice chest. A similar fate did not overtake oxytocin added to blood *in vitro*. It was therefore thought advisable to determine, by tests on the uterus of both the guinea-pig and mouse, whether the active extracts obtained in the present experiments had been similarly affected by storage. The results are given in Table III. It will be seen that no extract decreased in oxytocic activity when tested on the uterus of the mouse; but when tested on that of the guinea-pig there is definite evidence that the amount of oxytocic substance that can be demonstrated in a given extract decreases on standing.

TABLE III. Showing effect of storing in ice chest on content of oxytocic substance as tested on the mouse and the guinea-pig uteri. Values expressed as oxytocic units per litre.

	First day		Second day		Third day		Fourth day	
Animal	Mouse	Guinea-pig	Mouse	Guinea-pig	Mouse	Guinea-pig	Mouse	Guinea-pig
Rabbit RO 2	—	3	—	—	—	—	—	<2·4
Rabbit RO 3	—	8	—	—	—	<2·4	—	—
Rabbit RO 6	40	10	>20	8	>20	10	>20	<5
Rabbit RA 463	>20	—	7·5	—	15	—	—	—
Cow 6	20	5	10	<4	—	—	—	—
Woman 1	20	5	20	10	>20	5	—	—

A number of causes might account for such results but, on the supposition that the same active substance was responsible for causing the contraction of both the mouse and guinea-pig uteri, it seemed likely that the disappearance of activity when tested on the uterus of the guinea-pig might be due to the development in the extract of a factor which inhibited the response of the guinea-pig uterus to oxytocic substances, but exerted no such effect (or a much less effect) on the response of the mouse uterus. Experimental investigation of this hypothesis provided very adequate evidence for the existence of an inhibitory substance of this nature.

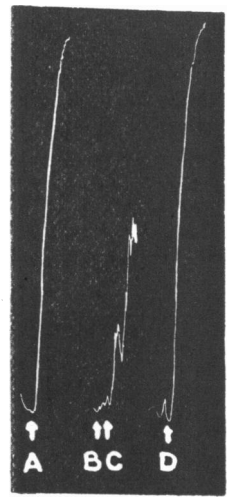

Fig. 1. Showing inhibition of the response of the guinea-pig uterus to oxytocin by a freshly prepared extract of cow blood. A, 0·006 unit of pituitrin added to bath; B, 1·0 c.c. of cow 8 blood extract added to bath; C, 0·006 unit of pituitrin added to bath; D, 0·006 unit of pituitrin added to bath. Solution changed after each addition of pituitrin.

It was found that an extract, even immediately after its preparation, may in some experiments inhibit to a certain extent the reaction of the guinea-pig uterus to a given dose of oxytocin. This is illustrated in Fig. 1, which shows the inhibition of the response to a dose of oxytocin by 1 c.c. of a freshly prepared extract added to the bath just previously. Such an extract, however, exerts no inhibitory action on the response of the uterus of the mouse to oxytocin.

When an extract is allowed to stand in the ice chest the amount of inhibitory substance in it appears to increase, so that the addition of 1 c.c. of it to the bath may completely abolish the response of the guinea-pig uterus to a supra-minimal dose of oxytocin and even 0·5 c.c. may have a marked inhibitory effect. This is illustrated in Fig. 2. Moreover, such an extract may exert

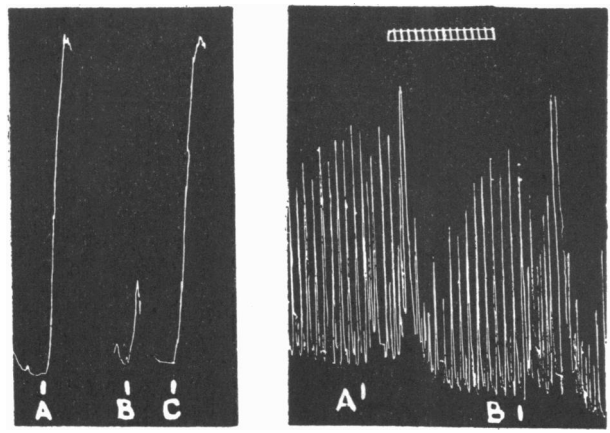

Fig. 2. Showing inhibition of the reaction of the guinea-pig uterus to oxytocin by small dose of stored blood extract and absence of inhibition in the mouse with a larger dose of the same extract. First tracing, guinea-pig uterus: A, 0·007 unit of pituitrin added to bath; B, 0·5 c.c. of cow 10 blood extract (stored for five days in ice chest) plus 0·01 unit of pitocin added to bath; C, 0·007 unit of pituitrin added to bath. Second tracing, uterus of parturient mouse: A, 0·02 unit of pituitrin added to bath; B, 1·0 c.c. of cow blood extract (as above) plus 0·02 unit of pituitrin added to bath. Solution changed after each addition of pituitrin. Time interval = 1 min. for both tracings.

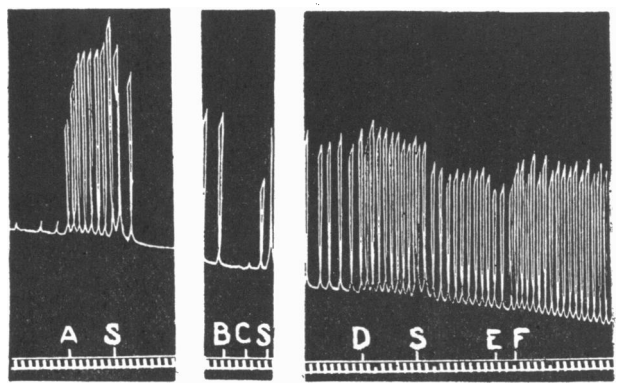

Fig. 3. Showing inhibition of the reaction of the parturient mouse uterus to oxytocin by large doses of stored blood extract. A, 0·01 unit pituitrin added to bath; B, 2 c.c. of cow 8 blood extract (stored for three days) added to bath; C, 0·01 unit pituitrin added to bath; D, 0·01 unit pituitrin added to bath; E, 1 c.c. cow blood extract as above added to bath; F, 0·01 unit of pituitrin added to bath. *S*, solution changed. Time interval = 1 min.

some inhibitory effect on the response of the mouse uterus too, although here the degree of inhibition is less marked. This is illustrated in Fig. 3.

Discussion.

The results of these experiments show definitely that an oxytocic substance capable of causing contractions of isolated uteri of the guinea-pig and of the mouse can occasionally be demonstrated in extracts of blood when the investigation is performed under the given conditions, and two main questions then arise, namely:

(1) Whether the appearance of this substance bears any time relation to the state of the animal, with special reference to gestation and parturition; and

(2) What the nature of the oxytocic substance is, and especially whether it is identical with the oxytocic substance of the posterior lobe.

An examination of the experimental data does not support the view that the occurrence of the oxytocic substance in question is a factor related to the initiation of parturition, though the results bear some superficial resemblance to those recorded by Bell and Morris [1934]. Considering first the evidence offered by the action of extracts of cow's blood on the guinea-pig's uterus, it will be seen that the sample obtained actually during parturition gave no positive effect; and further the only two samples that gave comparatively high values, namely 4 o.u. per litre given by cow 6 on the 12th day *ante partum* and cow 9 (6 days *ante partum*), gave negative results at subsequent stages of pregnancy. In the case of cow 6 indeed these negative results were obtained no less than four times on the 11th, 7th, 6th and 3rd days *ante partum*. Positive effects were obtained more frequently when the extracts were tested on the mouse uterus, but, nevertheless, the data do not support the view that these positive effects occur only with samples withdrawn at specific times. It is to be noted that three samples withdrawn from two cows not in the latest stages of gestation and from one non-pregnant cow gave negative results both on the mouse and guinea-pig uteri, but these data are not sufficient to warrant the conclusion that the oxytocic substance, when present, only occurs during the latest stages of pregnancy.

The evidence obtained from the examination of the human material on the whole supports the results obtained from the cow, for only one out of the six specimens obtained from patients in the second stage of labour gave any effect on the guinea-pig's uterus, while three out of six gave an action on the uterus of the mouse.

The results obtained on the rabbit show that no oxytocic substance

that is active when tested on the guinea-pig uterus is present in non-pregnant animals, but that such samples may occasionally yield a positive effect when tested on the mouse uterus (animal C 25).

The present series of experiments fully bears out the finding of Bell and Morris that active extracts when kept in the ice chest for some time fail to elicit contractions of the guinea-pig uterus, but in view of the additional evidence brought forward a new interpretation of these results appears necessary.

The facts to be considered are:

(1) That the oxytocic effect on the mouse uterus is not lost on standing; and

(2) That extracts which have stood for some time in the ice chest contain a factor which inhibits the response of the guinea-pig's uterus to oxytocin, and in larger quantities that of the uterus of the mouse.

The most likely conclusion appears to be that the observed loss of potency on standing is not due to the destruction or inactivation of the oxytocic substance but to the effect of the inhibitory factor which has developed.

Furthermore a careful examination of the experimental details offers an explanation for the previous finding of Bell and Morris that pituitrin added to blood does not become inactivated on standing. For in their experiments the quantity of pituitrin added (0·02 unit per c.c.) was so adjusted that the actual assay involved the addition to the bath of considerably less than 1 c.c. of extract. Now in the present experiment it has been found that a definite inhibitory effect on the guinea-pig's uterus can only be demonstrated with about 0·5 to 1 c.c. of extract while 2 c.c. or more are necessary for the mouse. Hence under the experimental conditions described by Bell and Morris no inhibitory action would come into play, and the activity on standing would therefore remain unchanged. On the other hand, in the case of the oxytocic substance in the blood the quantities are smaller, the amount of extract added to the bath in order to elicit a contraction larger, and the effect of the inhibitory substance becomes evident.

In so far as the nature of the oxytocic substance is concerned a number of possibilities can be eliminated. The properties of the mouse preparation exclude histamine; the substance is not a choline derivative as its effect persists after atropinization. A consideration of the quantities involved makes it highly unlikely that calcium or potassium is responsible. Control experiments with Ringer-Locke solution treated in exactly the same way as the plasma have eliminated osmotic phenomena. In

view of the marked differences in the titration values on the guinea-pig and mouse uteri it can be concluded that if one substance is responsible for both effects it cannot be identical with the oxytocin of the posterior pituitary lobe. Such a conclusion is supported by an examination of the contraction curve of the mouse uterus; this curve (Fig. 4) rises more steeply and falls more rapidly than that given by oxytocin.

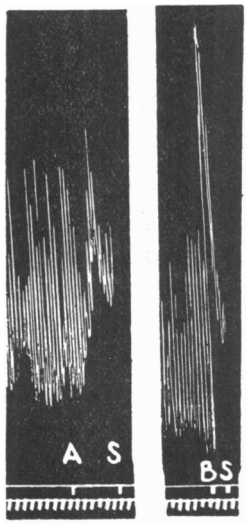

Fig. 4 *a*.

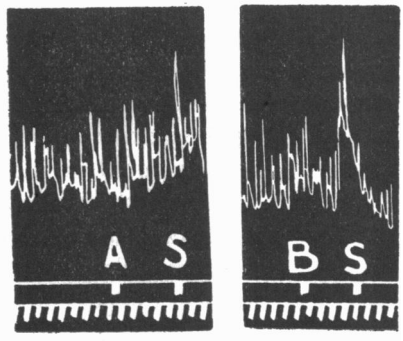

Fig. 4 *b*.

Fig. 4. Illustrating the difference in the character of the response of the mouse uterus to oxytocin and to an active blood extract. (*a*) A, 0·01 unit pituitrin added to bath; B, 2 c.c. cow 6 blood extract added to bath. (*b*) A, 0·02 unit of pituitrin added to bath; B, 1 c.c. cow 7 blood extract added to bath. *S*, solution changed. Time interval = 1 min.

Clark found [1924] that certain decomposition products of proteose cause contraction of the uterus in both the rat and the guinea-pig and that the titration values on the rat are considerably larger than those on the guinea-pig. This is of great interest, more especially as the active extracts used by Clark could be obtained by means of acid treatment. It appears possible that the substance dealt with in the present investigation is of a similar nature, its production being dependent on the acid treatment of the plasma.

Whatever the nature of the oxytocic substance, the possibility that the extract does contain small amounts of oxytocin cannot be excluded. It is indeed of interest to consider what quantities of oxytocin could be expected in a blood extract, arguing from *a priori* considerations. The

well-known clinical observation that intramuscular injection of 5 units or less of oxytocin causes contraction of the parturient uterus finds confirmation in the experiments of Moir [1934] on the early puerperal uterus. These data allow of the conclusion that a concentration considerably less than 1 unit per litre of the circulating blood causes a marked contraction of the human uterus. A similar conclusion follows from a consideration of experiments with uterine strips *in vitro* which, in the case of man, rabbit and mouse show minimum reactions to doses of oxytocin of 0·01 to 0·1 unit per litre when the strips are removed from parturient subjects [Robson, 1933*a* and *b*, 1934]. Moreover Schübel and Gehlen [1933], working on the early puerperal uterus of the cat and recording the contraction *in vivo*, showed that motor effects could be elicited with concentrations of oxytocin of less than 0·01 unit per litre of the circulating blood. All these considerations support the conclusion that if an oxytocic substance is involved in the process of parturition its concentration in the blood might not be more than a few tenths of a unit per litre and is highly unlikely to be more than one unit per litre. The demonstrations of such amounts would involve either the use of a reactor even more sensitive than the guinea-pig uterus, or some method of concentrating the active substance without incidentally producing oxytocic agents not previously present as such in the blood, and at the same time of eliminating the possible action of inhibitory factors.

Summary.

1. The oxytocic activity of extracts prepared from the blood of (*a*) pregnant cows, (*b*) women in the second stage of labour, (*c*) rabbits before and after injection of posterior pituitary extracts, was tested simultaneously on the isolated uterus of the guinea-pig and of the mouse.

2. A number of these was found to possess oxytocic properties; this does not appear to be associated with any particular phase of gestation. The uterus of the mouse was consistently found to be more sensitive to the active substance than that of the guinea-pig.

3. A factor capable of inhibiting the reaction of the uterus to oxytocin *in vitro*, more especially the uterus of the guinea-pig, has been demonstrated in blood extracts. The inhibitory power of these extracts appears to increase on standing.

4. The nature of the oxytocic substance and the significance of its occurrence are discussed.

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