tude of the Lactobacillus count and the relative rate of caries progression that one cannot consider the test as definitive within this group for the diagnosis or the prognosis of caries activity for the individual subject.

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17247. Nucleoproteins and the Cytological Chemistry of *Paramecium* Nuclei.*

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There are many exceptions to the classical definition of a cell as "a mass of protoplasm containing a nucleus."[1] Numerous examples are known of binucleate and multinucleate protoplasmic masses. In most cases the nuclei are all alike, but in binucleate ciliated protozoa such as *Paramecium*, the two nuclei are markedly different in size, and are therefore known as the macronucleus and the micronucleus. There are also considerable differences in behavior of these two nuclei. It is the general view that the macronucleus is a "somatic nucleus" serving the indispensable physiological needs of the organism in vegetative stages when no detectable effect of the micronucleus can be observed.[2] The micronucleus appears to be of chief importance as a potentially germinal structure, functioning in the nuclear interchange and reorganization which take place at conjugation or autogamy.[2] The present paper briefly reports the results of a study of the nucleoprotein composition of macro- and micronuclei in vegetative individuals of *Paramecium*; experiments were designed to answer the question whether in these stages the two nuclei are as unlike as other experimental evidence indicates them to be.

Experimental. The chemical constituents of nuclei are mainly nucleoproteins, and the quantitative technics of cytological chemistry are particularly suited to analysis and localization of these substances within individual nuclei. The procedures used in this investigation permit the computation of relative concentrations of nucleic acids and proteins from specific chemical reactions which have been shown to be applicable to properly fixed cytological preparations. Analyses were made by microscopic photometry of total and nonhistone protein from modifications of the Millon reaction,[3,4] of desoxyribose nucleic acid (DNA) from the Feulgen nucleal reaction,[5] of total nucleic acids and polynucleotides (removable with hot trichloroacetic acid) from the absorption in the ultraviolet (near 260 mμ) of their purine and pyrimidine bases[6,3] and of ribose nucleic acid (RNA) by difference in absorption at 260 mμ after enzymatic digestion with protease-free ribonuclease.[7]

Determinations were made on a culture of *Paramecium caudatum* which was raised on a baked lettuce medium inoculated with *A. aerogenes*[8] and kept under constant feeding

* Part of the research reported here was carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

1 Wilson, E. B., The Cell in Development and Heredity, 1928, 3rd edition, New York.

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3 Pollister, A. W., and Ris, H., *Cold Spring Harbor Symposium on Quant. Biol.*, 1947, **12**, 147.

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6 Caspersson, T., *Skand. Arch. Physiol.*, 1936, **73**, suppl. 8, 1.

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8 Sonneborn, T. M., and Dippell, R., *Physiol. Zool.*, 1946, **19**, 1.

TABLE I.
Comparison of Nucleic Acid and Protein Contents of the Macro- and Micronucleus of *Paramecium caudatum*.

	Total protein	Desoxyribose nucleic acid	Ribose nucleic acid
A. % macronuclear nucleoprotein determined	85.1	5.8	9.1
B. % micronuclear nucleoprotein determined	87.6	4.2	8.2
C. Ratio concentrations: Macronucleus / Micronucleus	0.9	1.2	1.0

and environmental conditions. At no time during the life of the culture was autogamy or conjugation observed; all individuals were in a "vegetative" state. The culture was concentrated by mild centrifugation, fixed in Carnoy's 1:3 acetic acid-alcohol, transferred to small agar blocks and imbedded in paraffin. Analyses were carried out on serial sections mounted on slides in the usual manner.

Mean extinction values ($\log_{10} I_0/I$) were computed from transmission measurements of cylindrical areas of a large number (30-50) of random sections of macronuclei, and of 8-12 entire micronuclei. The results were fairly uniform, as shown by the fact that the standard errors in no case exceeded 7% of the mean extinctions. From these mean values concentrations were computed by reference to standards measured on known dilute solutions in a Beckman Spectrophotometer. An approximation to the protein concentration was computed by assuming that the Millon reaction was due to a tyrosine derivative which constituted one-sixteenth (6.25%) of the protein. The total nucleoprotein concentration was computed by adding together the mean concentrations of protein, desoxyribose nucleic acid (DNA), and ribose nucleic acid (RNA). Table I (A and B) shows the percentages of the total concentration represented by each of these components.

Results. It is apparent from the data that protein is the major component of the nucleus (Table I), comprising over 85% of the nucleoprotein determined for each nucleus. A comparison of the extinctions of nuclei prepared to show total and non-histone proteins showed no significant difference and it followed that histone could not have comprised more than a few percent of the nucleoprotein. The protein/DNA ratio was from 15-20 to 1, in the range of high values reported by Pollister and Leuchtenberger[4] for metabolic nuclei of metazoa. The concentration of DNA present in the nuclei was quite comparable with determinations of other workers, being in the order of 10^{-12} mg per cubic micron. Most remarkable, however, are the results of digestion with purified crystalline ribonuclease[9] which show that about 60% of the total nucleotide extinction was lost after treatment and that a large quantity of RNA was consequently present. The RNA released by the enzyme amounted to almost twice the DNA present in the nucleus and comprised about 10% of the nucleoprotein moiety. That RNA might be contained in the macronucleus of *Paramecium* has already been indicated by the staining experiments of Shubnikova[10] and others who, with methyl green and pyronin (unpurified), showed pyronin coloring in the macronucleus removable with ribonuclease (unpurified). RNA is known to be a component of metazoan nuclei both in the nucleolus and in the chromosomes. Mirsky has indicated by his analyses of isolated mammalian chromosomes that the RNA which is a component of the residual chromosome, is high in active metabolizing cells and low in relatively inactive ones.[11]

Discussion. It can be seen from the ratios

[9] McDonald, M., *J. Gen. Physiol.*, 1948, **82**, 33.

[10] Shubnikova, E., *C. R. Acad. Sci. URSS*, 1947, **55**, 517.

of concentrations in Table I, row C, that the macro- and micronuclei are very much alike in nucleoprotein composition. It follows, then, that insofar as their chemical composition is indicative of their function, both nuclei are performing similar roles in the vegetative individual. Thus, the organism's dependence on the macronucleus and its apparent independence of the micronucleus during these stages[2] simply becomes a matter of the preponderance of the physiological-genetic material in the large macronucleus. There is a marked resemblance between these protozoan nuclei and highly metabolic nuclei of metazoa, as typified by the mammalian liver nucleus, which is strong evidence for the active participation of both macro- and micronucleus in metabolic functions of the cell. Any chemical differences which may serve to be correlated with the capacity of the micronucleus to differentiate into a special reproductive structure must be looked for at some other time in the organism's history. Presumably this is during the reproductive processes of conjugation and autogamy, when the behavior and morphology of the two nuclei are most markedly dissimilar.

Summary. Quantitative cytological chemical analyses of the nucleoprotein composition of the macro- and micronuclei of a clone of *P. caudatum* show that both nuclei contain protein, RNA and DNA in the ratio of 20:2:1. Twice as much RNA as DNA was found. From their resemblances to the nucleoprotein contents of metabolic metazoan nuclei, it is concluded that in the vegetative stage, both nuclei of *P. caudatum* are actively metabolic.

[11] Mirsky, A. E., *Cold Spring Harbor Symposium on Quant. Biol.*, 1947, **12**, 143.

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17248. Influence of Level of Adrenal Cortical Steroids on Sensitivity of Mice to X Irradiation.

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We reported previously that X radiation appeared to result in an increased demand for the adrenal cortical hormone.[1] Subsequent studies indicated that the adrenal changes noted after total-body X irradiation of rats (decreased cholesterol content and increased weight) were mediated by the pituitary.[2] The adrenal response could be prevented, in part, by suitable administration of adrenal cortical extract. However, such treatment failed to alter survival.[3] Since Ellinger[4] reported earlier that the daily injection of desoxycorticosterone increased survival of irradiated mice, it seemed desirable to extend these observations in order to clarify the role of the adrenal in the acute radiation syndrome. In these experiments we have determined the radiosensitivity of intact mice with and without whole adrenal cortical extract or desoxycorticosterone. Data on the sensitivity of adrenalectomized mice which were obtained as part of another study have been included for comparison.

Materials and Methods. Male and female CFI mice, weighing 18 to 28 g each, were the experimental animals. Mice of the same sex were used in individual experiments. For the irradiation, the animals were placed in individual cellulose acetate exposure cells and were given total-body exposures in groups of 16. The cells were rotated slowly on an electrically-driven turntable to insure equivalent irradiation of all the animals. Equal numbers of control and experimental mice

[1] Patt, H. M., Swift, M. N., Tyree, E. B., and John, E. S., *Am. J. Physiol.*, 1947, **150**, 480.

[2] Patt, H. M., Swift, M. N., Tyree, E. B., and Straube, R. L., *Science*, 1948, **108**, 475.

[3] Swift, M. N., Patt, H. M., and Tyree, E. B., *Fed. Proc.*, 1948, **7**, 121.

[4] Ellinger, F., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 31.