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THE EFFECT OF HORMONES ON THE PHYSICAL PROPERTIES AND COLLAGEN CONTENT OF THE RAT'S UTERINE CERVIX

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In a previous paper (Harkness & Harkness, 1959*b*) changes in the physical properties of the rat's cervix in pregnancy were described. These consisted first of a reorganization of the tissues of the walls of the cervical canals to give a larger natural circumference to the connective tissue framework, and secondly of a change in the properties of this framework which made it behave, not as normally like a continuous network, but as one connected by viscous links which slip slowly under comparatively low tensions.

It seemed of interest to determine whether these changes could be produced by hormonal treatment; and the effects of oestradiol, progesterone and relaxin individually, and in combination, on the cervix in spayed rats have now been investigated. It has been found that an increase in the circumference and weight of the cervix up to about the values found on the 17–18th day of pregnancy, together with a decrease in concentration of collagen similar to that found in pregnancy, can be produced by relaxin in combination with oestradiol and progesterone. Some increase in extensibility under prolonged loading is also produced. A preliminary account of this work has already been published (Cullen & Harkness, 1958).

METHODS

Animals. The rats used were albinos of the local strain, weighing 150–180 g and about 3 months old at the time of spaying. Ovaries were removed aseptically by the dorsal route under ether anaesthesia, and administration of hormones began about 3 weeks later.

Treatment. The following substances, all given subcutaneously, were used:

(*a*) Oestradiol cyclopentyl-propionate (Depo-Estradiol; Upjohn) in arachis oil.

(*b*) Progesterone, either dissolved in ethyl oleate (Progestin; Organon Laboratories) or progesterone B.P. in olive oil, any which would not dissolve being suspended by homogenization in a Potter–Elvehjem homogenizer.

(*c*) Relaxin powder in 5% (w/w) beeswax in peanut oil (Kroc, Steinetz & Beach, 1959). The preparation was 'Releasin' (Warner–Chilcott): (*A*) Batch no. W 1164 A, lot 53; (*B*) Batch W 1164 A, lot 66; (*C*) Batch W 1164 A, lot 43.

All these preparations have been assayed on the mouse and some on the guinea-pig against Warner-Chilcott Relaxin Reference Standard W1164A lot 8 (*Std*), of activity 150 guinea-pig units/mg. The figures with 95 % confidence limits (provided by Dr B. G. Steinetz) are given in Table 1.

TABLE 1

	Mouse assay	Guinea-pig assay
(*A*) Lot 53	0·2 mg *Std*/mg (79–127 %)	—
(*B*) Lot 66	0·99 mg *Std*/mg (72–140 %)	1·06 mg *Std*/mg (76–131 %)
	1·06 mg *Std*/mg (77–130 %)	—
(*C*) Lot 43	0·11 mg *Std*/mg (51–195 %)	0·21 mg *Std*/mg (64–157 %)
	0·12 mg *Std*/mg (71–141 %)	0·26 mg *Std*/mg (68–147 %)
	0·13 mg *Std*/mg (81–124 %)	—

We have recorded dosage in weight of the reference standard by mouse assay, e.g. for (*A*) 1 mg = 0·2 mg of reference standard. Thus, a dose of 1 mg on this scale is equivalent to 150 guinea-pig units.

Mechanical tests on cervix. The effect of tension on the walls of the cervix was examined by the same method as that used previously on rats at different times of pregnancy (Harkness & Harkness, 1959*b*), i.e. the excised cervix was suspended in oxygenated Ringer–Locke solution and stretched between two parallel steel rods 0·6 mm in diameter, one through each canal. Some experiments were done at 22° C, some at 37° C. Two types of test were made. *a*. The force pulling the rods apart was increased stepwise by 25 g every 15 sec until the tissues ruptured (22° C).

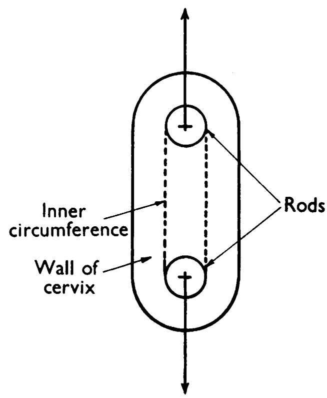

Fig. 1. Diagram of transverse section of cervix under tension. The bar of tissue which separates the two cervical canals and runs between the rods is not represented in the figure.

b. The force pulling the rods apart was kept constant for a time and subsequently increased as in (*a*) until the tissues ruptured (37° C).

As before (Harkness & Harkness, 1959*b*) the measurement used to describe the effect of tension is the distance round and between the rods (Fig. 1) and is referred to as the 'inner circumference'. For calculations of tensions per unit cross-sectional area the tissue was regarded as if it were a straight piece of this length and constant cross-sectional area, with density of 1 for whole tissue and 1·4 for collagen. The use of this length is not precise, as the mean circumference may be as much as 50 % higher than the inner circumference and there is a corresponding over-estimate of the cross-sectional area. The errors, are, however, generally similar in the different groups, and their effect is only to produce small alterations in the absolute magnitude of the differences between groups. For calculation of breaking stress, the mean was taken between the load which broke the tissue and the previous one which just did not. Tests of the first sort (*a*) give length–tension curves of which a typical example is shown in Fig. 2. The circumference increased rapidly at first but more slowly as the load was increased. Then over a wide range of load the curve became approximately a straight line until a little before the point of rupture when the slope usually increased again. When the load was taken off between each addition, there was immediate diminution in circumference, increasing with load and amounting to 10–15 % just before break. This effect was much the same in most groups as in normal non-pregnant animals (Harkness & Harkness, 1959*b*) and will not be discussed. Only the following parameters of the curves were measured: first the circumference (l_z) of the cervix at zero load, obtained by extrapolation back from the straight part of the curve; secondly, the slope (k) of the linear part of the curve of length against tension; thirdly, the conditions at breaking point.

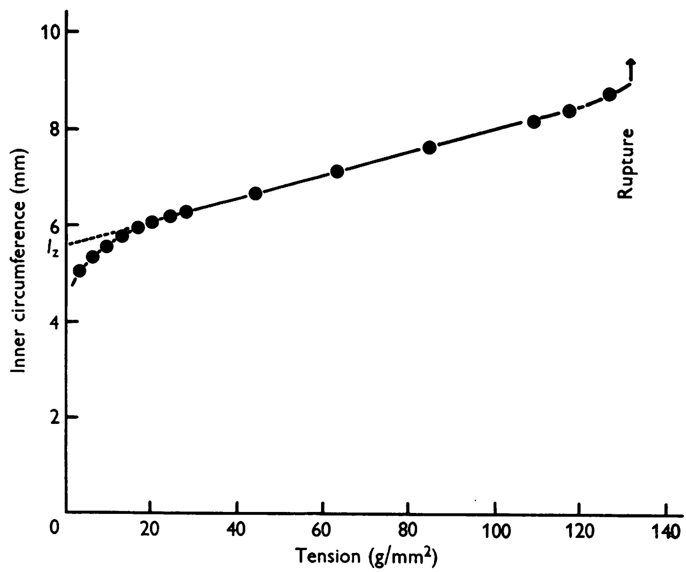

Fig. 2. Example of the effect of tension on inner circumference of cervix.

It is clear that in tissues of the same material and shape the constant k is related to size of the sample, i.e. to l_z, and in order to remove this effect we have recorded k/l_z rather than k itself. It is also clear that other things being the same, the value of k is related inversely to the amount of material present which resists stress. Even if one does not know the nature of this material, a measure of it may be obtained from the breaking stress. For measurement of k, therefore, tension has been expressed in units of breaking tension (= 1). The value of k/l_z in these units is the fractional change in length between zero and breaking tension.

The results of these tests have generally shown variations of k/l_z between different treatment groups which are small and inconsistent compared to differences in l_z. The main use of these tests is therefore to give a measure of the size (circumference, l_z) of the connective-tissue framework of the cervix after different treatments. The comparison between these is hardly affected if the recording is still further simplified by taking only the single measurement of circumference at half breaking tension, as was done in the preliminary communication (Cullen & Harkness, 1958).

Smooth muscle does not appear to play any important part in these tests (Harkness & Harkness, 1959*b*).

In tests of the second sort (*b*), when a constant load is applied for a long time, it has previously been found (Harkness & Harkness, 1959*b*) that the cervix on the last day of pregnancy, after an initial rapid extension, shows a slower prolonged increase in length at constant rate. This rate was measured by the ratio of the slope (K) of the linear part of the curve to the length at zero time (l_0) obtained by extrapolation (Fig. 3) and the same procedure is used here. K is measured in mm/min. This type of test gives information on the properties of a component of the tissues which behaves as a series viscous element. It is probable that this is an interfibrillary cementing material, and the prolonged extension takes place by the fibrils slipping past each other (Harkness & Harkness, 1959*a*). The rate of this slow extension increases with temperature and the experiments were therefore performed at 37° C to make the measurement easier.

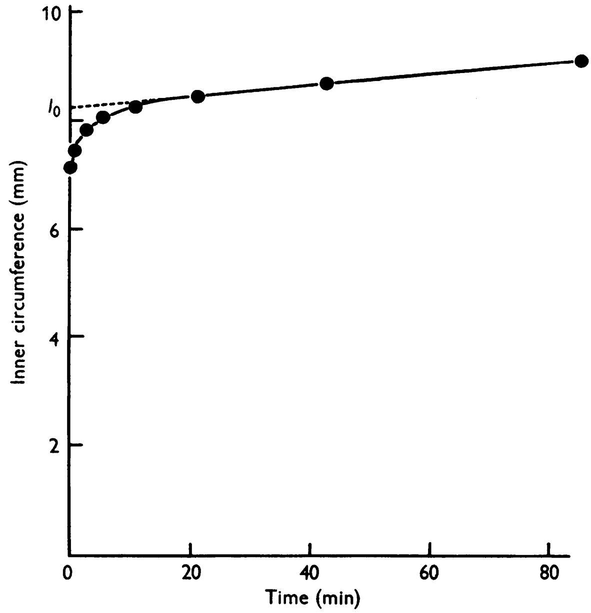

Fig. 3. Example of the effect of prolonged loading on cervix. The cervix, from a rat previously treated with oestradiol, progesterone and relaxin (doses, Table 3), was loaded with 100 g weight.

Chemical methods. Hydroxyproline was estimated by the method of Neuman & Logan (1950) on acid hydrolysates (5 ml. 6N-HCl, 4 hr, 40 lb./sq.in. or 2·8 kg/cm^2 in autoclave) of the whole tissue removed from the apparatus at the end of the experiment. The collagen content of the tissue was obtained by multiplying the hydroxyproline value by 7·46. A number of blanks were done, in which hydrogen peroxide was omitted, to test for the presence in these crude hydrolysates of chromogens, other than hydroxyproline, which might react with the *p*-dimethylamino-benzaldehyde in the last stage of the estimation. No appreciable amounts were found.

RESULTS

We began by investigating the time course of change in the cervix after administration of oestradiol and progesterone individually and of relaxin in combination with oestradiol. Relaxin was not used alone at this stage because it is known to have little effect by itself on the symphysis pubis. In these experiments only tests of the first sort (*a*), in which load was increased progressively, were used. On the basis of these experiments others using the same type of test were done with combinations of hormones given for a standard time. On the results of these experiments others were done, again with combinations of hormones given for a standard time, but the cervix was tested by the second method (*b*), with a single load applied for a long time.

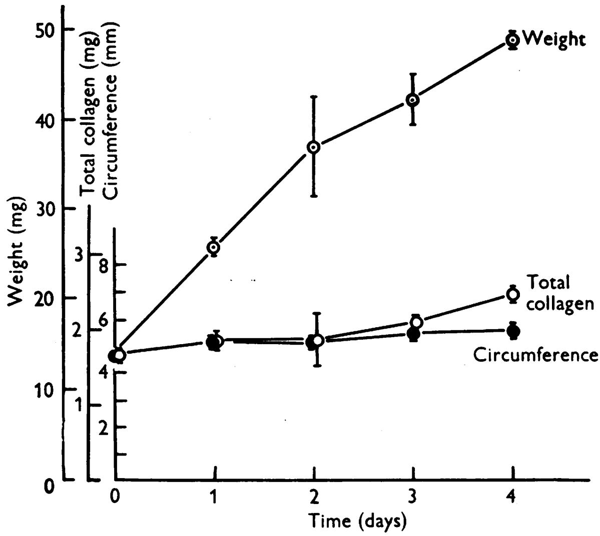

Fig. 4. Effect of oestradiol on weight, total collagen content and inner circumference of cervix. 5 μg of oestradiol cyclopentyl-propionate was given on day 0. The circumference is that at half breaking tension. The length of the vertical line through each point is twice the standard error of the mean.

Time course of change under treatment

Oestradiol. A single dose of oestradiol (5 μg) produced a rapid increase in weight of tissue and, like oestrone (Harkness, Harkness & Moralee, 1957), a slow increase in the total collagen content of the cervix (Table 2). There was a rise in k/l_z and in the circumference at breaking point. In later experiments the main effect of oestradiol alone was on l_z. The principal point of interest shown by these preliminary experiments was that the total effect on circumference was small, being more like that on collagen content than on weight. The relations between the changes in weight and collagen content, and the results of the mechanical tests summarized by taking circumference at half the breaking tension, are shown in Fig. 4. Breaking stress per unit cross-sectional area of tissue decreased to about half, but this could be accounted for simply by the fall in concentration of collagen resulting from its slow growth compared with the increase in

TABLE 2. Effect of oestradiol and progesterone on cervix of spayed rat

Treatment	Body wt. (g)	Wt. of cervix (mg)	Total collagen (mg)	Concn. collagen (g/100 g)	l_z (mm)	k/l_z	Circumference at break (mm)	Stress at break (g/mm²)	Stress at break (kg/mm² coll.)
Oestradiol (5 μg)									
Untreated	228 ± 5	13·9 ± 0·8	1·67 ± 0·09	12·0 ± 0·5	4·02 ± 0·16	0·36 ± 0·02	6·07 ± 0·21	91 ± 3	1·07 ± 0·03
1 day	203 ± 12	26·0 ± 1·0	1·85 ± 0·14	7·1 ± 0·6	3·96 ± 0·09	0·52 ± 0·04	6·50 ± 0·17	73 ± 9	1·44 ± 0·13
2 days	211 ± 6	37·2 ± 5·5	1·87 ± 0·37	6·7 ± 0·7	3·87 ± 0·10	0·63 ± 0·09	6·63 ± 0·48	54 ± 5	1·55 ± 0·24
3 days	205 ± 13	42·3 ± 3·0	2·11 ± 0·08	6·8 ± 0·6	4·26 ± 0·14	0·46 ± 0·12	7·25 ± 0·67	52 ± 3	2·14 ± 0·07
4 days	210 ± 2	47·6 ± 0·8	2·48 ± 0·11	7·0 ± 0·3	4·22 ± 0·20	0·60 ± 0·04	7·35 ± 0·20	40 ± 4	1·07 ± 0·14
Progesterone (10 mg/day)									
A. Untreated	206 ± 15	15·6 ± 1·2	1·84 ± 0·09	11·7 ± 0·4	3·45 ± 0·17	0·40 ± 0·02	5·05 ± 0·25	92 ± 6	1·07 ± 0·04
1 day	212 ± 11	16·1 ± 0·5	1·84 ± 0·06	11·4 ± 0·5	3·62 ± 0·30	0·42 ± 0·02	4·98 ± 0·47	101 ± 2	1·25 ± 0·27
2 days	217 ± 11	21·1 ± 2·1	2·44 ± 0·31	11·5 ± 0·6	3·58 ± 0·11	0·44 ± 0·04	5·15 ± 0·19	151 ± 8	1·22 ± 0·10
3 days	207 ± 5	19·1 ± 1·5	2·26 ± 0·20	12·1 ± 1·3	3·42 ± 0·11	0·38 ± 0·02	6·70 ± 0·58	99 ± 12	1·13 ± 0·03
9 days	219 ± 9	16·7 ± 2·5	2·14 ± 0·13	13·1 ± 1·4	3·30 ± 0·06	0·51 ± 0·13	5·22 ± 0·37	114 ± 19	1·25 ± 0·22
B. Untreated	264 ± 8	18·5 ± 1·8	2·08 ± 0·23	10·7 ± 0·2	3·15 ± 0·15	0·28 ± 0·06	4·97 ± 0·12	86 ± 4	1·12 ± 0·07
9 days	264 ± 14	53·5 ± 6·0	2·90 ± 0·34	8·5 ± 0·1	4·84 ± 0·14	0·39 ± 0·06	7·91 ± 0·55	43 ± 6	1·09 ± 0·15

There were three rats in each group. Treatment with oestradiol was with 5 μg cyclopentyl-propionate in 0·1 ml. arachis oil on day 0, progesterone 10 mg/day in 0·5 ml. olive oil. The meaning of l_z and k are given in text. The estimate of variation is the standard error of the mean.

weight of the whole tissue. When breaking stress was expressed in terms of collagen, in this, as in subsequent experiments, no evidence of change was found (Table 2).

Progesterone. A dose of 10 mg/day was used, as this is about the minimum required to maintain pregnancy after spaying (Alexander & Frazer, 1954). In the first experiment (Table 2, *A*) a commercial preparation (Progestin), in which 10 mg were dissolved in 0·4 ml. of ethyl-oleate, had no effect. In a second experiment (Table 2, *B*) progesterone homogenized

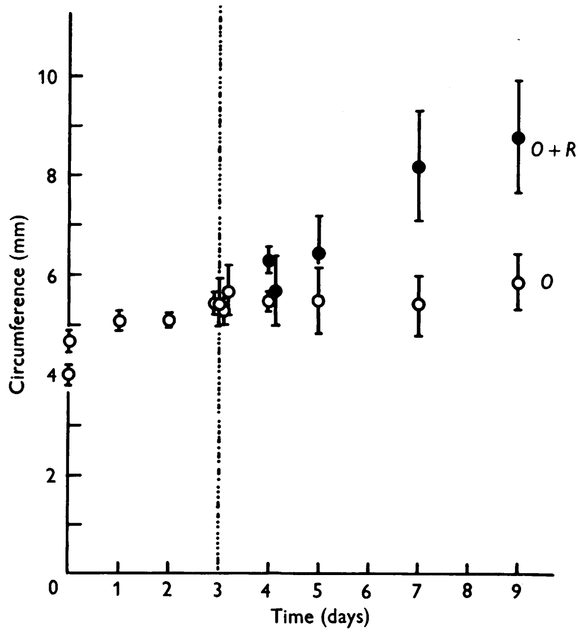

Fig. 5. Summary of effect of oestradiol (*O*) and relaxin (*R*) on circumference of cervix. This figure summarizes all results for animals treated with oestradiol (*O*), with and without relaxin (*R*) (Tables 1, 2 and 3). The circumference is that at half breaking tension. The estimate of variation shown by the vertical lines is the standard error of the mean. Treatment with oestradiol began on day 0, with relaxin on day 3.

in olive oil, partly in solution and partly in suspension, produced a small but significant increase in weight of cervix and in l_z.

Relaxin. In the first experiment the animals were previously treated with oestradiol and 3 days later given a single dose of 0·5 mg of relaxin (*A*). They were examined 24 hr later and no significant effect was found (Table 3, *A*). The experiment was repeated with twice and four times the original dose of relaxin, again without significant effect (Table 3, *B*); but when administration of relaxin was prolonged (4 days) a significant increase in l_z was produced (Table 3, *C*; Fig. 5).

TABLE 3. The effect of relaxin on the cervix in spayed rats previously treated with oestradiol

Dose of relaxin (mg *Std*)*	Day killed	Body wt. (g)	Wt. of cervix (mg)	Total collagen (mg)	Concn. collagen (g/100 g)	l_z (mm)	k/l_z	Circumference at break (mm)	Stress at break (kg/mm² coll.)
A. Control	4	200 ± 24	63 ± 9	3·6 ± 1·0	6·0 ± 0·3	4·33 ± 0·28	0·51 ± 0·06	7·00 ± 0·43	1·01 ± 0·08
Relaxin 0·5	4	224 ± 16	63 ± 10	3·5 ± 0·9	5·4 ± 0·8	4·89 ± 0·35	0·59 ± 0·11	8·42 ± 0·60	1·04 ± 0·16
B. Control	4	227 ± 17	63 ± 16	4·4 ± 1·1	6·9 ± 0·1	4·43 ± 0·40	0·39 ± 0·09	6·95 ± 0·35	1·01 ± 0·28
Relaxin 0·5	4	231 ± 10	71 ± 16	4·7 ± 1·2	6·6 ± 0·3	4·57 ± 0·37	0·43 ± 0·12	7·82 ± 1·42	1·04 ± 0·09
Relaxin 1·0	4	266 ± 10	48 ± 7	3·0 ± 0·3	6·3 ± 0·6	4·97 ± 0·47	0·32 ± 0·06	6·90 ± 0·42	0·86 ± 0·06
Relaxin 2·0	4	214 ± 48	71 ± 20	5·1 ± 1·0	6·9 ± 0·5	4·92 ± 0·23	0·36 ± 0·08	7·39 ± 1·09	0·85 ± 0·12
C. Control	3	246 ± 9	78 ± 11	7·4 ± 1·9	9·2 ± 1·3	4·65 ± 0·55	0·37 ± 0·05	6·40 ± 0·67	0·62 ± 0·10
Control	5	261 ± 6	74 ± 5	4·2 ± 0·5	5·7 ± 0·4	4·20 ± 0·06	0·58 ± 0·11	7·95 ± 0·77	0·82 ± 0·06
Control	7	285 ± 6	59 ± 1	3·9 ± 0·4	6·6 ± 0·5	4·28 ± 0·30	0·51 ± 0·03	8·04 ± 0·38	0·83 ± 0·09
Relaxin 0·5 × 1	5	293 ± 5	60 ± 1	3·2 ± 0·4	5·4 ± 0·5	4·83 ± 0·31	0·64 ± 0·08	8·31 ± 0·93	0·84 ± 0·08
Relaxin 0·5 × 2†	7	271 ± 19	94 ± 11	5·6 ± 3·0	5·5 ± 1·6	6·66 ± 0·82	0·52 ± 0·14	10·75 ± 0·24	1·14 ± 0·15

There were three rats in each group. The estimate of variation is the standard error of the mean. All animals were given oestradiol cyclopentyl-propionate (5 μg) on day 0; relaxin (*A*) was given on day 3, or days 3 and 5 (†). **Std*, see Table 1. The meanings of l_z and k are stated in the text.

TABLE 4. The effect of combinations of oestradiol, progesterone and relaxin on the cervix of spayed rats. Experiment 1

	1	2	3	4	5	6	7	8	9	10
Treatment	Body wt. (g)	Wt. of cervix (mg)	Total collagen (mg)	Concn. collagen (g/100 g)	l_z (mm)	k/l_z	Breaking load (g)	Circumference at break (mm)	Stress at break (g/mm²)	Stress at break (kg/mm² coll.)
None	281 ± 7	18·7 ± 2·2	2·53 ± 0·12	13·8 ± 0·9	3·47 ± 0·17	0·32 ± 0·02	804 ± 148	4·95 ± 0·29	105 ± 10	1·11 ± 0·18
Oestradiol (*O*)	268 ± 26	66·0 ± 14·0	4·45 ± 0·96	6·7 ± 0·2	4·96 ± 0·40	0·37 ± 0·05	729 ± 36	8·40 ± 1·21	48 ± 3	1·02 ± 0·07
Progesterone (*P*)	258 ± 3	15·3 ± 4·1	2·13 ± 0·24	15·5 ± 3·0	3·55 ± 0·15	0·37 ± 0·07	587 ± 50	5·13 ± 0·31	108 ± 18	1·01 ± 0·07
Relaxin (*R*)	248 ± 3	14·0 ± 1·5	1·28 ± 0·48	8·7 ± 1·7	3·90 ± 0·21	0·32 ± 0·04	654 ± 178	5·49 ± 0·13	127 ± 33	2·39 ± 0·96
PR	258 ± 4	19·7 ± 2·4	2·21 ± 0·40	11·0 ± 0·7	3·85 ± 0·31	0·36 ± 0·10	679 ± 54	5·45 ± 0·33	94 ± 21	1·19 ± 0·19
OP	251 ± 5	47·0 ± 8·7	3·37 ± 0·16	7·5 ± 1·0	5·30 ± 0·34	0·57 ± 0·03	662 ± 72	8·40 ± 0·73	63 ± 13	1·05 ± 0·10
OR	238 ± 4	84·3 ± 5·7	4·03 ± 0·24	4·8 ± 0·4	7·30 ± 1·00	0·44 ± 0·06	637 ± 9	11·15 ± 0·91	43 ± 7	1·28 ± 0·18
OPR	255 ± 7	97·0 ± 13·0	4·70 ± 0·76	4·9 ± 0·2	8·70 ± 0·59	0·61 ± 0·16	362 ± 29	14·22 ± 0·67	27 ± 6	0·80 ± 0·17

There were three rats in each group. The estimate of variation is the standard error of the mean. *O* = oestradiol cyclopentyl-propionate 5 μg on day 0. *P* = progesterone 10 mg/day, *R* = relaxin (*A*) 0·5 mg *Std* on days 3, 5 and 7. The meanings of l_z and k are stated in the text.

Combinations of hormones

In the next experiment we increased the duration of relaxin treatment by a further 2 days and used all three hormones, oestradiol, relaxin and progesterone, in all possible combinations. The results are shown in Table 4 and an analysis of variance on them in Table 5. Both oestradiol and

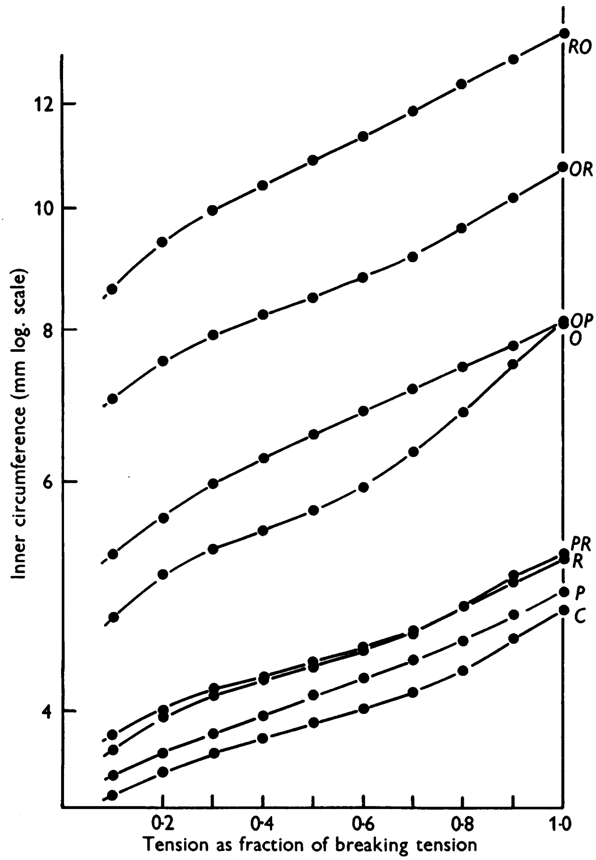

Fig. 6. The effect of tension on circumference of cervix of rats treated in differen ways. Each curve is the mean for all rats in the group. The treatment is given at the end of each curve, C = no treatment, O = oestradiol, P = progesterone, R = relaxin. The doses are given in Table 3.

relaxin produced effects. These were (*a*) an increase in l_z and in circumference at time of rupture (Table 4, cols. 5 and 8), the whole length–tension curve being shifted bodily upward without much change in its shape (Fig. 6); (*b*) an increase in the wet weight of the cervix (Table 4, col. 2); (*c*) a diminution in the concentration of collagen (Table 4, col. 4). There is also a significant interaction in the statistical sense (Table 5) between oestradiol and relaxin, which means that the combined effect was greater than was to be expected from simple addition of the individual effects, relaxin given alone having no significant effect. The largest effect on circumference in this experiment was obtained when progesterone was combined with oestradiol and relaxin. Though the effect of progesterone was not significant statistically at the 5 % level, it appears to be a real one as it was found again in later experiments. It will be seen in Table 6 that l_0 obtained by extrapolation to zero time with constant load, like l_z in Table 4, is greater in the *OPR* than in the *OR* group. The effects of different combinations of hormones on l_0 and l_z are obviously similar (compare Tables 4 and 6) and if the results are combined the difference between the *OR* and the *OPR* groups is significant ($0{\cdot}02 > P > 0{\cdot}01$).

TABLE 5. Significance of results of Experiment 1 with oestradiol, progesterone and relaxin (Table 4)

	Primary effects			Interactions			
	O	*R*	*P*	*PR*	*OP*	*OR*	*OPR*
Weight of cervix	0·001	0·01	—	—	—	0·01	—
Total collagen	0·001	—	—	0·05	—	—	—
Concn. of collagen	0·001	0·01	—	—	—	—	—
l_z	0·001	0·001	—	—	—	0·01	—
K/l_z	0·05	—	0·05	—	—	—	—
Circumference at break (mm)	0·001	0·001	—	—	—	0·001	—
Stress at break (g/mm³)	0·001	—	—	—	—	—	—

The figures in the table are values of *P* in analysis of variance, and are maxima, i.e. *P* of 0·05 means $0{\cdot}05 > P > 0{\cdot}01$, *P* of 0·01 means $0{\cdot}01 > P > 0{\cdot}001$.

Effect of prolonged loading. The tests used in the previous experiments were conducted at 22° C with rapidly increasing load. They would be expected to bring out any effects of hormones on the dimensions of the cervix, but would not give evidence whether any of them would cause the tissues to show the prolonged slow extension under low load seen at the end of pregnancy (Harkness & Harkness, 1959*b*). We therefore repeated the last experiment with the three hormones in all possible combinations with the tissues in Locke's solution at 37° C, maintaining a constant load for $1\frac{1}{2}$ hr and measuring the rate of increase of the circumference over the last half of the period. In Table 6 this slope (K) is recorded as a proportion of the circumference at zero time (l_0) obtained by extrapolation. In none of the groups of animals was there any prolonged extension comparable in rate to that seen at the end of pregnancy, nor in a second experiment (Table 7) did increasing the dose of relaxin eight times produce any increase in K/l_0, values still being lower than at the end of pregnancy for comparable conditions. Increasing the dose of relaxin did, however, increase the value of l_0. In this second experiment only animals treated with oestradiol and

TABLE 6. The effect of combinations of oestradiol, progesterone and relaxin on the cervix of spayed rats. Experiment 2

Treatment	Body wt. (g)	Wt. of cervix (mg)	Total collagen (mg)	Concn. collagen (g/100 g)	l_0 (mm)	K/l_0 for 100 g load	Stress* at l_0 (g/mm² coll.)	Circumference at break (mm)	Stress at break (g/mm²)	Stress at break (kg/mm² coll.)
None	207 ± 20	20·7 ± 1·5	1·97 ± 0·40	9·6 ± 0·9	3·36 ± 0·09	< 0·1	112 ± 9	4·82 ± 0·19	119 ± 12	1·74 ± 0·01
Oestradiol (*O*)	199 ± 3	53·3 ± 2·7	3·41 ± 0·59	4·6 ± 1·1	4·84 ± 0·07	0·53 ± 0·09	103 ± 20	7·90 ± 0·78	56 ± 1	1·26 ± 0·17
Progesterone (*P*)	228 ± 33	21·8 ± 4·7	1·85 ± 0·29	9·0 ± 0·6	3·25 ± 0·51	0·29 ± 0·29	124 ± 37	4·73 ± 0·95	100 ± 15	1·55 ± 0·13
Relaxin (*R*)	214 ± 12	25·7 ± 4·0	2·36 ± 0·18	9·4 ± 0·8	3·70 ± 0·04	< 0·1	110 ± 7	5·50 ± 0·24	90 ± 4	1·39 ± 0·10
PR	216 ± 20	22·3 ± 3·7	1·99 ± 0·08	9·3 ± 0·2	3·57 ± 0·04	< 0·1	119 ± 3	4·79 ± 0·42	104 ± 29	1·55 ± 0·13
OP	216 ± 8	60·0 ± 11·7	3·33 ± 0·65	5·6 ± 0·1	4·28 ± 0·50	0·20 ± 0·20	92 ± 8	7·02 ± 0·71	66 ± 5	1·67 ± 0·13
OR	234 ± 8	96·6 ± 3·6	3·55 ± 0·03	3·7 ± 0·1	7·22 ± 0·30	1·27 ± 0·63	143 ± 5	11·02 ± 0·18	43 ± 3	1·68 ± 0·08
OPR	225 ± 28	122·4 ± 1·4	4·55 ± 0·40	3·7 ± 0·3	10·55 ± 0·35	1·24 ± 0·37	164 ± 9	16·00 ± 1·10	37 ± 11	1·43 ± 0·43

The estimate of variation is the standard error of the mean. There were two rats in each group. The dose of oestradiol was 10 μg cyclopentyl-propionate on day 0; progesterone 10 mg/day from day 3 onwards; relaxin 0·5 mg *Std* on days 3, 5, 7 and 9. The rats were killed on days 10 and 11, the cervices of those killed on day 10 being loaded initially with 50 g, those on day 11 with 100 g. The value of K/l_0 for 50 g load was multiplied by 2 to combine with values for 100 g load. The meanings of l_0 and K are given in the text. * For 100 g load.

progesterone, with or without relaxin, were used. We have also recorded in Table 7 the value l_1 in the equation $l = l_1 + a \log t$, where l is circumference and t is time (1 unit = 5 sec). This describes fairly well the initial effects of loading, and allows the results to be compared with those reported previously for normal and pregnant animals (Harkness & Harkness, 1959*b*). In further experiments in which samples of relaxin of different degrees of purity were used and the dosage taken up to 40 times the original, some further increase in l_0 was found, and a small increase in K/l_0, but still not to values as high as at the end of pregnancy (Table 8, Fig. 7).

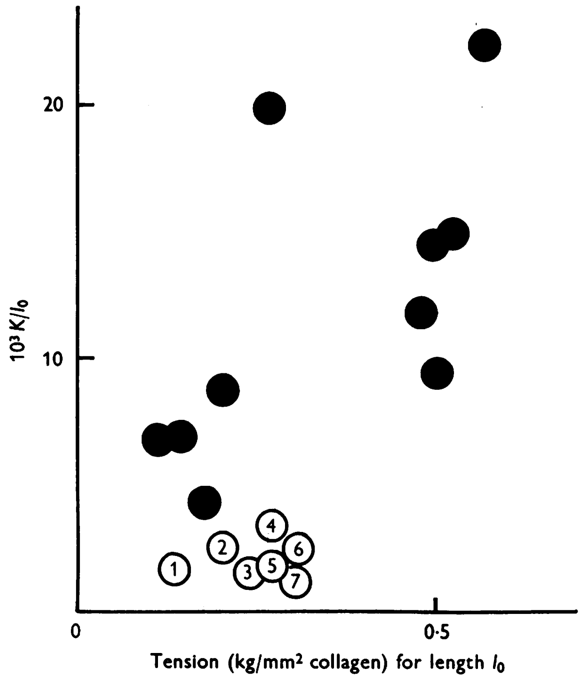

Fig. 7. Relation between rate of extension under prolonged loading (ordinate, K/l_0) and tension per unit cross-sectional area of collagen calculated for length l_0 (abscissa). ● 21st day of pregnancy (from Harkness & Harkness, 1959*b*) and ○ relaxin-treated, means from Table 7. Dose (mg) and type of relaxin: 1, 0·5 *C*; 2, 1 *C*; 3, 1 *A*; 4, 10 *C*; 5, 0·5 *B*; 6, 0·5 *A*; 7, 1 *B*.

DISCUSSION

Relation of results to investigation of 'dilatability' of cervix

One method which has been used to investigate the 'dilatability' or softening of the cervix is to introduce a conical probe into the canal, to push it in until resistance is felt and then to record the diameter (de Vaal, 1946). Crelin (1958) has made this method more quantitative as regards the

TABLE 7. The effect of different doses of relaxin on cervix in rats treated with oestradiol and progesterone

	Dose of relaxin (mg *Std*/day)						
	Sample *A* (30 g-p u./mg)				Sample *B* (150 g-p u./mg)		
	0	0·5	1·0	2·0	0·5	1·0	2·0
Weight of cervix (mg) ...	61 ± 5	129 ± 7	164 ± 12	141 ± 36	113 ± 11	148 ± 20	146 ± 7
Total collagen (mg)	3·68 ± 0·13	4·60 ± 0·06	4·77 ± 0·42	3·58 ± 0·30	4·53 ± 0·89	4·72 ± 0·06	4·12 ± 0·48
Concn. of collagen (g/100 g)	6·12 ± 0·24	3·59 ± 0·14	2·95 ± 0·47	2·67 ± 0·49	3·99 ± 0·43	3·25 ± 0·48	2·81 ± 0·03
l_1 (mm)	4·2 ± 0·8	7·6 ± 1·3	11·5 ± 0·5	10·7 ± 1·9	8·3 ± 1·2	10·4 ± 0·3	11·2 ± 0·6
l_z (mm)	4·8 ± 0·9	9·2 ± 1·8	13·9 ± 1·0	12·3 ± 2·4	9·3 ± 1·3	11·9 ± 0·1	11·8 ± 0·4
$10^3\ K/l_0$	0·42 ± 0·14	1·19 ± 0·11	0·83 ± 0·27	1·01 ± 0·15	0·69 ± 0·36	0·40 ± 0·05	0·67 ± 0·15
Stress at l_0 (g/mm² collagen)	91 ± 14	131 ± 24	205 ± 32	237 ± 27	154 ± 50	176 ± 3	240 ± 17
Stress at break (kg/mm² collagen)	1·16 ± 0·07	1·23 ± 0·07	1·75 ± 0·11	1·10 ± 0·11	1·10 ± 0·13	1·44 ± 0·29	1·44 ± 0·13
Circumference at break (mm)	6·4 ± 0·6	12·8 ± 1·4	17·7 ± 0·5	15·7 ± 2·0	12·4 ± 0·1	15·4 ± 0·6	17·4 ± 1·6

The estimate of variation is the standard error of the mean. There were two rats in each group. All animals were given oestradiol cyclopentyl-propionate (10 μg on day 0), and progesterone (10 mg/day from day 3). Relaxin was given every day from day 3 in concentration 5 mg *Std*/ml. in beeswax and oil. The meanings of l_1, l_z, l_0 and K are given in the text. Load 100 g. g-p u. = guinea-pig unit.

TABLE 8. Effect of samples of relaxin of different purity

		Dose of relaxin (mg *Std*/day)						
	Control	Sample *B* (150 g-p u./mg)		Sample *A* (30 g-p u./mg)		Sample *C* (18 g-p u./mg)		
	0	0·5	1·0	0·5	1·0	0·5	1·0	10·0
Wt of cervix (mg)	62 ± 1	90 ± 4	104 ± 5	92 ± 3	159 ± 3	110 ± 1	121 ± 14	142 ± 12
Total collagen (mg)	3·49 ± 0·01	3·22 ± 0·42	3·22 ± 0·12	2·98 ± 0·01	4·27 ± 0·45	4·55 ± 0·26	4·06 ± 0·07	4·11 ± 0·29
Concn. of collagen (g/100 g)	5·6 ± 0·1	3·6 ± 0·6	3·1 ± 0·1	3·2 ± 0·1	2·7 ± 0·2	4·2 ± 0·3	3·4 ± 0·3	2·9 ± 0·2
l_1 (mm)	4·7 ± 0·2	10·6 ± 0·8	12·0 ± 0·1	11·6 ± 1·0	13·6 ± 1·6	7·6 ± 0·5	10·3 ± 2·3	13·1 ± 0·9
l_0 (mm)	5·5 ± 0·3	12·1 ± 1·2	13·7 ± 0·3	12·9 ± 1·1	15·4 ± 1·6	8·1 ± 2·4	11·6 ± 2·5	16·0 ± 1·0
$10^3\ K/l_0$	1·09 ± 0·02	1·85 ± 1·03	1·24 ± 0·11	1·95 ± 0·16	1·52 ± 0·46	1·68 ± 1·08	2·59 ± 0·94	3·59 ± 0·52
Stress at l_0 (g/mm² collagen)	110 ± 3	263 ± 9	298 ± 16	303 + 26	236 ± 52	127 ± 43	200 ± 40	274 ± 25
Circumference at break (mm)	7·3 ± 1·3	17·6 ± 1·9	17·5 ± 0·6	16·9 ± 1·4	20·3 ± 2·3	14·3 ± 1·9	17·3 ± 5·2	23·2 ± 1·6
Stress at break (kg/mm² collagen)	1·34 ± 0·39	1·44 ± 0·18	2·02 ± 1·07	2·10 ± 0·62	1·55 ± 0·10	1·29 ± 0·13	1·61 ± 0·10	1·36 ± 0·12

The estimate of variation is the standard error of the mean. There were two rats in each group except the control and 10·0 mg *Std* (sample *C*) which had three. Animals were treated as in Table 6. The meanings of l_1, l_0, and K are given in the text. g-p u., see Table 7.

force exerted by incorporating a spring gauge so that the probe can be pushed in with a standard pressure. This type of test is similar in principle to our first type (examination of the relation of circumference to tension) but the lack of a measure of force or time during which it is applied makes the results difficult to interpret with certainty. In particular, it is not clear to what extent the activity of smooth muscle affects this test. However, it seems probable that the probe would be arrested when the tissue had stretched to a circumference which in our experiments would lie somewhere near the beginning of the relatively flat part of the length–tension curves. Using this method Graham & Dracy (1952), Zarrow, Neher, Sikes, Brennan & Bullard (1956), Steinetz, Beach & Kroc (1956) and Kroc *et al.* (1959) have found that relaxin increases the 'dilatability' of the cervix in sow, rat and mouse. That relaxin has an effect on the cervix must therefore be regarded as clearly established. The smaller effect of oestrogen is less clear. Thus Smith & Nalbandov (1958) recorded a constriction of the cervical canal by oestrogen in the sow. Such a finding with the probe test might be the result of swelling of the inner lining only, and it is difficult to distinguish the various possibilities without more information on the forces used and time for which they acted. A number of observations on the effects of relaxin on the human cervix are recorded in the literature but the results have been so variable that it seems premature to discuss the subject. It is worth while to point out that in some of the experiments in which no effect was found only short periods of treatment (hours) were used. Experiments on animals indicate that much longer treatment is needed to produce effects.

Nature of effect of hormones on the cervix

The effect of oestradiol alone was to increase the weight of the cervix to about the normal non-pregnant level. The total quantity of collagen in the cervix increased proportionately less than weight so that concentration fell. This confirms previous results with oestrone (Harkness *et al.* 1957). The principal effect of oestradiol, shown in physical tests, was to cause an increase in the circumference of the collagenous framework, and this resembled the change in total collagen in that it was less extensive and took place more slowly than increase in weight. Thus the increase in total collagen content and in circumference appear to be related in both extent and time course. This suggests that both changes have a common origin in simple growth in size of the connective-tissue framework without change in its shape. Circumference is a linear dimension and should increase as the cube root of the total amount of tissue concerned if there is no change in shape. For animals other than those treated with relaxin combined with oestradiol, the circumference is, in fact, approximately proportional to the cube root of the total collagen content (Fig. 8). We may conclude then that in these animals increase in circumference is to be accounted for by growth of the collagenous framework without change in shape. This conclusion, however, does not apply to the relaxin-treated (oestrogen-primed) animals, which show an increase in circumference with little increase in total collagen above the value found with oestradiol alone (Tables 4 and 6, Fig. 8). We may conclude that relaxin produces a change in the shape of the collagenous framework. In addition, it can increase the extensibility of the framework under prolonged loading.

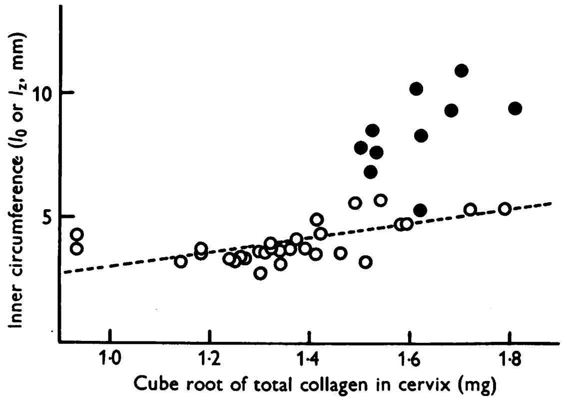

Fig. 8. Relation between inner circumference and total collagen content of horns. ●, animals treated with oestradiol and relaxin with or without progesterone; ○, other treatments, the dotted line going through the mean of abscissal and ordinate value for these, and having slope required for value of ordinate to be proportional to that of abscissa.

Comparison of the effect of relaxin on the cervix with changes found in pregnancy. During pregnancy in the rat the cervix becomes heavier in weight and its total collagen content is increased by about a half though the concentration is reduced (Harkness & Harkness, 1959*b*). The circumference of the connective-tissue framework also becomes greater and this is not accounted for by simple growth but involves change in shape. In addition to these changes there is an increase in the extensibility of the framework under prolonged loading (Harkness & Harkness, 1959*b*). All these changes can be produced by relaxin, though we have not so far found the latter to cause changes as great as those found at the end of pregnancy. The tests made on cervices of pregnant animals are not all exactly the same as those carried out in the present investigation, but a close enough comparison can be made to enable one to say that the maximum increase in circumference found in the present series of experiments corresponded to the value at about the 17–18th day of pregnancy. Kroc *et al.* (1959)

also found maximum values of circumference by the probe method to correspond with the values obtained by the same method at this time of pregnancy. It is impossible to say yet whether the inability of relaxin so far to reproduce the complete changes found in pregnancy in the cervix is the result of inadequate dosage or because other hormones are involved. That other hormones are involved is suggested by the fact that Kroc *et al.* (1959) found that relaxin given to rats oophorectomized in pregnancy would prevent fall in circumference of the cervix (probe method) and allow it to develop to the size found normally at the end of pregnancy. Factors other than relaxin appear to be of relatively greater importance in mice than in rats in producing the increase in the circumference of the cervix found by the probe test in pregnancy (Steinetz *et al.* 1956; Kroc *et al.* 1959).

Comparison of effects of relaxin on cervix and symphysis pubis

In the rat the symphysis pubis is scarcely affected by pregnancy or relaxin, so far as is known. In other animals, e.g. guinea-pig, two sorts of changes can be produced. The first is an oedematous swelling of the tissue which is detectable within a few hours of the time of administration (Abramowitz, Money, Zarrow, Talmage, Kleinholz & Hisaw, 1944; Zarrow & Money, 1948), but we did not find any evidence of such a rapid change in the cervix. The second change produced by more prolonged administration is a slow reorganization of the tissues of the symphysis in which bone and cartilage are absorbed and replaced by loose connective tissue, so that the originally short and compact structure is replaced by a long, loose ligament between the bone ends, which become widely separated. The changes in the cervix which we have investigated also take place slowly and clearly involve considerable reorganization of the tissue. For the actions of relaxin both on the symphysis and on the cervix, previous treatment with oestrogen is needed, relaxin alone having no effect.

Whether the same component of the relaxin preparation affects the cervix as the symphysis cannot be stated for certain. However, in our experiments with preparations of different potency by assay on the symphysis their potency correlated well with their effect on the circumference of the cervix. Our results are therefore compatible with the hypothesis that the same component of the preparation produces both effects.

SUMMARY

1. The effect has been studied of previous treatment of spayed rats with oestradiol, progesterone and relaxin, individually and in combination, on the reaction of the excised cervix to tension, the tissues being extended under load between two parallel rods, one inserted through each cervical

canal. The differences between animals, detected by these tests, were (*a*) in the circumference of the connective-tissue framework and (*b*) in the extensibility of the framework under prolonged loading.

2. Oestradiol (10 μg cyclopentyl-propionate) caused an increase in the circumference of the cervix to approximately the normal non-pregnant level. This effect could be accounted for by simple growth of the whole cervix without change of shape.

3. Relaxin in beeswax and oil given to rats previously primed with oestradiol caused a further increase in circumference of the cervix which could not be accounted for by simple growth, but appeared to involve change in the shape of the collagenous framework. Relaxin also caused some increase in the extensibility of the cervix under prolonged loading. The changes produced resemble those found in pregnancy but were not as great as at the end of the latter. The maximum circumference corresponded to that found on 17–18th day of pregnancy. Relaxin caused a reduction in concentration of collagen in the tissue by an increase of material other than collagen and this effect also is similar to that found in pregnancy.

4. To produce changes of the type investigated the action of hormones for several days at least was required.

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REFERENCES

ABRAMOWITZ, A. A., MONEY, W. L., ZARROW, M. X., TALMAGE, R. V. N., KLEINHOLZ, L. H. & HISAW, F. L. (1944). Preparation, biological assay and properties of relaxin. *Endocrinology*, **34**, 103–114.

ALEXANDER, D. P. & FRAZER, J. F. D. (1954). The effect of spaying in the pregnant rat. *J. Physiol.* **124**, 36–37*P*.

CRELIN, E. S. (1958). The developmental mechanism of pelvic sexual dimorphism in mice. *Anat. Rec.* **130**, 401.

CULLEN, B. M. & HARKNESS, R. D. (1958). Effect of oestradiol, progesterone and relaxin on the physical properties of the uterine cervix. *J. Physiol.* **140**, 46–47*P*.

DE VAAL, O. M. (1946). Extensibility of ostium uteri in rat. *Acta brev. neerl. Physiol.* **14**, 79–81.

GRAHAM, E. F. & DRACY, A. E. (1952). Effect of relaxin and mechanical dilatation on the bovine cervix. *J. Dairy Sci.* **36**, 772–777.

HARKNESS, M. L. R. & HARKNESS, R. D. (1959*a*). Effect of enzymes on mechanical properties of tissues. *Nature, Lond.*, **183**, 1821–1822.

HARKNESS, M. L. R. & HARKNESS, R. D. (1959*b*). Changes in the physical properties of the uterine cervix of the rat during pregnancy. *J. Physiol.* **148**, 524–547.

HARKNESS, M. L. R., HARKNESS, R. D. & MORALEE, B. E. (1957). The effect of the oestrus cycle and of hormones on the collagen content of the uterus of the rat. *J. Physiol.* **135**, 270–280.

KROC, R. L., STEINETZ, B. G. & BEACH, V. L. (1959). The effects of estrogens, progestagens and relaxin in pregnant and non-pregnant laboratory rodents. *Ann. N.Y. Acad. Sci.* **75** 942–980.

NEUMAN, R. E. & LOGAN, M. A. (1950). The determination of hydroxyproline. *J. biol. Chem.* **184**, 299–306.

SMITH, J. C. & NALBANDOV, A. V. (1958). The role of hormones in the relaxation of the uterine portion of the cervix in swine. *Amer. J. vet. Res.* **19**, 15–18.

STEINETZ, B. G., BEACH, V. L. & KROC, R. L. (1956). The influence of estrogen, progesterone and relaxin on the response of the nineteen-day pregnant mouse to oxytocin. *Anat. Rec.* **124**, 365.

ZARROW, M. X. & MONEY, W. L. (1948). Some studies on the pharmacology of relaxin. *J. Pharmacol.* **93**, 180–187.

ZARROW, M. X., NEHER, G. M., SIKES, D., BRENNAN, D. M. & BULLARD, J. F. (1956). Dilatation of the uterine cervix of the sow following treatment with relaxin. *Amer. J. Obstet. Gynec.* **72**, 260–264.