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Cytoplasmic Fractions of Rat Myometrium

2. LOCALIZATION OF SOME CELLULAR CONSTITUENTS IN THE PREGNANT AND OVARIECTOMIZED STATES

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(*Received* 5 *November* 1959)

Wakid (1960) gave a qualitative description of the various cytoplasmic fractions isolated from the myometrium of the pregnant rat. The present study is devoted to some quantitative aspects of changes in intracellular distribution that accompany the physiological changes in this muscle. Since the uterus at the end of pregnancy and after ovariectomy is at its two extremes in size and functional activity the comparison has been carried out on these two states in the hope of showing maximum contrast.

Telfer (1953) and Longwell & Reif (1955) have demonstrated that after oestradiol injection the increase in succinoxidase activity of the uterus of castrate rats is faster than the rate of growth of the organ on a dry-weight basis. Telfer surmises that her observed effects could 'well be due to an increase in the mitochondrial mass through duplication or enlargement of these bodies'. It was apparent in this connexion that isolation of the mitochondria by differential centrifuging would

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help to elucidate this point. Succinic dehydrogenase was determined in the present work instead of succinoxidase, since in the latter system several stages are involved and hormonal effects could be on one or more of these.

Adenosine-triphosphatase activity is present in the cytoplasmic particles of all three types of mammalian muscle (Perry, 1952; Needham & Cawkwell, 1956; Holton, Hülsmann, Myers & Slater, 1957). It is apparently present in partially 'unmasked' form, since activity is readily elicited by the mere addition of Mg^{2+} ions without the necessity of prior aging of the particles. Initially in this work on the myometrium adenosine-triphosphatase activity was studied in an assessment of the morphological integrity of mitochondria. However, the finding of a very high activity in the uterine cytoplasmic particles, particularly in the microsomal fraction (Wakid, 1960), has prompted further study of this enzymic activity at different physiological states of the organ.

In their work on nucleic acids of rat uterus Needham & Cawkwell (1957), using buffered 0·1 M-potassium chloride, found that many extractions with repeated grindings did not remove more than 30–40 % of the ribonucleic acid from the residue containing the myofilaments and nuclei. Hendler (1956), working on an analogous organ, the hen oviduct, has also noticed that the greater part of the ribonucleic acid is associated with the easily sedimentable fraction. Jervell, Diniz & Mueller (1958) have made another similar observation with rat uterus. The question of localization of ribonucleic acid in myometrial tissue has been taken up further in the present work.

MATERIALS AND METHODS

Reagents. Unless otherwise stated all reagents were of analytical grade and were made up in water distilled in glass. The source of most of the special reagents used has been described by Wakid (1960). Gelatin was from Harrington Bros. Ltd.

Preparation of the cytoplasmic fractions. This has been described in the preceding paper. Each fractionation was performed on a single uterus at the end of pregnancy and on batches of 10–12 uteri after ovariectomy.

Enzyme assays

Succinic dehydrogenase. This was assayed at 28° with ferricyanide as electron acceptor in a system similar to that of Pressman (1955). In a final volume of 6 ml. the concentrations were: 0·04 M-succinate brought to pH 7·4 with NaOH, mM-$K_3Fe(CN)_6$, 25 mM-2-hydroxymethylpropane-1:3-diol (tris) chloride buffer, pH 7·4, 0·67 mM-orthophosphate, 6·7 mM-$MgSO_4$, 0·01 M-KCN (freshly prepared and neutralized), mM-adenosine triphosphate (ATP), mM-ethylenediaminetetra-acetate (EDTA) and 0·1 % of neutralized, freshly boiled gelatin. The gelatin is necessary to keep a linear relationship with activity at low enzyme concentrations. Adenosine monophosphate (AMP) and ATP behave as activators of succinic dehydrogenase in this system (see also Nordmann, Nordmann & Gauchery, 1951). Although AMP causes a twofold increase in activity, ATP is about 50 % more effective than AMP. Low concentrations of phosphate, Mg^{2+} ions and EDTA, though less effective than the nucleotides, are also found necessary for maximum activity of the enzyme. Also, warming the particles before assay usually increases the activity by 25 %. In routine analysis the tissue suspension was allowed to stand at room temperature for 15 min. before assay. The reaction was started with tissue suspension containing 0·1–0·2 mg. of nitrogen and was stopped with 5 % (v/v) of perchloric acid (final concentration). Samples were taken after 5 and 40 min. of incubation, during which time the reduction of ferricyanide proceeded linearly. After centrifuging the decrease in extinction of the supernatants was measured at 420 mμ in a Unicam SP. 500 spectrophotometer. Controls in the absence of succinate showed no reduction of ferricyanide.

Inorganic pyrophosphatase. This was assayed at 20° in the presence of 5 mM-$Na_4P_2O_7$, 0·05 M-tris chloride buffer, pH 7·4, and 5 mM-$MgSO_4$. The reaction was stopped after 15 min. with a final concentration of 8 % (w/v) of trichloroacetic acid, and inorganic P was determined in the acid supernatant after centrifuging.

Adenosine triphosphatase. The assay of this was described by Wakid (1960).

Analytical methods

Nucleic acids and phospholipids. These were determined by a combination of the Schmidt–Thannhauser and Schneider procedures (Volkin & Cohn, 1954). After removal of the acid-soluble phosphorus with 8 % (w/v) of trichloroacetic acid, phospholipids were extracted with water–ethanol, ethanol, ethanol–chloroform and finally ether. All extractions until the ethanol–chloroform stage were at 0°. The total P in the combined extracts of these fat solvents was taken as lipid P.

The residue was then hydrolysed by incubation for 16–18 hr. at 35° in 0·3 N-KOH. Proteins and deoxyribonucleic acid (DNA) were precipitated from the hydrolysate with a final concentration of 5 % (w/v) of perchloric acid. The supernatant was reserved for total and inorganic P estimations, the difference between the two being taken as ribonucleic acid (RNA) P. Inorganic P was determined here after precipitation with calcium according to Delory (1938) to avoid residual interfering proteins. The perchloric acid precipitate was extracted for 15 min. with 5 % (w/v) of perchloric acid at 90°, total P in the extract being taken as DNA P.

Other methods. The methods used for estimation of inorganic P, of nitrogen and of collagen have been given by Wakid (1960).

RESULTS

Total nitrogen

The distribution of total nitrogen in the fractions isolated by differential centrifuging is given in Table 1. The cytoplasmic particles constitute a small percentage of the total nitrogen of the cell. The two main components are the myofilaments and connective tissue of fraction N, and the soluble

Table 1. *Distribution of total nitrogen*

Homogenates in 0·3 M-sucrose were centrifuged at 800 *g* for 10 min. to give fraction N (nuclei, myofilaments and connective tissue). The supernatant (S_1) was centrifuged at 12 000 *g* for 15 min. to give fraction M (mitochondria). The resulting supernatant was centrifuged at 90 000 *g* for 60 min. to yield fraction P (microsomes) and the final supernatant S (see Wakid, 1960). The results of ten fractionations for pregnant and seven fractionations for ovariectomized animals are expressed as means ± S.E.M. per uterus. Each fractionation was performed on a single uterus at the end of pregnancy or on batches of 10–12 after ovariectomy. The values given for homogenate represent the sum of the values for individual fractions.

	Pregnant rat		Ovariectomized rat	
Fraction	N (mg.)	Percentage of homogenate	N (mg.)	Percentage of homogenate
N	18·97 ± 1·61	54·6 ± 1·3	1·047 ± 0·243	59·4 ± 1·1
M	1·13 ± 0·12	3·2 ± 0·2	0·125 ± 0·039	7·1 ± 0·2
P	2·63 ± 0·22	7·6 ± 0·2	0·190 ± 0·054	10·8 ± 0·4
S	11·94 ± 0·76	34·4 ± 1·6	0·401 ± 0·027	22·7 ± 0·8
Homogenate	34·67 ± 2·12		1·763 ± 0·120	

Table 2. *Distribution of succinic dehydrogenase*

See Table 1 for fractionation procedures and the Materials and Methods section for conditions of assay. Activities are in μmoles of ferricyanide reduced/hr. and are expressed as means ± S.E.M. per uterus. These are the results of six centrifugal fractionations for each physiological state. The values given for S_1 are merely the sum of the activities of subsequent fractions. Recovery of activity from all the fractions was not estimated as a routine, owing to the small amount of material available. In two experiments on the pregnant rat the recovery of activity was 80 and 95% that of S_1.

	Pregnant rat			Ovariectomized rat		
Fraction	μmoles/hr.	Percentage of S_1	μmoles/hr./mg. of N	μmoles/hr.	Percentage of S_1	μmoles/hr./mg. of N
M	38·9 ± 7·1	59·9 ± 2·5	35·9 ± 1·6	1·24 ± 0·2	41·2 ± 2·8	9·7 ± 1·3
P	20·3 ± 1·8	31·3 ± 2·8	7·9 ± 0·8	1·56 ± 0·1	51·8 ± 2·1	7·3 ± 0·5
S	5·7 ± 0·8	8·8 ± 1·3	0·5 ± 0·0	0·21 ± 0·07	7·0 ± 2·3	0·5 ± 0·2
S_1	64·9 ± 9·0	—	4·0 ± 0·2	3·01 ± 0·0	—	3·9 ± 0·3

nitrogen in the final supernatant S. Whether in pregnancy or after ovariectomy, the microsomal N is always greater than that of the mitochondria.

After ovariectomy the uterus weighs one-twentieth to one-thirtieth of its weight at the end of pregnancy. The difference in total nitrogen, though not as great, still parallels roughly the decrease in weight. In spite of this drastic change there is no major difference in the general pattern of distribution (see Table 1). Minor differences are an increase in the percentage of the total nitrogen to be found in the particles and a diminution in the soluble fraction S.

Succinic dehydrogenase

In the pregnant animal succinic-dehydrogenase activity is located predominantly in the mitochondrial fraction of rat myometrium (Table 2). Its concentration per milligram of total nitrogen (the specific activity) is also highest in this fraction. Some activity is present in fraction P, since it is impracticable to obtain a clear-cut separation between the two fractions of cytoplasmic particles. There is also a trace of activity in the final supernatant S. The succinic-dehydrogenase activity in fraction N was not measured as a routine, since this fraction is difficult to resuspend. However, by adding the activities of fractions N_b and S_{1b} in Table 5 it is possible to estimate that the activity normally present in fraction N would be about 9% of the total.

After ovariectomy there is a fall in activity that accompanies the decrease in size of the organ. The specific activity of the enzyme in the mitochondrial fraction decreases considerably (to about one-quarter), but that in the microsomal fraction does not. The greater part of the activity is actually now found in the small-granule fraction P, indicating that the particles bearing succinic-dehydrogenase activity have become smaller in size and have therefore required a higher centrifugal force for a longer time in order to sediment. It is not likely that this is the result of greater fragmentation of mitochondria, since all tissues were homogenized in the same manner. It is, however, possible that after ovariectomy the mitochondria become more fragile.

The presence of appreciable amounts of collagen, an inert protein, among the cytoplasmic particles results in lowered specific activities. Correction can

be made for this by subtracting the collagen nitrogen of the fractions from their total nitrogen. The collagen content of the fractions has been given by Wakid (1960). After correction the mean value for the specific activity of succinic dehydrogenase in fraction M becomes $35{\cdot}9/(1-0{\cdot}24) = 47{\cdot}2$ μmoles/hr./mg. of nitrogen at the end of pregnancy and $9{\cdot}7/(1-0{\cdot}36) = 15{\cdot}2$ μmoles/hr./mg. of nitrogen after ovariectomy. The difference in specific activity between the two physiological states still exists although now it is not as great as with the uncorrected values. Since, however, the succinic-dehydrogenase activity is spread over two fractions, M and P, especially after ovariectomy, it would be pertinent to consider the specific activity of the enzyme in the two fractions taken together. Thus (observed activities of M + P in μmoles/hr.)/(the total nitrogen of M + P minus their respective collagen nitrogen) = 18·2 μmoles/hr./mg. of nitrogen at the end of pregnancy, and 10·9 μmoles/hr./mg. of nitrogen after ovariectomy. On this basis also there is decreased specific activity of the succinic dehydrogenase in the castrate as compared with the pregnant state.

Magnesium-activated adenosine triphosphatase

The distribution of Mg-activated adenosine triphosphatase is shown in Table 3. Fraction N was not assayed because it contains the myosin adenosine triphosphatase. At the end of pregnancy the greater part of the adenosine-triphosphatase activity, and also the highest specific activity, are found in the microsomal fraction P. After ovariectomy, although there is the usual loss in activity accompanying the decrease in size of the organ, there is a marked increase in the specific activity of the enzyme in fraction P. If the specific activity of adenosine triphosphatase in the particles is again considered after correction for collagen, as with succinic dehydrogenase, the values in μmoles/hr./mg. of nitrogen for fraction M are: 166 for pregnancy and 120 for ovariectomy. The corresponding values for fraction P are 382 and 683. The contrast between the two microsomal fractions is thus increased.

The Mg-activated adenosine triphosphatase of rat myometrium is not identical with inorganic pyrophosphatase, since over 90% of the activity with $Na_4P_2O_7$ as substrate is located in the final supernatant S.

Nucleic acids and phospholipids

Table 4, on the distribution of nucleic acids, shows that over half of the RNA is found in the easily sedimentable fraction N, the next richest fraction being the microsomal particles P. The DNA, which in most tissues is considered to be located in the nucleus of the cell, sediments with fraction N. However, as in cases reported for mouse liver (Schneider, Hogeboom & Ross, 1950)

Table 3. *Distribution of magnesium-activated adenosine triphosphatase*

Assay was at 20° in the presence of 5 mM-ATP, 0·05 M-tris chloride buffer, pH 7·4, and 0·04 M-KCl. The results of four centrifugal fractionations for each physiological state are expressed as means ± S.E.M. per uterus. The values given for S_1 are merely the sum of the activities of the other fractions. Recovery of activity from all the fractions (as determined in two experiments on the pregnant rat) was 100 and 130% that of S_1.

	Pregnant rat			Ovariectomized rat		
Fraction	μmoles of P/hr.	Percentage of S_1	μmoles of P/hr./mg. of N	μmoles of P/hr.	Percentage of S_1	μmoles of P/hr./mg. of N
M	148·3 ± 31	12·9 ± 2·1	126·0 ± 10	8·4 ± 1·8	6·2 ± 1·4	77·1 ± 14
P	841·0 ± 148	73·0 ± 4·7	365·5 ± 27	102·2 ± 4·4	75·6 ± 3·8	601·0 ± 37
S	162·5 ± 48	14·1 ± 4·1	15·4 ± 4	24·5 ± 7·6	18·1 ± 5·1	60·9 ± 19
S_1	1151·8 ± 150	—	88·2 ± 12	135·1 ± 8·0	—	224·0 ± 19

Table 4. *Distribution of nucleic acids*

The results of four centrifugal fractionations for each physiological state are expressed as means ± S.E.M. per uterus. The values given for homogenate represent the sum of the values of individual fractions.

	Pregnant rat			Ovariectomized rat		
	RNA			RNA		
Fraction	(μg. of P)	(μg. of P/mg. of N)	DNA (μg. of P)	(μg. of P)	(μg. of P/mg. of N)	DNA (μg. of P)
N	379·9 ± 31·5	18·3 ± 0·7	220·2 ± 26·2	10·7 ± 1·1	10·6 ± 0·5	44·4 ± 3·1
M	15·0 ± 4·2	12·6 ± 3·1	35·2 ± 3·2	1·26 ± 0·2	9·1 ± 0·6	10·5 ± 1·6
P	92·9 ± 13·5	33·2 ± 4·7	—	3·76 ± 0·8	19·2 ± 3·5	—
S	68·9 ± 19·7	5·0 ± 0·7	—	0·50 ± 0·3	1·4 ± 0·9	—
Homogenate	556·7 ± 60·8	14·8 ± 0·7	255·4 ± 26·4	16·22 ± 1·3	9·4 ± 0·5	54·9 ± 2·6

Table 5. *Repeated homogenization of rat myometrium*

Myometrium from pregnant rats was homogenized in 0·1 M-KCl and 0·039 M-boric acid adjusted to pH 7 with borax (Perry & Grey, 1956) and centrifuged at 800 *g* for 10 min. The resulting supernatant (with washings) was labelled S_{1a}. The residue was rehomogenized with additional medium and recentrifuged to give a final residue N_b and its supernatant S_{1b}.

	N_b	S_{1a}	S_{1b}
Total nitrogen (mg.)	11·1	19·6	3·6
Succinic dehydrogenase (μmoles of ferricyanide reduced/hr.)	1·6	64·2	4·6
RNA P (μg.)	238	199	42·0
Lipid P (μg.)	282	550	37·8
Lipid P/RNA P	1·18	2·76	0·9

Table 6. *Distribution of phospholipids*

The results of three centrifugal fractionations for pregnant and four for ovariectomized animals are expressed as means ± S.E.M. per uterus. The values given for homogenate represent the sum of the values for individual fractions. Lipid P/RNA P ratios were calculated by using data from Table 4.

	Pregnant rat			Ovariectomized rat		
Fraction	(μg. of P)	(μg. of P/mg. of N)	Lipid P / RNA P	(μg. of P)	(μg. of P/mg. of N)	Lipid P / RNA P
N	458 ±9	22·2±4	1·21	15·1±1·7	14·4±0·9	1·41
M	84·9±38	60·4±13	5·66	3·4±0·5	26·6±7·0	2·67
P	291·3±74	96·0±17	3·13	12·4±1·6	68·8±4·7	3·30
S	81·7±21	6·1±2	1·19	8·0±1·0	20·6±2·8	16·0
Homogenate	916 ±125	23·4±2	1·64	38·9±3·4	22·9±2·7	2·4

and for skeletal muscle (Perry, 1952), there is also a small amount present in fraction M. The amount of DNA in fraction P was not determined since in one experiment it was found to be less than 1 %. The presence of DNA in fractions subsequent to N indicates incomplete sedimentation or partial fragmentation (or both) of some nuclei.

Between the two physiological states there is only a fivefold difference in the DNA content of the organ, whereas the RNA content falls after ovariectomy to a value less than 3 % of that at the end of pregnancy. Yet except for a relatively lower content in the final supernatant, the general pattern of RNA distribution is much the same; the greater part is found in fraction N and the microsomes also have a high content. The ratio of RNA P to total nitrogen is lower in all fractions after ovariectomy.

The possibility that the high RNA content of fraction N is due to incomplete cell breakage was tested by rehomogenizing this fraction repeatedly. With each homogenization a supernatant corresponding to S_1 (Wakid, 1960) was extracted and analysed for succinic dehydrogenase and RNA. Succinic dehydrogenase was taken as an index of the release of mitochondrial particles. The results of a typical experiment are shown in Table 5. A fractionating medium was selected which, according to Dr S. V. Perry (personal communication; see also Perry & Grey, 1956), has better potentialities than sucrose for extracting particles and protein from striated muscle. It is seen that about 40 % of the RNA is extracted with the first homogenization, and only a further 9 % with the second. That 90 % of the succinic-dehydrogenase activity is extracted during the first (routine) homogenization is indicative of a fair degree of cell breakage.

It may be argued, however, that homogenization has reached its limit with the type of homogenizer used, or that there is excessive agglutination of the microsomal elements. Now the microsomal fraction has the highest phospholipid concentration per milligram of nitrogen (Table 6); cytoplasmic granules are indeed known to be characteristically high in phospholipid content (Claude, 1943; Perry, 1952). If then fraction N and the cytoplasmic particles have about the same proportions of phospholipid and RNA it would be reasonable to deduce a high contamination of fraction N with these particles. The results in Tables 5 and 6, however, show that this may not be justifiable. The lipid P/RNA P ratio is actually lower in fraction N than in the cytoplasmic particles. Most of the RNA of the easily sedimentable fraction presumably must exist in a form different from the cytoplasmic particles as isolated. This is considered further in the Discussion.

The phospholipids are distributed in all fractions of rat myometrium. Their concentration per milligram of nitrogen is lower after ovariectomy in all fractions except S. The high concentration in the supernatant of the castrate organ is made obvious by the lipid floating at the surface of this fraction in the centrifuge tube.

DISCUSSION

In their review on the isolation of cell components Schneider & Hogeboom (1951) point out that in liver and kidney the mitochondria and the microsomes are of about equal bulk. With brain tissue the total nitrogen of the large granules exceeds that of the smaller granules (Wakid & Kerr, 1955; Jordan & March, 1956). In contrast, myometrium and skeletal muscle (rat sartorius in unpublished experiments) have a microsomal bulk about twice that of the mitochondria.

The small amount of succinic-dehydrogenase activity in fraction S is probably due to the liberation of the enzyme in soluble form by partial fragmentation of mitochondria during homogenization. The use of ferricyanide as electron acceptor has presumably permitted its detection in the soluble fraction. Kearney & Singer (1956) have observed that the native soluble succinic dehydrogenase will react directly with ferricyanide in the absence of all other components of the succinoxidase system.

In the experiments of Telfer (1953) and of Longwell & Reif (1955) oestradiol treatment resulted, after about 12 hr., in increased succinic-oxidase activity of the whole homogenate per gram dry wt. In the present experiments the whole homogenate was not tested; however, in fraction S_1 there is little change between the two physiological states in the specific activities of succinic dehydrogenase. Since fraction N has only small amounts of succinic-dehydrogenase activity (see Table 5), and since the percentage of nitrogen is only some 10% higher in fraction N after ovariectomy, it seems clear that there can be only a comparatively small difference in specific activity between the two whole homogenates. Yet, because the conditions are different, no real contradiction between these results and those of the above-mentioned authors is indicated: a short-term effect of oestrogen treatment of an oestrogen-deprived uterus appears to be increase in succinic oxidase, taking place at a greater rate than the general increase in protein.

That the specific activity of succinic dehydrogenase in S_1 does not vary between the two physiological states can be interpreted as follows. When the uterus at term, also under the influence of oestrogen, is compared with the castrate uterus we find that the hormonal conditions have been such that the greater activity of the dehydrogenase in the mitochondria is more or less balanced by the relatively increased amount of other proteins in the myometrium. Thus in S_1 of the gravid-uterus fraction M constitutes about 5% of the total nitrogen, whereas in S_1 from the castrate uterus fraction M makes up 18%; this difference in concentration of mitochondrial material would be even more marked if we take into account the fact that after ovariectomy a higher proportion of the mitochondrial material has sedimented in fraction P.

When the mitochondrial fraction in the uterus after ovariectomy is compared with the same fraction in the pregnant uterus, the following differences may be discerned. The mitochondria have different chemical constitutions (indicated by the changed relative content of nitrogen, RNA and phospholipid); there is possibly decrease in size of some of the mitochondria; the mitochondria are more crowded in the cell. The rapid increase in succinic-dehydrogenase activity in the whole organ which might be expected on injection of oestrone could depend on increase in mitochondrial size and numbers; from the present experiments it seems likely that increase in specific activity in the mitochondria themselves also plays an important part.

Phospholipid distribution, in general, is similar to that of rat liver (excluding fraction N, which is not analogous to the nuclear fraction). Ada (1949) found a high percentage in the small-granule fraction, and Huseby & Barnum (1950) also found this fraction to have a high concentration per gram wet weight.

The characteristically high RNA content of fraction N is perhaps not an artifact. The possibility that the nuclei themselves should contain such large amounts of RNA is not tenable, since the nuclei of most tissues have a RNA/DNA ratio less than one (Thomson, Heagy, Hutchison & Davidson, 1953; Schneider & Hogeboom, 1951). Two possible explanations, not mutually exclusive, may be considered. In the first place, electron micrographs (Shoenberg, 1958) show very close association between myofilaments and sarcoplasmic reticulum, and it would not be surprising if parts of this remained attached to the sedimenting filaments. Perry & Zydowo (1959*a*, *b*) have observed with rabbit muscle that upon fractionation in 0·25 M-sucrose about 60% of the RNA sediments at low centrifugal force in the nuclear–myofibrillar fraction. They suggest that the sarcoplasmic reticulum may be responsible for a large part of this RNA. In the second place, Shoenberg has drawn attention to dark patches present in the substance of the uterus myofilaments. On higher magnification these dark patches are resolved into dense granules identical in size with those present in the sarcoplasmic reticulum. Palade & Siekevitz (1956*a*, *b*) have shown these dense granules of the cytoplasmic reticulum in liver to consist of ribonucleoprotein. Although the possibility also exists, according to Shoenberg, that the dense granules may be glycogen particles, the greater probability is that they are ribonucleoprotein contributing to the high RNA content of the fraction

Table 7. *Relative amounts per cell*

The total values for RNA P, lipid P, succinic-dehydrogenase activity, total nitrogen and adenosine-triphosphatase activity are all expressed per milligram of DNA P for each physiological state. Data are from Tables 1, 2, 3, 4 and 6.

	Pregnant rat (*a*)	Ovariectomized rat (*b*)	(*a*)/(*b*)
Total RNA P (μg.)	2180	295	7·4
Total lipid P (μg.)	3590	708	5·1
Succinic dehydrogenase (μmoles/hr.)	254	55	4·6
Total nitrogen (mg.)	136	32	4·2
Adenosine triphosphatase (μmoles/hr.)	4510	2460	1·8

containing the myofilaments. These dense granules appear so embedded in the substance of the myofilaments that to attempt to liberate them by mere homogenization would be superfluous. The lower phospholipid/RNA ratio in fraction N compared with S_1 (Table 5) might be explained partly by the presence of the granular patches just mentioned and partly by the detachment of RNA particles from the phospholipid membranes of the sarcoplasmic reticulum during homogenization and their subsequent agglutination to the myofilaments.

It is already established from microscopic observation that the increase in size of the myometrium occurs essentially through an increase in cell dimensions, although a slight hyperplasia does occur without cell growth in early pregnancy (Reynolds, 1949). If the DNA content of the cell remains constant under various physiological conditions (Vendrely & Vendrely, 1949; Thomson *et al.* 1953), then referring all values to the total DNA of the organ would provide an index of the relative amounts per individual cell. From the values given by Needham & Cawkwell (1957) it can be calculated that the DNA content of the non-pregnant uterus (in oestrus) is intermediate between the values for pregnancy and castration. This not only implies some hyperplasia during pregnancy but also hypoplasia after ovariectomy. Thus Table 7 shows that, between the two extremes in physiological state, the individual cell does not suffer changes as drastic as those of the whole organ. RNA and phospholipids which are involved in the growth and metabolism of the cell (Jervell *et al.* 1958; Sinclair, 1934) diminish relatively more than the total nitrogen. On the other hand, although succinic-dehydrogenase activity diminishes to about one-fifth, adenosine-triphosphatase activity is not even halved. The function of the microsomal adenosine triphosphatase is unknown, and the explanation of its particularly high activity in the uterus of castrate animals remains for further research to settle.

SUMMARY

1. Cytoplasmic fractions obtained by differential centrifuging were studied in the rat myometrium, both at the end of pregnancy and 3 weeks after ovariectomy.

2. The general pattern of total-nitrogen distribution was similar in the two states; the main changes were that after ovariectomy a smaller percentage of the total nitrogen occurred in the final supernatant and a higher percentage in the particle fractions.

3. In the uterus at term succinic dehydrogenase was mainly found in the mitochondrial fraction (M). After ovariectomy the specific activity in M had fallen markedly whereas the specific activity in the microsomal fraction (P) was unchanged. The specific activity in the particles M and P taken together was lower than in pregnancy. The specific activity of Mg–adenosine triphosphatase was much higher after ovariectomy in fraction P.

4. Both in pregnancy and after ovariectomy over half of the total ribonucleic acid is centrifuged down with fraction N, the next richest fraction being P. It is probable that the high ribonucleic acid content of fraction N is not due solely to incomplete cell breakage, but that some of it at least is intimately associated with a cell component sedimentable at low speed.

5. After ovariectomy M and P both have lower ribonucleic acid-P/N and lipid-P/N ratios than those found at the end of pregnancy.

6. When compared on a cellular basis, ribonucleic acid, phospholipids, succinic dehydrogenase, total nitrogen and magnesium–adenosine triphosphatase all suffer a decrease after ovariectomy, the magnitude of this fall decreasing in the order given.

We are grateful to Dr S. V. Perry and Dr E. C. Webb for valuable discussions; to Dr H. B. F. Dixon for help in statistical analyses; and to Miss R. Leader for care of the rats. One of us (N.W.W.) wishes to thank the British Council for a scholarship and for research expenses during the period of this work. Thanks are also due to the Agricultural Research Council for grants (to D.M.N.).

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Some Aspects of the Metabolism of 5-Hydroxytryptamine

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(*Received* 31 *December* 1959)

The conversion of 5-hydroxytryptamine into 5-hydroxyindolyl-3-acetic acid by kidney and liver preparations is initiated by monoamine oxidase (Blaschko, 1952; Titus & Udenfriend, 1954), but there are indications that other enzymes may also be concerned with the metabolism of this base. For example, Dalgliesh & Dutton (1957) detected a number of unidentified indole derivatives after the perfusion of rat liver with 5-hydroxytryptamine and a number of compounds other than 5-hydroxyindolyl-3-acetic acid have been found in the urines of patients with malignant carcinoid disease characterized by high circulatory levels of 5-hydroxytryptamine (Snow, Lennard-Jones, Curzon & Stacey, 1955). Some of these substances might have arisen by entirely different metabolic pathways, and the present work was undertaken to investigate these possibilities.

A preliminary account of the results obtained when rat-liver preparations were incubated with 5-hydroxytryptamine has already appeared (Chadwick & Wilkinson, 1958).

EXPERIMENTAL

5-Hydroxytryptamine base. A solution of 5-hydroxytryptamine creatinine sulphate (May and Baker Ltd., 300 mg.) in 0·5 M-sodium borate buffer (pH 9–8; 30 ml.) was saturated with sodium chloride and the base extracted three times with butanol (20 ml.). The solvent was removed under 3 mm. Hg pressure and the residue dried at 20°. Yield: 90 mg.

Metabolism of 5-hydroxytryptamine by rat-liver homogenates. Male Wistar albino rats (100–150 g.), fed on M.R.C. cubed diet no. 41 (Bruce & Parkes, 1949) and tap water without restriction, were killed by neck dislocation. The venae cavae were cut to exsanguinate the livers, which were quickly removed and weighed in Sørensen 0·067 M-phosphate buffer (pH 7·4; 5 ml.) at 0–5°. Sufficient buffer was then added to give a buffer:liver ratio of 2:1 and the mixture was homogenized in a cold, Monel-metal Ato-Mix 100 homogenizer for 1½ min. The whole procedure occupied about 10 min.

The homogenate (18 ml.) was shaken with 0·01 M-5-hydroxytryptamine creatinine sulphate (2 ml.) in a Braun rotary Warburg apparatus at 37° in O_2 for 2 hr. The mixture was cooled to 0° and acidified to pH 4–5 with 50% (v/v) acetic acid. After 5 min. the mixture was centrifuged at 500 *g* for 15 min. Control preparations containing boiled homogenates and 5-hydroxytryptamine creatinine sulphate were similarly treated.

Indole derivatives were extracted from the supernatant by an adaptation of the method used by Dalgliesh (1956) for the isolation of urinary indoles. Charcoal (1 g.), partially deactivated with stearic acid, was shaken with the supernatant for 15 min. and separated by filtration. It