in the medium, of acetoacetate. This process is known to be catalysed by amino groups and it was found that during incubation considerably more protein escaped into the medium from rat-liver slices than from sheep-liver slices. On the other hand, this may represent another metabolic difference between rat and sheep liver. It should be noted that, since the incorporation of isotope into ketone bodies was always much less than into carbon dioxide, only a small proportion of the $^{14}CO_2$ produced during incubation of the rat-liver slices would be derived from the decarboxylation of acetoacetate.

SUMMARY

1. Propionate, in equimolar concentration, largely abolished the production of $^{14}CO_2$ from carboxyl-labelled acetate by rat-liver slices. Butyrate, *iso*valerate, valine, isoleucine and methionine inhibited acetate oxidation to varying degrees. The oxidation of propionate was not decreased by acetate.

2. Acetate oxidation by slices of other rat tissues was inhibited by propionate, but to a lesser degree than with rat-liver slices.

3. The uptake of acetate and the incorporation of its carboxyl-carbon into ketone bodies by rat-liver slices were also strongly inhibited by propionate.

4. The oxidation of carboxyl-labelled butyrate by rat liver was not inhibited by propionate; the oxidation of $C_{(2)}$-labelled pyruvate was affected to a much smaller extent than that of acetate.

5. The effects of propionate on acetate metabolism by sheep-liver slices were much smaller than those observed with rat liver.

6. The endogenous ketone-body production of sheep liver was less than that of rat liver.

The author wishes to acknowledge the careful technical assistance of Mr J. Appleton and Mr R. Green.

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The Protein Composition and Nucleic Acid Content of the Rat Uterus in Different States

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(*Received* 13 *August* 1956)

This work arose out of our interest in the proteins of the contractile mechanism of the uterus. Csapo (1950*a*) has reported that more myosin and actomyosin were present in extracts of human, rabbit and rat uteri at the end of pregnancy than in extracts from the non-gravid uterus. In the rabbit some weeks after ovariectomy there was a marked fall in the amount of these proteins present per gram wet weight of uterus, and injection of oestrone into castrated animals led to a gradual rise towards the normal value (Csapo, 1950*b*). Csapo used exclusively the viscosimetric method worked out by Balenovic & Straub (1942; see also Szent-Györgyi, 1945) for estimation of the purified proteins, and it is possible that in tissue extracts interfering substances might be present. The value for the myosin–actomyosin

content of human striated muscle (44 mg./g. wet wt.) reported by him is low compared with the value found by thorough extraction of rabbit muscle with salt solution and precipitation of the myosin and actomyosin by dilution: 53% of the total protein (Hasselbach & Schneider, 1951), i.e. 82 mg./g. wet wt. (see Dische, 1926). Further, the myosin–actomyosin fraction of Csapo makes up only 7–14% of the total protein of the uterus. For these reasons it seemed of interest to attempt a fractionation of the proteins of rat uterus on the lines used by Robinson (1952*a*) for embryo muscle, and to follow the changes in all these fractions in different states of the uterus. The changes in the myosin–actomyosin fraction, precipitated by dilution of the extract, could thus be compared with the changes in the other fractions and set against a proper background. Three different states of the uterus were chosen: in mid-oestrus, at the last day of pregnancy and at 3 weeks after ovariectomy.

Since deoxyribonucleoprotein is extracted by salt solutions and precipitated by dilution under conditions similar to those used for actomyosin, it became necessary to investigate also the amounts of deoxyribonucleic acid (DNA) in the tissue and in the dilution precipitates; ribonucleic acid (RNA) was also estimated.

A preliminary account of this work has already been published (Needham & Cawkwell, 1955).

METHODS AND MATERIALS

Fractionation of the proteins. The method used was based on that of Robinson (1952*a*).

(i) Preparation of the extract. The tissue was extracted with 1·25M-KCl containing 0·066M potassium phosphate, pH 7·9, and 0·0015M adenosine triphosphate (ATP). Since the uterus after ovariectomy is difficult to disintegrate, being fibrous and slippery, the conditions of extraction were conditioned by the need to ensure that the organ in this state was thoroughly extracted. The routine adopted was as follows. Immediately after the death of the animals by rapid bleeding, the uteri were dissected out and cooled to about 4°. In the cold room, the organs were slit open on cold glass plates and the epithelial layer and any uterine secretion were scraped off by means of a flexible plastic pen. The analyses thus refer to the endometrium and myometrium together. The uteri were then chopped; the weighed tissue was placed in a cold mortar and left to cool in air at −12 to −15°. After 3 hr. it was ground for 10 min. in the room at −12° with 10 vol. of the extracting fluid and one-tenth of its weight of acid-washed sand. The mortar was removed to 4° and all subsequent operations were carried out at this temperature. After thawing was complete (about 30 min.) the contents were centrifuged for 10 min. at 600 *g* on the International refrigerated centrifuge. The residue was ground twice more, each time with 5 vol. of extracting solution. Next day the combined supernatants were again centrifuged for 10 min. as before, and then for 1 hr. at 41000 *g* in the Spinco ultracentrifuge, model L, to remove cell particles which might be carried down with the dilution precipitate. All values given for *g* are average values.

(ii) Precipitation of the proteins insoluble at low ionic strength. A known volume of the supernatant was diluted 2·5 times by the gradual addition of water and the pH was brought to 7·0 with acetate buffer, pH 4·5, by means of the glass electrode. Dilution was continued by slowly adding 4 vol. of 19% (v/v) ethanol with constant stirring. The white precipitate quickly flocculated; it was centrifuged down after some hours and dissolved in 0·6M-KCl; the solution was made up to a known volume.

(iii) Extraction of the residue with 0·1M-NaOH. The residue was ground in the mortar with the alkali (5 ml./g. of tissue), and centrifuged. This procedure was repeated twice more, the third extraction being left to stand overnight. The combined supernatants were made up to a known volume.

(iv) The residue was treated with an equal vol. of 10% (w/v) trichloroacetic acid and centrifuged. It was washed twice with 5% (w/v) trichloroacetic acid, then warmed with 2 ml. of conc. H_2SO_4. After the protein had dissolved, the sand was centrifuged down and the supernatant transferred to a Kjeldahl flask. The sand was washed three times with dilute H_2SO_4 and the washings were added to the flask. Estimations showed that only about 1·5% of the residue N remained with the sand.

Nitrogen estimations. These were made by the method of Chibnall, Rees & Williams (1943), with a Markham (1942) distillation apparatus on known amounts of fractions (i) to (iii), after removal of non-protein N by three extractions with 5% trichloroacetic acid; in fraction (iv) known samples were taken after incineration. Total protein N was estimated in triplicate on 50–100 mg. samples; these were well stirred with 5% (w/v) trichloroacetic acid in the centrifuge tubes and allowed to stand overnight. After centrifuging, the residue was washed twice with the trichloroacetic acid. The recovery in the four fractions was 96–98% of the total.

Estimation of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). This was done by a modification of the method of Schmidt & Tannhauser (1945), with removal of phospholipids by means of ice-cold *n*-butanol (P. D. Mitchell & J. Moyle, personal communication; see Needham & Cawkwell, 1956). When nucleic acid estimations were to be made on the whole tissue, the chopped material was prepared as usual, then freeze-dried to constant weight and finely ground. Weighed amounts of the powder were suspended in water (about 50 mg./3 ml.) for extraction. The DNA P and RNA P were estimated by Allen's method (1940) or, if the quantities were below 20 μg., by a modification of the method of Weil-Malherbe & Green (1951; see Needham & Cawkwell, 1956). Since the method of extraction with butanol had not previously been used for animal tissues, a number of parallel analyses were made in which the method of Reichert (1944) with the modifications of Mitchell & Moyle (1951) was used for extraction of the phospholipids. Good agreement was found.

Estimation of collagen. This was estimated by means of the hydroxyproline content, by the method of Neuman & Logan (1949, 1950). Hydroxyproline (L. Light and Co. Ltd., Colnbrook, Bucks) was used for the standard; this gave the same standard curve as a specimen five times recrystallized by Dr G. R. Tristram. Occasional difficulties with fading of the colour to be measured were overcome by redistillation of the A.R. *iso*propyl alcohol used. Since the collagen content of the uterus is high, the small error due to tyrosine was

Table 1. *Distribution of protein N in the rat uterus in different states*

Results (mg./g. wet wt.) are expressed as means ± S.E.M. The experiments were done on groups of four to six uteri in oestrus, on single uteri in pregnancy and on groups of nine to twelve uteri after ovariectomy. The number of groups or of single animals used is shown in parentheses.

	Oestrus	End of pregnancy	After ovariectomy
Total N	25·03 ± 0·28 (8)	21·04 ± 0·39 (9)	29·41 ± 0·13 (2)
Soluble in 1·25 M-KCl	14·40 ± 0·30 (5)	13·00 ± 0·40 (8)	14·30 ± 0·18 (4)
Pptd. on dilution of the extract	5·68 ± 0·30 (5)	4·24 ± 0·19 (8)	5·95 ± 0·12 (4)
Residue soluble in 0·1 N-NaOH	2·17 ± 0·02 (5)	2·34 ± 0·07 (8)	3·21 ± 0·20 (4)
Insoluble residue	8·03 ± 0·51 (5)	5·55 ± 0·17 (8)	11·85 ± 0·13 (4)

Table 2. *Distribution of protein N as percentage of the total N*

	Oestrus	End of pregnancy	After ovariectomy
1. Soluble in 1·25 M-KCl	57·3 ± 1·9	62·0 ± 1·0	48·4 ± 0·5
2. Pptd. on dilution of the extract	22·5 ± 1·1	20·0 ± 1·0	21·4 ± 1·0
3. Residue soluble in 0·1 N-NaOH	8·4 ± 0·6	11·1 ± 0·4	10·7 ± 0·7
4. Insoluble residue	32·7 ± 1·6	26·2 ± 0·7	40·3 ± 0·5

Values of P are as follows for significance of differences in the fractions between any two groups. NS, Not significant.

Protein fraction	Oestrus – pregnant	Oestrus – ovariectomized	Pregnant – ovariectomized
1	NS	<0·05	<0·01
2	NS	NS	NS
3	<0·05	NS	NS
4	<0·05	<0·05	<0·001

For total N the value of P for significance of differences between groups is <0·001.

Table 3. *Ribonucleic acid and deoxyribonucleic acid of the rat uterus in different states*

Results (mg. of RNA P or DNA P/g. wet wt.) are expressed as means ± S.E.M. The experiments were done on two groups of four uteri in oestrus; on four single uteri at the end of pregnancy; and on three groups of five uteri after ovariectomy.

	Oestrus	End of pregnancy	After ovariectomy
RNA P	0·49 ± 0·033	0·34 ± 0·004	0·38 ± 0·049
DNA P	0·38 ± 0·004	0·15 ± 0·003	0·97 ± 0·01
RNA/DNA	1·29	2·34	0·39

neglected. The chopped tissue (weighed samples of about 100 mg. wet wt.) was heated in sealed tubes with 3 ml. of 6 N-HCl for 48 hr. at 100° without preliminary separation of the collagen.

Animals. The rats were piebald Norwegians weighing 170–250 g. Only virgins were used for the normal, non-pregnant uteri and the uteri after ovariectomy. The former were selected all at the stages of mid to late oestrus, by means of vaginal smears characterized by abundant cornified cells (Long & Evans, 1922). The ovariectomized animals were used 20–22 days after the operation; vaginal smears were taken daily for 5 days before use, to ascertain that no oestrous cycle occurred.

RESULTS

In Table 1 the protein N of the four fractions is given per g. wet wt. of the organ in different states. Since the total protein N is different in the three states, a more revealing picture of the changes is obtained by expressing the results as percentages of the total protein N, and this is done in Table 2. It is then seen that the amount of protein extractable by salt solution is significantly greater in the oestrous and pregnant uteri than in the uteri after ovariectomy. The connective tissue residue is greatest after ovariectomy and least in pregnancy, the differences being significant.

Table 3 shows the content in RNA P and DNA P; the marked changes in amount of DNA present/g. wet wt. would be expected to lead to differences in the DNA content of the dilution precipitate made from uteri in different states. For this reason a number of estimations were made of the total nucleic acid P in these precipitates. The values obtained for oestrus, end of pregnancy and after ovariectomy were 0·475 ± 0·014, 0·20 ± 0·019 and 0·83 ± 0·04 respectively. This nucleic acid was mainly DNA; when a separation was made, only 10–20 % appeared in the RNA fraction. It seemed desirable to make a closer assessment of the part of the dilution precipitate contributed by actomyosin and the part contributed by nucleoprotein. Two methods were tried.

(1) If the tissue is thoroughly extracted with a dilute salt solution (0·1 M-KCl buffered with 0·03 M potassium phosphate or sodium borate, pH 7·1) the water-soluble sarcoplasmic proteins are extracted together with the cell particles. An extract of the residue made with 0·1 N-NaOH would then contain the N of the nuclei and of the myofibrillar protein. If the N contribution from the nuclei is known, and this could be determined separately (see Robinson, 1952*b*), it should be possible to arrive at a figure for the actomyosin content. This method was unsuccessful, since many extractions with repeated grindings did not remove more than 30–40 % of the RNA from the residue. Thus in one case from uteri in oestrus the residue contained 0·38 mg. of DNA P and 0·158 mg. of RNA P after seven extractions and grindings. It is likely that some of this remaining RNA was contained in small cell particles, in which

Table 4. *Assessment of the actomyosin-like protein of the dilution precipitate by correcting for nucleoprotein*

Method of obtaining the correction is described in the text. The experiments were done on four groups of four to six uteri in oestrus, on six single uteri in pregnancy and on three groups of nine to twelve uteri after ovariectomy. Results (as means ± S.E.M.) are expressed in mg./g. wet wt. of original tissue.

	Oestrus	End of pregnancy	After ovariectomy	Statistical significance of differences between groups (*P* values): Oestrus – ovariectomized	Pregnant – ovariectomized
Nucleic acid P in dilution ppts.	0·48 ± 0·014	0·20 ± 0·019	0·83 ± 0·04	—	—
Acid-insol. N in dilution ppts.	5·91 ± 0·13	4·31 ± 0·22	5·95 ± 0·12	—	—
Acid-insol. N after correction for nucleoprotein N	4·72 ± 0·14	3·82 ± 0·28	3·91 ± 0·26	—	—
Protein N after correction, in percentage of total protein N	18·8 ± 0·55	18·2 ± 1·3	13·3 ± 0·93	<0·01	<0·05

the protein N associated with the P might be as high as 17:1 (Hogeboom, Schneider & Pallade, 1948) on analogy with liver-cell particles. The error on the actomyosin due to this would be too large and uncertain and this method was abandoned.

(2) The method adopted for correcting for the nucleic acid in the dilution precipitates was as follows. Nucleoprotein precipitation, in the form of stringy clots, was observed to begin at much lower dilution than was necessary to obtain the main bulk of the precipitate, which was softer and finely divided. In several experiments therefore the usual routine was followed but dilution was carried to the point (0·4–0·3 M-KCl) at which the clots appeared in amount just enough to make their analysis possible. About 50 % of the total nucleic acid was obtained in such clots, which were removed and analysed for total N (protein plus nucleic acid) and for nucleic acid P. The ratio N/P was found to be about 2·5. In some cases this precipitation was carried out in the presence of 5×10^{-3} M ATP, to minimize the precipitation of actomyosin (cf. Hoffmann-Berling, 1956). If it is assumed that this same ratio applies throughout precipitation, and that the protein of the nucleoprotein precipitated contains no actomyosin, then the amount of actomyosin N in a dilution precipitate can be taken as

(mg. of total N of precipitate) – (mg. of N equal to 2·5 × mg. of nucleic acid P of precipitate).

This correction is a maximum one. If only actomyosin itself combines with the nucleic acid, then the correction would consist of the nucleic acid N only – about 1·6 times the nucleic acid P (Chargaff, 1955). The true correction may lie between these extremes. In Table 4 experiments are shown in each of which the nucleic acid P was estimated in the dilution precipitate, and in each the maximum correction has been applied. When this was done, the actomyosin content after ovariectomy was about 72 % of that in oestrus or pregnancy; with smaller corrections the difference would be less.

Table 5. *Collagen content of the rat uterus in different states*

Results (mean values ± S.E.M.) are calculated from the hydroxyproline content of the whole tissue, as described in the text. Four groups of three uteri were used in oestrus, two single uteri in pregnancy; after ovariectomy, two groups of two and one single uterus were used.

	Oestrus	End of pregnancy	After ovariectomy
Collagen (mg./g. wet wt.)	38·3 ± 1·89	23·8 ± 0·75	65·9 ± 1·33
Collagen (% of the connective tissue)	83	71	92

Telfer (1953) has remarked upon the effect of oestradiol injection in bringing about increase in the protein fraction extractable by water from the uterus of the castrated rat. This increase in water-soluble proteins on passing from the castrate to the oestrous and pregnant uterus is seen in our experiments, when the protein precipitated on dilution is subtracted from the protein soluble in salt solution and the result is expressed in percentage of the total proteins; the values come out as 35, 42 and 28 % respectively. The correction to be applied for any water-soluble protein precipitated as nucleoprotein is small, as can be seen from the calculations given above, and would not appreciably alter the picture.

A few experiments were done, with oestrous and pregnant uteri, in which the tissue was extracted three times with 0·5 M-KCl containing 0·05 M potassium phosphate, pH 7·5, the extractant commonly used for extraction of actomyosin from skeletal muscle. The dilution precipitate was obtained as is also usual in actomyosin preparations from skeletal muscle: by dilution with water only to 0·03 M-KCl at pH 6·5–6·8. The results, after correction for nucleoproteins in the way described, were about 75 % of the values obtained by precipitation with 15 % alcohol at 0·1 M-KCl. The precipitate in water was very finely divided, and did not settle or

centrifuge down easily; it was much more difficult to deal with in a quantitative manner than the more flocculent precipitate in 15% alcohol.

Some estimations were made of the collagen content of the uterus in the three states, and these are shown in Table 5.

DISCUSSION

When the results are expressed as percentages of the total nitrogen (Table 2) the main differences to be seen in the protein fractions, by comparing the different states of the uterus with the oestrous state, are: (1) a decrease in the amount of protein extracted by the salt solution after ovariectomy and an increase at the end of pregnancy; (2) a marked increase in the insoluble connective-tissue residue after ovariectomy and a decrease at the end of pregnancy. A systematic study of the changes in wet and dry wt. of the uterus was not made in this work; but the average weight of the uterus in mid-oestrus was 0·4 g. (dry wt. 20%), at the end of pregnancy about 2 g. (dry wt. 18%) and after ovariectomy 0·08 g. (dry wt. 23%). It is thus clear that there were large absolute changes in all the fractions.

The amount of collagen found at the end of pregnancy (23·8 mg./g. wet wt.) is in agreement with that calculated from the figures of Harkness & Harkness (1954) (23·3 mg./g. wet wt.). Our figure for the uterus in oestrus (38·3 mg./g. wet wt.), on the other hand, is higher than the value from the data of Harkness & Harkness (28·3 mg./g. wet wt.). This discrepancy may be due to the use of uteri at different stages of the oestrous cycle. According to Astwood (1939), both the wet wt. and the dry wt. of the rat uterus fall by about 20% during the period between proestrus–oestrus and late oestrus. If, as seems likely, the collagen content per uterus is unchanged during the oestrous cycle, then the amount of collagen/g. wet wt. would be markedly lower early in the oestrous cycle than later, at the time when our specimens were taken.

The collagen content, calculated on the assumption that all the hydroxyproline is derived from collagen, falls short of the amount of insoluble connective-tissue residue (see Table 5). This is probably to be explained by the presence of some elastin. The hydroxyproline content of rat elastin is 2·3%, to be compared with 13·4% in collagen (Neuman & Logan, 1950).

The fraction of protein insoluble in salt solution but soluble in 0·1 N-NaOH is of unknown composition. It probably contains material from nuclei remaining in the residue, since only about 80% of the DNA is extracted by the salt solution. In the uteri from ovariectomized animals a large part of the fraction could be accounted for in this way. This explanation cannot apply to the uteri in oestrus, and still less to the pregnant uteri, because of the smaller nucleic acid content/g. wet wt. Unexpectedly, the fraction soluble in sodium hydroxide in percentage of the total nitrogen is significantly higher in pregnancy than in oestrus, indeed as high as after ovariectomy. Robinson (1952*a*), in his study of the proteins of embryonic chick muscle, found this fraction to be as much as 25% of the total nitrogen at hatching; he has suggested that it might include mucopolysaccharides.

It is clear from Table 4 that with the methods used here the changes in 'actomyosin' content of the rat uterus are not large even when expressed as percentage of the total protein. Csapo found a greater change in the rat by the viscosimetric method (a rise of 100%/g. wet wt. by the end of pregnancy). In the rabbit this increase was not so marked, but after ovariectomy a fall from 13 to 6 mg. of actomyosin/g. wet wt. was found. Further investigations are needed to explain the differences in these results.

The degree of homogeneity of the dilution precipitates from the uterus, and the degree of correspondence between the actomyosin in them and skeletal-muscle actomyosin are matters still uncertain (see Needham & Cawkwell, 1956). These questions are under study with uteri from larger animals.

The absolute values given by Telfer for RNA and DNA in the ovariectomized rat are 4·1 and 14·8 mg./g. wet wt. respectively. Conversion of our figures for nucleic acid phosphorus into nucleic acid, on the basis of a phosphorus content of 9·4% in RNA and 9·9% in DNA (Leslie, 1955), gives 4·05 and 9·81 mg./g. wet wt. respectively. The discrepancy in the value for DNA probably depends on the different methods of estimation used. It has been pointed out (Drasher, 1953*b*) that in some tissues too low results may be obtained by the Schmidt & Tannhauser method, owing to appearance of some degraded DNA in the RNA fraction. However, if this were so in the present work, one would expect the RNA value to be high. On the other hand, too high values for DNA can be obtained by the Dische method (used by Telfer) in the presence of protein (Dounce, 1952). A similar discrepancy between the results on the mouse, obtained by Drasher (using the Schmidt & Tannhauser method) and by Jeener (using the Dische method), leads us to suspect that the former is the more reliable with these tissues.

The experiments of Jeener (1948) and Drasher (1952) on the mouse and of Telfer (1953) on the rat show that injection of oestradiol into the castrated animal leads to a marked increase in the RNA/DNA ratio of the uterus. In Jeener's work injection of 20 μg. led in 24 hr. to an increase from 0·28 to 0·62; the wet wt. rose by 200% and the dry wt. by 100%

but there was little mitosis. Thus growth of the uterus after such treatment, as well as during the oestrous cycle (Drasher, 1953*a*), seems to depend largely on increase in cell size reflected in increase in cytoplasmic RNA relatively to nuclear DNA.

Increase in the cell dimensions is also recognized as the main basis of growth of the uterus during pregnancy (Reynolds, 1949), and in this connexion the work of Drasher (1953*b*) may be mentioned. In her experiments following the changes in RNA, DNA and protein nitrogen throughout pregnancy in the mouse, the ratios in oestrus, after ovariectomy and at the end of pregnancy were 1·58, 0·43 and 1·12 respectively. The first two values are similar to those of the present work (1·29 and 0·39), but the last is much lower than the 2·34 found by us at the end of pregnancy. It is interesting to notice that in available curves for increase in weight during pregnancy in the rat uterus (Siegmund, 1930; Harkness & Harkness, 1954) growth is shown as continuing at a steady rate from the 13th to the 20th day. In Drasher's curves for the mouse there is a sudden increase in protein nitrogen at the 11th day, and from the 13th to the 20th day further increase is much slower. It is unlikely that there is any fundamental difference in the growth pattern of the pregnant uterus between the rat and the mouse, but it is possible that the rate of growth during the last week can be affected by conditions such as the nutritional state, number of foetuses, etc. A study for the rat of the RNA/DNA ratio in the uterus, correlated with growth rate, would be of great interest.

SUMMARY

1. Four protein fractions, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) have been estimated in the rat uterus in mid-oestrus, at the end of pregnancy and after ovariectomy. The fractions were: (i) the protein soluble in 1·25M potassium chloride solution; (ii) the protein precipitated on dilution of this extract; (iii) the fraction of the residue soluble in 0·1N sodium hydroxide solution; (iv) the insoluble residue.

2. When the results are expressed as percentages of the total protein, the most important changes in the protein fractions were a significant fall in (i) and rise in (iv) after ovariectomy, as compared with the oestrous state; and a significant rise in (i) and fall in (iv) by the end of pregnancy, as compared with the oestrous state. When certain corrections were made there was significantly less of fraction (ii) after ovariectomy than in the other two states.

3. The RNA/DNA ratios in mid-oestrus, at the end of pregnancy and after ovariectomy were 1·29, 2·34 and 0·39 respectively.

Our thanks are due to Miss Pauline Alexander and Professor A. St G. Huggett for their instruction in carrying out the ovariectomies; to Miss Ruby Leader for her care of the animals; to Dr D. H. Cruickshank for her collaboration in the early stages of this work; to Dr P. D. Mitchell and Miss J. Moyle for making details of their method available to us; to Mrs C. F. Shoenberg for histological examinations; to Dr T. H. Hollingsworth and Dr H. B. F. Dixon for help with the statistical analyses; and to Dr K. Bailey for helpful discussions. We acknowledge with gratitude grants from the Broodbank Fund of Cambridge University, the Medical Research Council and the Agricultural Research Council.

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