Glycine, Alanine, Valine, Leucine, Cystine	Hydrophobic
Hydroxyproline, Glutamic, Aspartic, Arginine, Lysine	Hydrophilic

No increase in reactive sulfhydryls upon activation could be shown.[4] The presence of the other amino acids is not easily tested with the protein intact. Arginine and lysine are the principal amino acids supplying reactive amino groups. A decrease in reactivity of these might be evidenced by the reaction of HCHO with protyrosinase and not with tyrosinase. This is not borne out, however, by the reaction with nitrous acid, where both protyrosinase and tyrosinase are affected (unpublished results).

Summary.—Differences in solubility of protyrosinase and tyrosinase in $(NH_4)_2SO_4$ exist. Protyrosinase is soluble in 0.9 per cent NaCl. Tyrosinase, however produced, is insoluble in 0.9 per cent NaCl and is differentially soluble in $(NH_4)_2SO_4$

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OVARIAN HORMONES AND THE IONIC BALANCE OF UTERINE MUSCLE

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Csapo and Corner[1] studied the tension developed by rabbit uterine strips in vitro as a function of the frequency of stimulation. They observed that the tension increased with increasing frequency of stimulation (positive staircase) if the uterus was taken from an estrous animal or from a castrate treated with estrogen. The uteri of early pregnant animals and those of castrates treated with progesterone following estrogen exhibited what they termed "negative staircase;" that is, the tension decreased with increasing frequency of stimulation. Csapo[2] proposed the

following hypothesis to explain the difference between estrogen- and progesterone-dominated muscles. The staircase of the estrogen-dominated uterus is similar to that of the frog heart, described by Bowditch and studied by Hajdu and Szent-Györgyi,[3] and the same mechanism as that proposed by the latter authors may be assumed to account for it. Like the frog heart, the estrogen muscle is thought of as having more intracellular K when at rest than is optimal for developing tension. On stimulation, K is lost and the tension increases. As an explanation of the negative staircase of the progesterone-dominated uterine muscle, Csapo[2] assumed that its intracellular K concentration is less than optimal at rest and will be even lower when K is lost on stimulation. Since changes in the intracellular K concentration are generally accompanied by similar, but opposite, changes in Na concentration, this theory implies a higher intracellular K:Na ratio under estrogen than under progesterone domination. Aside from adducing proof of this hypothesis, the determination of Na and K in the uterine muscle seemed to be of interest because of the scarcity of such data in the literature (Reynolds; also Rossenbeek; Wilkins; Talbot, Lowry, and Astwood).[4] The existing data refer more often to the whole uterus rather than to uterine muscle under specified hormonal conditions.

New Zealand White rabbits were used in all experiments.[5] The animals were either castrated mature females or infantile females not castrated. They were divided into three groups, labeled "estrogen," "estrogen withdrawal," and "progesterone." All three groups received daily injections of 25 μgm. natural estrogens in oil (Squibb) or 25 μgm. estradiol benzoate (Ciba) in 0.2-ml. volume intramuscularly. This treatment was continued for 7–8 days in the case of the castrated mature animals and for 12–15 days in the case of the infantile females. The estrogen animals were killed at the end of this treatment period. The estrogen-withdrawal group was killed 3–4 days after the last estrogen injection, during which time the animals were left untreated. The progesterone group received 1 mg. progesterone in oil (Squibb or Ciba) daily in 0.2-ml. volume for 3–4 days after the estrogen treatment was completed.

Uteri or uterine muscle samples will be referred to as "estrogen," "estrogen-withdrawal," or "progesterone" uteri or uterine muscle samples, according to the group of treated animals they came from.

The animals were anesthetized intravenously with Nembutal, and the uterus was removed, opened along the mesometrial attachment, wiped gently to remove blood and mucus, and divided into 0.1–0.4-gm. samples. Two to three of these samples served for the determination of dry weight and Na and K concentration. Another two to four were used for the determination of the inulin space. Results obtained on this material are tabulated under the heading "Whole Uterus," as contrasted with another set of data tabulated under the heading "Isolated Myometrium." This latter material, the uterine muscle proper, was obtained by removing the endometrium with a pair of fine scissors and forceps. This operation was carried out at room temperature within 3 minutes or less. The main part of the uterus was kept in a moist chamber while working on a sample. The endometrium was discarded, and the muscle divided again into 0.1–0.7-gm. samples. One larger sample served for the determination of Na and Cl space, two to four smaller samples for the determination of dry weight and Na and K content. Samples for the determination of inulin space were saturated with inulin before removing the endometrium.

For the determination of dry weight the samples were placed in a glass container with a ground-glass stopper. After noting the initial wet weight, drying was carried out in an electric oven at 105–115° C., the dry weight noted and expressed as the percentage of the wet weight. The same muscle samples were used for Na and K determinations.

The dried residues were extracted either with hot HNO_3 for about 30 minutes or with water or with a 30-mM $LiNO_3$ solution for 3–8 days in the refrigerator under occasional agitation. After appropriate dilution these extracts were read against a set of standards in either the Beckman flame attachment or in a Jenkee flame photometer using the Li internal standard. The standard stock solution had the following composition: 10 mM NaCl, 10 mM KH_2O_4, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$. The working standards were made by diluting this stock so as to obtain the following series: 0.1, 0.3, 0.5, 0.8, 1.0 mM K and Na. The use of standards containing both Na and K served to minimize error due to interference. Standards were read both before and after reading the unknowns, and the average of the two readings was plotted against the concentration. Also, the unknowns were read twice, from first to last and backward, and the average of the two readings was read off the standard curve prepared as described. This procedure was suggested by Gilbert Ling (oral communication) to minimize error due to the drift of the instrument. Results were tabulated as millimoles Na or K per kilogram wet weight.

Determination of the extracellular water (ECW) was carried out by three methods which were modifications of the technique described by Boyle *et al.*[6] For inulin-space determinations samples were soaked in inulin–Krebs solution. This solution contained Na-lactate instead of glucose, and correspondingly less NaCl. Recrystallized inulin was dissolved in the NaCl to yield a final concentration of 0.2 per cent after adding the other components of the Krebs solution. The solution was oxygenated with a mixture of 95 per cent O_2 plus 5 per cent CO_2 at room temperature. At the end of the soaking period the endometrium was removed. One sample of the muscle served for dry-weight determination to make correction for the eventual change in water content possible. Other samples were soaked in inulin-free Krebs, again containing Na-lactate instead of glucose, and correspondingly less NaCl. This solution was of a measured volume, and the gas mixture used to oxygenate it was previously saturated with water vapor to minimize evaporation. In preliminary experiments it was learned that 10–13 hours were necessary to obtain in the inulin-free solution all the inulin that was taken up by the muscle during the initial soaking. Therefore, both soaking periods were chosen to be 13 hours or longer. The inulin was determined by the method of Higashi and Peters[7] using the Coleman Junior or the Klett-Summerson colorimeter.

Determination of the Na and Cl space was carried out by estimating the amount of Na or Cl given off by a known weight of endometrium-free muscle to a given volume of 4 per cent glucose containing 30 mM $LiNO_3$. This solution was found to be isotonic with the uterine muscle in preliminary experiments using the percentage dry weight as a measure of movement of water through the cell boundaries. By taking samples of the bath at definite intervals and plotting the Na and Cl given off by the muscle against time, the amount of Na or Cl present at zero time may be estimated by extrapolating the second slope to zero. The volume of the extracellular water is then found by dividing this figure by the Na or Cl concentra-

tion of the serum to which the Donnan correction has been applied (Boyle *et al.*[6]). Na was estimated by the flame photometer (hence the necessity of the 30 mM $LiNO_3$ in the glucose). Chlorine was measured by the Vollhard titration. After the appropriate corrections, all three methods yielded the same extracellular water for the myometrium of a given uterus (Table 1).

TABLE 1
EXTRACELLULAR SPACE OF RABBIT UTERINE MUSCLE
COMPARISON OF METHODS

TREATMENT	RABBIT NO.	METHOD (PER CENT ECW) Inulin	Na	Cl	Average
Estrogen	121	36.5	..	..	36.5
	130	31.9	..	..	31.9
	131	36	..	..	36
	3	..	35.1	42.2	38.6
	4	..	36.1	..	36.1
Estrogen withdrawal	11	25.2	23.4	33.8	27.5
	12	(37.1)	27.8	..	27.8
	13	34.2	32.6	31.1	32.6
Progesterone	14	30.4	30.5	..	30.5
	15	24.2	25.9	..	25.0
	16	30.4	34	32.4	32.3

TABLE 2
NA AND K CONCENTRATIONS (MM/KG)

	WHOLE UTERUS Na	K	Per Cent ECW	ISOLATED MYOMETRIUM Na	K	Per Cent ECW
Estrogen:*						
$\bar{x}$	74.1	67.0	31.8	71.1	76.1	35.9
n	24	25	23	9	10	6
s_x	4.58	6.13	3.57	2.4	3.34	2.0
CVs_x	6.2	9.2	11.2	3.4	4.4	5.6
Progesterone:						
$\bar{x}$	66.7	70.8	35.0	70.6	73.0	29.2
n	29	29	9	16	16	10
s_x	5.9	6.64	1.72	5.23	5.55	3.56
CVs_x	8.8	9.4	4.9	7.4	7.6	12.3
Estrogen withdrawal:						
$\bar{x}$	...	...	...	60.4	70.8	27.0
n	...	...	...	6	7	8
s_x	...	...	...	7.96	3.38	2.74
CV_x	...	...	...	13.2	4.8	10.2

$\bar{x}$ = Arithmetic mean Na, K, and ECW, respectively, of samples from one and the same animal; n = Number of animals; x = Arithmetic mean, $\Sigma x/n$; s_x = Standard deviation, $\sqrt{\frac{\Sigma(x-\bar{x})^2}{n}}$; CV_x = Coefficient of variation, $\frac{100 s_x}{\bar{x}}$.

Calculation of the intracellular concentrations was carried out in the usual way, as follows: Total water per kilogram = 10 × (100 − per cent dry weight); ECW per kilogram = 10 × per cent ECW; ICW (intracellular water) per kilogram = Total water per kilogram − ECW per kilogram; amount of Na in the ECW = [(ECW per kilogram) × (concentration Na per liter ECW)]/1000; amount of Na in the ICW = Na per kilogram wet weight − amount of Na in the ECW; concentration of Na per liter ICW = amount of Na in the ICW per volume of the ICW. Concentration of K per liter ICW = mM K per kilogram wet weight over volume of ICW.

Experimental values of dry weight, ECW, Na, and K obtained on samples from the same animal were averaged to yield representative figures for that animal. These figures were in turn averaged for animals belonging to each of the three

groups: estrogen, estrogen withdrawal, and progesterone. These group averages ($\bar{x}$) are listed in Table 2, together with the number of animals they were obtained from (n). The table also contains the corresponding standard deviations (s_x) and coefficients of variation (CV_x). Dry-weight values are not tabulated, so as not to make Table 2 too crowded and also because the variation of this parameter is the smallest and has the least influence on the calculated intracellular concentrations. Actually, the dry-weight group averages (dry weight expressed as percentage of the wet weight) are 15.8 and 17.6 for estrogen and progesterone uteri, respectively. For the isolated myometrium the dry-weight group averages are 16.1, 17.0, and 15.7 for estrogen, estrogen-withdrawal, and progesterone animals, respectively.

Statistical analysis of Table 2 reveals the following: As far as the whole uterus goes, and difference between the ECW, Na, and K of the estrogen and progesterone uteri is significant within 97.5 per cent confidence limits by the method of t. By the same method, it is found that the ECW of the estrogen-withdrawal and progesterone myometrium is not significantly different, but both are lower than the ECW of the estrogen uterine muscle. This difference is significant within 99 per cent confidence limits. The difference in Na between estrogen and progesterone myometrium is not significant, but the Na concentration of the estrogen-withdrawal muscle is significantly lower within the 99 per cent confidence limits. The potassium concentration of the estrogen-withdrawal muscle is significantly different from that of the progesterone myometrium but is lower than the K concentration of the estrogen myometrium. The difference in the K concentration between estrogen and progesterone myometrium is without statistical significance.

TABLE 3

INTRACELLULAR ION CONCENTRATIONS

GROUP	mM/LITER ICW			RATIO [K/Na] ICW
	Na	K	K + Na	
Estrogen	30	158	188	5.3
Estrogen withdrawal	32	126	158	3.9
Progesterone	45	132	177	2.9

The figures of Table 2 tabulated under "myometrium" may now be used for the calculation of intracellular ion concentrations. The results of these calculations are found in Table 3. It may be seen from Table 3 that the intracellular Na concentration is 30 mM for the estrogen and estrogen-withdrawal myometrium and is 45 mM for the progesterone myometrium. The K concentrations are 158, 126, and 132 mM for the estrogen, estrogen-withdrawal, and progesterone muscle, respectively. From what was said in the previous paragraph about the significance of differences, it follows that the intracellular K concentrations of the estrogen-withdrawal and progesterone muscles must be regarded as respectively equal to and lower than the intracellular K concentration of the estrogen muscle. By the same reasoning, the intracellular Na concentration of the progesterone muscle is indeed higher than the intracellular Na concentration of the estrogen muscle, whereas the small difference between the estrogen and the estrogen-withdrawal muscle is probably insignificant.

It may be concluded, then, that progesterone muscles have a higher intracellular Na concentration and a lower intracellular K concentration than estrogen muscle. The decrease in the intracellular K concentration may conceivably be due to the

withdrawal of estrogen. The same cause does not account for the high intracellular Na concentration of the progesterone muscle.

The intracellular K:Na ratios are also shown in Table 3. This ratio is appreciably higher (5.3) for the estrogen than for the progesterone muscles (2.9).

The findings on the intracellular Na and K concentrations of the uterine muscle are in agreement with the hypothesis of Csapo, proposed as an explanation of the positive and negative staircase phenomena.

It is interesting to note that exactly the opposite conclusion would have been reached had the ECW, Na, and K data obtained on the whole uterus been used for calculation of the intracellular ion concentrations. Actually, comparison of the whole uterus with the isolated myometrium permits rough calculations for the endometrium.

Comparing the whole uterus with the isolated myometrium by the method of *t*, we get the following information: The Na concentration of the estrogen endometrium is the same as that of the myometrium within the errors of random sampling. Both the ECW and K of the endometrium are significantly less than those of the myometrium. By the same reasoning, the ECW of the progresterone endometrium is larger, its Na concentration is smaller than the corresponding parameters of the myometrium, and the K concentration is the same in both endometrium and myometrium.

If the proportionate weight of the endometrium were known, one could calculate exactly the ECW, Na, and K values for the endometrium from the data in Table 2. Since the proportionate weight of the endometrium is not known, different values were assumed for it. Calculating intracellular concentration directly from the "Whole Uterus" data of Table 2 amounts to assuming the limiting value of 1 for the weight fraction of the endometrium, i.e., calculating as if the whole uterus were endometrium. Substituting lower values also for the weight fraction, the following results were obtained, expressed at once as millimoles per liter ICW in the endometrium: Estrogen: Na, 45.6; K, 128; progresterone: Na, 24.1; K, 150.

It may be concluded, then, that whatever the assumed proportional weight of the endometrium may be, the estrogen endometrium always contains more Na and less K than the progesterone endometrium.

The ratio of intracellular K to Na is 2.8 or less for the estrogen, and 6.2 or more for the progesterone, endometrium. Using this ratio as a measure of the selective K accumulation of a tissue, it may be stated that the endometrium accumulates K more efficiently under progesterone than under estrogen domination. The reverse is true for the myometrium. One is led to the conclusion, therefore, that, although the ovarian hormones have a definite influence on the ionic balance of the uterine tissues, they do not determine invariably the selective K accumulation of the tissue. This function seems to depend just as much on the qualities of the tissue as on the dominating ovarian hormone.

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THE PARTICULATE ORGANIZATION OF THE CHROMOSOME

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In the simplest view, the organization of the genetic system should find its counterpart in the organization of the chromosome. If there exist discrete, particulate gene loci, the chromosome should correspondingly contain discrete, bonded particles which represent them.[1] If, as is implied in the classical view, genes are separable from each other and "point" effects have a different mechanism from "rearrangements," then there should exist chemical arrangements between the units that differ from those within them. In the opposing view of the chromosome as a genetic continuum in which discrete functional particles cannot be defined,[2] there is no reason to anticipate that the chromosome is composed of discrete and separable particles, though this is not excluded. It could be a biochemical continuum in the sense that no class of bonds characterizes the boundaries between units that differs from the bonds existing within the units. The distinction is experimentally meaningful when it is possible to define the conditions under which the chromosome can be broken down to smaller units and the units themselves can be examined. The most obvious way of determining how the chromosome is put together is to investigate the means of taking it apart.

Evidence for a Protein Continuum.—In common experience, the chromosome is a rather stable structure when viewed as a sample of matter. It can be manipulated within the living cell[3] and can be isolated from it by various means.[4] There is no reason to suppose that its continuity and integrity depend on dynamic conditions in the living cell, even though it is obvious that these conditions affect its appearance.

The possibility of dispersing the chromosome by means of chemical agents has been investigated rather extensively. Strong alkali[5, 6] and half-saturated urea in 0.5 *N* NaOH[6, 7] cause interesting changes, but the chromosome survives as an identifiable body. Very strong salt solutions remove a great deal of the DNA and protein, but a "residual chromosome," retaining the essential structural characteristics of the original, remains.[4]

Enzymes as tools for taking the chromosome apart have been exploited extensively since the earlier work of Caspersson[8] and Mazia and Jaeger[9] suggested that the continuity of the chromosome could be disrupted by means of proteolytic enzymes but not by enzymes that degrade and remove nucleic acids. The conditions of the rigorous use of this method have been defined by Kaufmann and co-workers.[10, 12]