found between males and females. In health, the bodies of women contain proportionately more fat and less muscle tissue than those of men(7); the lower values for Ke/wt in normal females are attributed to this factor. When thyrotoxicosis occurs in women, fat is catabolized in preference to muscle tissue, so that the relative fat content decreases; hence, the Ke/wt value initially would tend to remain unchanged or actually to increase. With more severe or protracted thyrotoxicosis, which exhausts the store of fat, a proportionately greater quantity of muscle tissue is eventually catabolized and the Ke/wt value decreases to subnormal levels. Such may have been the case in subject 7.

The results of the present study suggest that the alterations in the exchangeable potassium content noted in thyrotoxicosis can be adequately explained on the basis of the nonspecific catabolic effects of increased thyroid activity. There is no evidence in the present study to suggest a defect in the intracellular metabolism of potassium.

Summary. 1. A radioactive isotope of potassium (K^{42}) was used to determine the exchangeable potassium content (Ke) in 13 subjects with untreated thyrotoxicosis. The Ke values were lower in untreated thyrotoxic males than in normal male subjects. It is concluded that the low Ke values could be attributed to a decrease in the total muscle mass of the body, resulting from increased catabolism of body tissue. 2. In contrast to the results found in men, the values for Ke/wt in untreated women were normal, with one exception. This sex difference has been attributed to the dissimilarity in body composition normally found between males and females. It is postulated that in women mild or early thyrotoxicosis decreases the relative fat content of the body. Muscle tissue is extensively catabolized only after the store of fat has been depleted.

1. Corsa, L., Olney, J. M., Steenburg, R. W., Ball, M. R., and Moore, F. D., *J. Clin. Invest.*, 1950, v29, 1280.

2. Aikawa, J. K., Harrell, G. T., and Eisenberg, B., *ibid.*, 1952, v31, 367.

3. Edelman, I. S., Olney, J. M., James, A. H., Brooks, L., and Moore, F. D., *Science*, 1952, v115, 447.

4. Aikawa, J. K., Felts, J. H., Tyor, M. P., and Harrell, G. T., *J. Clin. Invest.*, 1952, v31, 743.

5. Mosher, R. E., Boyle, A. J., Bird, E. J., Jacobson, S. D., Batchelor, T. M., Iseri, L. J., and Myers, G. B., *Am. J. Clin. Path.*, 1949, v19, 461.

6. Aikawa, J. K., Felts, J. H., and Harrell, G. T., *J Clin. Invest.*, 1953, v32, 15.

7. Edelman, I. S., Haley, H. B., Schloerb, P. R., Sheldon, D. B., Friis-Hansen, B. J., Stoll, G., and Moore, F. D., *Surg., Gyn. and Ob.*, 1952, v95, 1.

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Aging Changes in Nucleic Acid and Protein-Forming Systems of the Virgin Mouse Uterus.* (20724)

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Several investigators(1-3) have established that nucleic acid concentration is much higher in rapidly growing embryonic tissue than in the corresponding adult tissue. Schulz(4) attempted to follow such a change in the mouse liver with aging but was unable to detect any changes in the concentration of either ribonucleic acid (RNA) or desoxyribonucleic acid (DNA). He expressed his results in terms of concentration of the nucleic acids per mg tissue. Since the extreme chromosomal polyploidy of liver tissue makes the use of RNA/DNA ratios of little use in so far as using them as an estimate of relative RNA per cell, it is possible that any change, if it did occur at the

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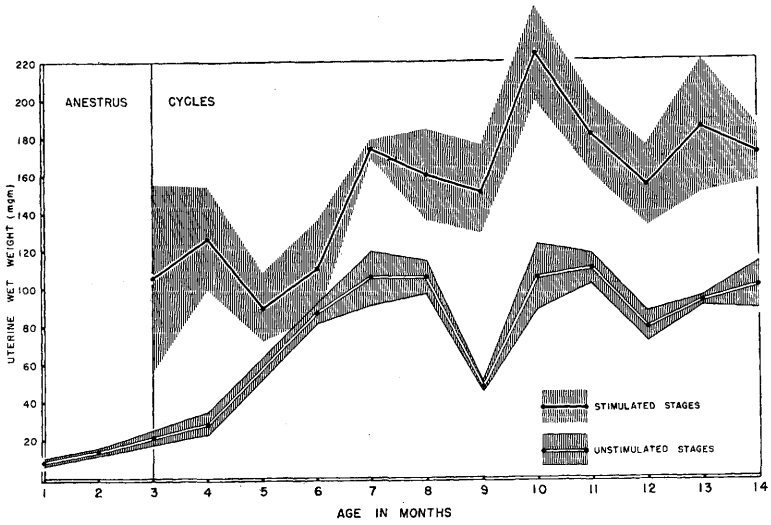

FIG. 1. Changes in wet weights of uteri from 1 to 14 mo of age. Stand. errors of the means represented by shaded areas.

cellular level, may have been masked. Earlier work(5-7) has shown that the use of the ratio between ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) gives useful information on the growth activity of the uterus in certain experimental and normal physiologic conditions. It was considered of interest to make a systematic study of these changes during the early maturation and subsequent aging of the mouse uterus to determine whether or not the aging process itself might be accompanied by a decrease in the amount of RNA per cell. In the course of this experiment data have been collected which indicate that there is a progressive decrease in the amount of RNA per cell in the uterus with age, and also that this cellular RNA, as expressed by the RNA/DNA ratio, bears a definite relationship to the uterine water content and to the amount of protein synthesized.

Methods. To eliminate any possible effects of pregnancy on the maturation process only virgin females of strain 129 mice were used in the following study. Groups of mice were sacrificed at monthly intervals from 1 to 14 months of age, a total of 247 animals being used. Animals were maintained on Purina Fox Checkers and water *ad libitum* and were routinely sacrificed between 8:30 and 10:30 A.M. to minimize any possible effects due to the time of feeding. At the time of sacrifice the stage of the estrous cycle was determined by a vaginal smear and the animals classified as being in proestrus, estrus, early metestrus, late metestrus or diestrus. Since not enough animals at any one stage of the estrous cycle were obtained at each age, the animals were grouped into two major classes, stimulated stages (proestrus, estrus, and early metestrus) and unstimulated stages (late metestrus and diestrus). For these reasons each of these two major groups was rather heterogeneous and so no attempt at a statistical analysis of differences between the stimulated and unstimulated stages at each age was made. The uteri were removed immediately after sacrifice and dissected free of adherring connective tissue. After weighing on a Roller-Smith torsion balance, they were homogenized in cold 5% trichloroacetic acid and processed prior to analysis as has been described previously(7). Individual uterine samples were then weighed on an analytical balance. Water content was calculated from the initial wet weight and the weight of these dehydrated and delipidated uteri. Prior to 3 months the uteri were too small for complete analysis on individual uteri and so uteri were pooled. Sub-samples of the dried residue were removed for protein nitrogen analysis by a micro-Kjeldahl method(8) and nucleic acids extracted from the remainder by the Schneider procedure(9). DNA in the extract was determined by the Dische(10) reaction and RNA by the Mejbaum(11) method.

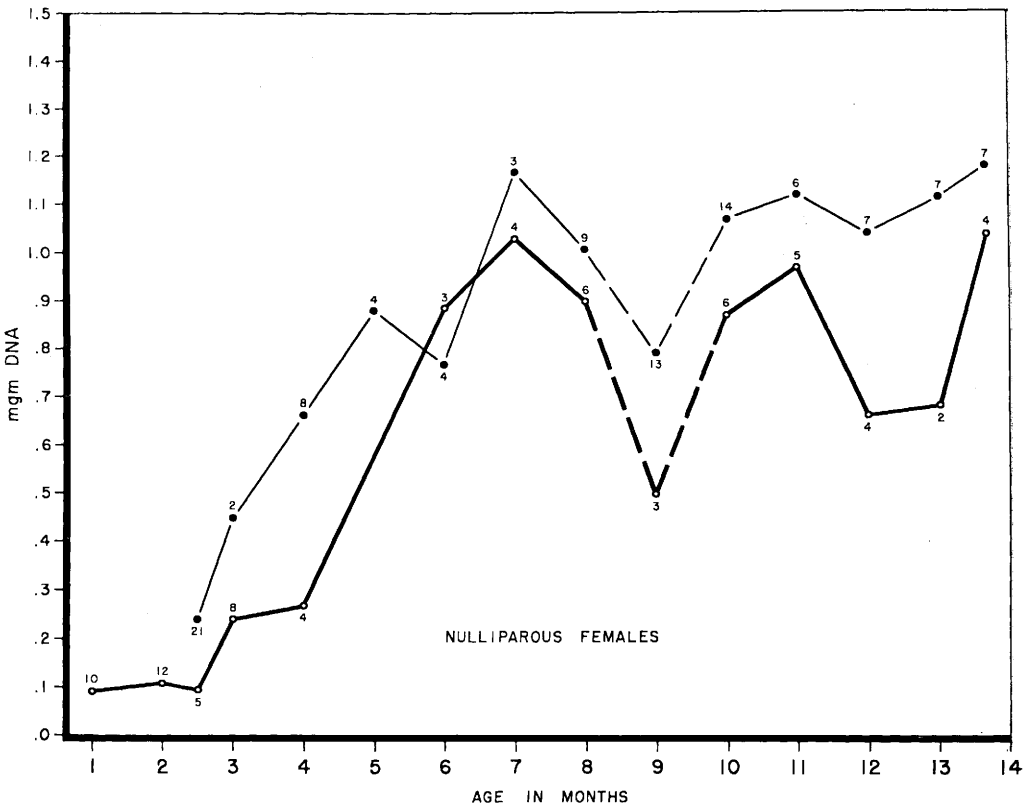

FIG. 2. Increase in amount of total uterine DNA from 1 to 14 mo of age in virgin mice. Heavy line—DNA at unstimulated stages; light line—DNA at stimulated stages. Numbers above each point indicate number of analyses at each point. Drop in the curve evident at 9 mo is at present unexplainable and is probably of no significance.

Results. Changes in mean uterine weight are presented in Fig. 1. However, it was felt that growth of the uteri could be followed more accurately by means of total uterine DNA as an indication of relative numbers of cells. Relative amounts of RNA per average cell were estimated by the use of the RNA/DNA ratio as proposed by Davidson(12). This assumes that the nuclear DNA remains constant within the cells and can be used validly in tissue where there is no large amount of polyploidy. That the DNA content of the cells of the rodent uterus remains constant during various conditions of hormonal stimulation has been shown by Alfert and Bern(13) using microspectrophotometric technics.

The increment of total uterine DNA is shown in Fig. 2. The lighter line represents the amount of DNA at the stimulated stages and the heavy line the amount at the unstimulated stages. At all ages the DNA values at the stimulated stages are somewhat greater than at the unstimulated stages. This is probably due to some extent to the infiltration of leukocytes during the late estrus and metestrus stages as well as to a slight increase in the number of uterine cells at these stages. From one to 2½ months the animals were in an anestrous condition so that only the values for unstimulated uteri are shown in this early period. It will be noted that the period of most rapid growth is from 2½ to 6 months.

Changes in the RNA/DNA ratio of these uteri during this same period of time are shown in Fig. 3. In all cases the ratio is higher at the stimulated stages than at the unstimulated stages. It is interesting that the greatest values of this ratio occur at the stimulated stages during this period of early maturation (*i.e.* from 2½ to 4 months). This coincides with the early period of most rapid uterine growth as evidenced by the increase in total DNA (Fig.

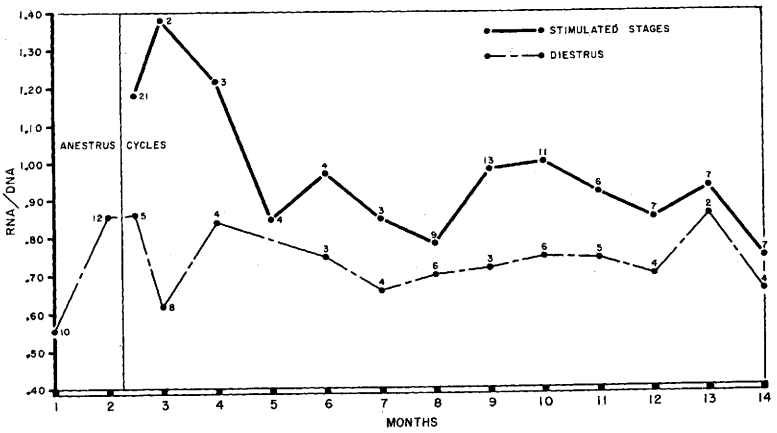

FIG. 3. Changes in RNA/DNA ratio of uterus at stimulated stages (solid line) and unstimulated, "diestrus" stages (broken line) of estrous cycle from 1 to 14 mo of age. Numbers above each point indicate number of analyses at each point.

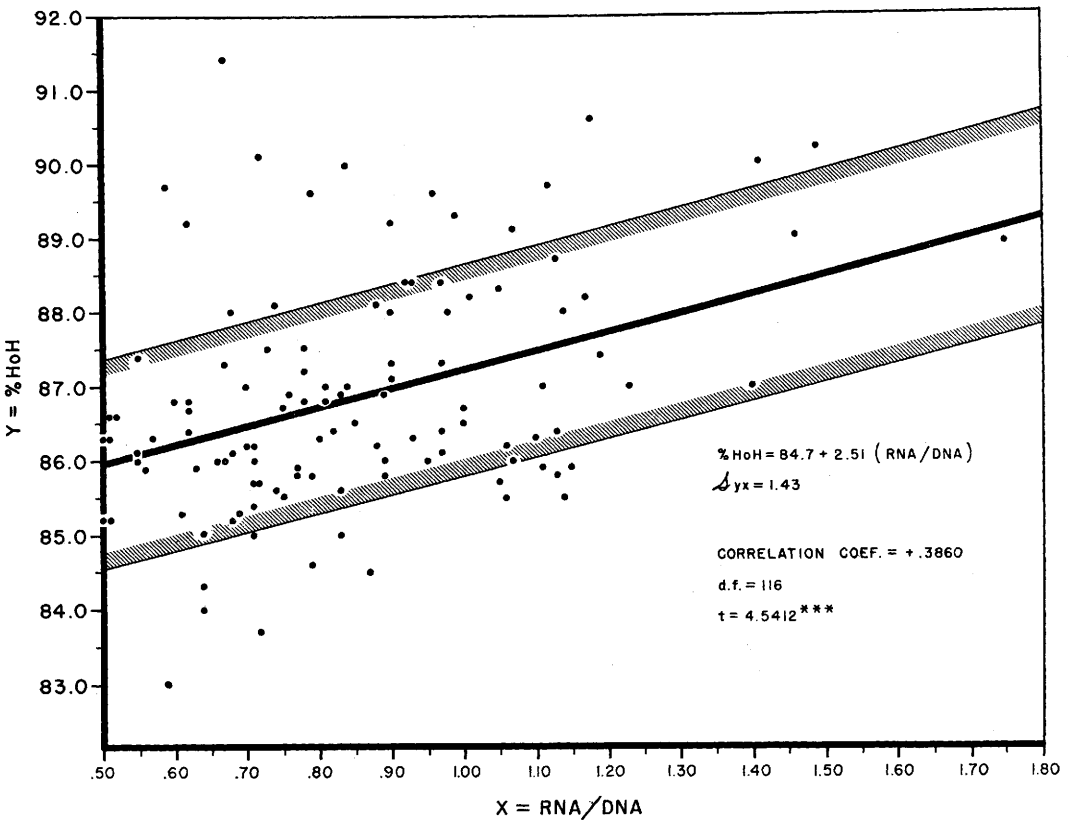

FIG. 4. Regression line of uterine water content on RNA/DNA ratio of uteri of mice from 3 to 14 mo of age. Stand. error of regression line is indicated by shaded outlines.

2). These animals showed vaginal cycles after 2½ months of age. However, corpora lutea were not found in the ovaries and ova not found in the oviducts upon histologic examination until this period of early rapid growth of the uterus was completed at 5 months of age(14). After this early period of rapid growth, the elevation of the RNA/

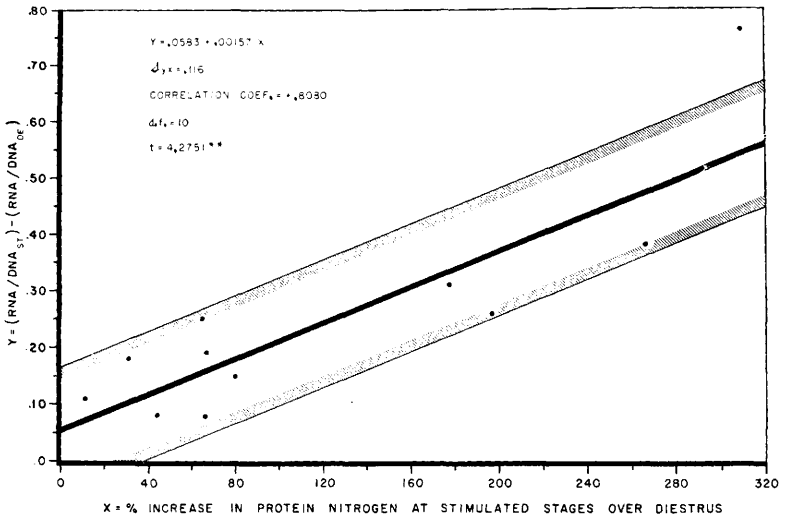

FIG. 5. Regression line of amount of increase in RNA/DNA ratio of uterus on percent increase in protein nitrogen at stimulated stages over the unstimulated "diestrus" stages. Stand. error of regression line is indicated by shaded outlines.

DNA ratio at the stimulated stages is not so pronounced and remains fairly constant until near the end of the age span studied here when it again appears to fall.

These changes in uterine RNA/DNA are paralleled by the changes in the uterine water content and in the amount of protein synthesized during the stimulated stages of the estrous cycle at any age. This results in the appearance of 1) a high degree of correlation between uterine water content and the uterine RNA/DNA ratio (Fig. 4), and 2) a correlation between *absolute increase* in the RNA/DNA ratio at the stimulated stages over the diestrus or unstimulated stages at a particular age and the amount of protein nitrogen synthesized (the percent increase of total protein nitrogen at the stimulated stages over that present at the unstimulated stages) (Fig. 5). For the correlation of RNA/DNA with uterine water content, values obtained on individual uterine samples of animals from 3 to 14 months were used. It was not possible to do this when the amount of increase in the RNA/DNA ratio at stimulated stages over unstimulated stages was to be correlated with the percentage increase in protein nitrogen. In this latter case, the average RNA/DNA ratio at the unstimulated stages was subtracted from the average ratio at the stimulated stages at each age to give the absolute amount of increase of the ratio. This value was then paired with the percentage increase of protein nitrogen at stimulated stages over unstimulated stages at each age, again utilizing average values. Such an analysis shows that a positive relationship exists between the RNA/DNA value of a single uterus and its water content ($r = +.3860$) and that the "t"-value of this correlation coefficient has a probability of less than .001. Likewise, the absolute increase in the RNA/DNA ratio is related to the percent of protein nitrogen synthesized ($r = +.8080$) and that the "t"-value of this correlation coefficient has a probability of less than .01.

Discussion. Since the early work of Caspersson(15) and Brachet(16) much evidence has been accumulated to show that ribonucleic acid in particular is linked in some manner with the synthesis of protein. The synthesis of new protein is undoubtedly one of the true requisites of growth and is therefore of importance in the maturation of aging of any organ. The uterus is a particularly interesting organ in which to study these changes since it is subjected to cyclic growth stimuli by the ovarian hormones. The data presented above seem to indicate that the early maturation and growth of the uterus of the mouse (from 2½ to 5 months in strain 129) occurs cyclically under the repeated stimuli of the early estrous cycles with their accompanying high levels of

estrogen. This is to be assumed from the fact that during this early period the very high levels reached by the RNA/DNA ratio of the uterus characteristic of these early months are reached only during the stimulated stages of the estrous cycle. The RNA/DNA values for the unstimulated stages of the cycle remain constant at about .70 to .80 throughout the entire period studied here (from 2½ to 14 months). The correlation obtained here between the increase in the amount of RNA per cell (the RNA/DNA ratio) and the percentage increase in protein nitrogen during the stimulated stages is merely further confirmation of the increase of cellular RNA at times of protein synthesis which has been noted previously by many other investigators in other types of biologic materials. That there might be a correlation between cellular RNA and water content might be expected from our general recognition of the fact that increase in water content of a tissue generally occurs during active growth(17,18). However, such a correlation between the RNA/DNA ratio and water content has not been demonstrated specifically previously.

Summary. Changes in the total DNA, protein nitrogen, the RNA/DNA ratio and water content have been studied in the uteri of mice of strain 129 from 1 to 14 months of age. RNA/DNA (an estimate of relative RNA per cell) at the stimulated stages of the estrous cycle is maximal at the time of maximal uterine growth, between 2½ and 5 months of age. The RNA/DNA of the uterus at the stimulated stages is higher than at diestrus at all ages. However, the differences between the diestrous values and that reached by the ratio at the stimulated stages slowly decreases with age. The cellular RNA content is significantly correlated with the water content of the uterus under the conditions studied here and is also positively correlated with the amount of protein nitrogen synthesized.

1. Davidson, J. N., and Waymouth, C., *Biochem. J.*, 1944, v38, 379.
2. Robertson, T., and Dawbarn, M., *Australian J. Exp. Biol. Med. Sci.*, 1929, v6, 261.
3. Andreason, E., *Acta Path. Microbiol. Scand. Suppl.*, 1943, v49, 11.
4. Schulz, D. M., *Arch. Biochem.*, 1950, v27, 57.
5. Stein, R. J., and Stuermer, U. M., *Am. J. Obstet. Gynecol.*, 1951, v61, 414.
6. Drasher, M. L., *J. Exp. Zool.*, 1952, v119, 333.
7. ———, *ibid.*, 1953, v122, 385.
8. Markham, R., *Biochem. J.*, 1942, v36, 790.
9. Schneider, W. C., *J. Biol. Chem.*, 1945, v161, 293.
10. Dische, Z., *Mikrochemie*, 1930, v8, 4.
11. Mejbaum, W., *Z. physiol. Chem.*, 1939, v36, 117.
12. Davidson, J. N., *Nature*, 1949, v164, 984.
13. Alfert, M., and Bern, H. A., *Proc. Nat. Acad. Sci.*, 1951, v37, 202.
14. Fekete, E., personal communication.
15. Caspersson, T., *Naturwiss.*, 1941, v29, 33.
16. Brachet, J., *Enzymologia*, 1941, v10, 87.
17. Lowrey, L. G., *Anat. Rec.*, 1913, v7, 143.
18. Brachet, J., *Chemical Embryology*, 1950, Interscience Publishers, Inc., New York.

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Inactivation of Histamine by Lung Tissue from Histamine-Sensitive and Histamine-Resistant Mice.* (20725)

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Mice are naturally resistant to histamine. Inoculation with *H. pertussis* vaccine greatly increases the sensitivity of these animals to histamine(1,2), to bacterial vaccines(2), and to anaphylactic shock(2,3). It appeared possible that *H. pertussis* vaccine might sensitize mice to the lethal effects of histamine by altering the ability of the animals to inactivate

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