Chromosome Size in Normal Rat Organs in Relation to B Vitamins, Ribonucleic Acid, and Nuclear Volume

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Rat chromosomes vary considerably in volume from one normal cell type to another. The relationship of their size to the concentration of the B vitamins and ribonucleic acid, and to nuclear volume, should aid in explaining the significance of the morphological differences between chromosomes of cancer cells and those of normal tissues (2, 6, 7) that are already evident by the third day of epidermal carcinogenesis in mice (5).

There is a direct relation between average chromosome volume and concentration of B vitamins in normal rat organs that promises to make more vivid our conception of the role played by the chromosomes as vital organelles in somatic cells. The study of cytoplasmic concentration of ribonucleic acid should allow us to determine whether excess amounts of nucleic acid in the cell and on the chromosomes could be responsible for the increased size noted in cancer chromosomes (2, 5, 6, 7). Since the greater size seems rather to be a reflection of a multiple structure, it is of interest to determine whether chromosomes of the same double nature as cancer chromosomes occur in normal organs. To this end a study of plasmosome numbers and nuclear volumes was made, although nuclear volume turned out to be not strictly proportional to the total volume of the mitotic chromosomes.

MATERIALS AND METHODS

The organs used in this study were taken from 4 rats,1 of which Rat A was a 2 day old male of the Wistar strain, Rats B and C were adult Osborne-Mendel males bearing transplants of hepatoma 31, and Rat D was a Wistar male 84 days old.

In order to determine metaphasic chromosome volumes, nuclear volumes and plasmosome numbers of resting nuclei, acetocarmine preparations were made after fixation in Carnoy’s fluid. The 25 best mitotic figures of metaphase or late prophase found in the preparations of each organ were drawn under oil immersion with a camera lucida at a drawing magnification of 3,000. An average chromosome volume was computed according to the method previously described (6, 7) for each of 350 approximately metaphasic figures. Depending on the variability of nuclear volume, 50 or 100 resting nuclei were measured with the aid of a camera lucida, millimeter ruler, and the fine adjustment of the microscope, and their volumes were calculated as the average of the volumes of a short cylinder and an ellipsoid of the dimensions found (7). When possible, the number of plasmosomes was counted in each resting nucleus measured. In so far as occasional fusion of plasmosomes was revealed by lobing of large plasmosomes, each lobe was counted as a single plasmosome. In addition, volumes were computed and plasmosomes were counted in 200 resting nuclei of regenerating liver and 400 nuclei of control adult livers that furnished the chromosomes of another study (3).

Ribonucleic acid was demonstrated in tissues by the histochemical method of Brachet (9). First, pieces of a number of organs of Rat D were preserved in Helly’s fixative. Experimental slides of each organ were incubated in a solution of ribonuclease2 of a concentration of 0.2 mgm. per cm.2 in McIlvaine’s

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1 For this ribonuclease, which was salt-free and 5 times recrystallized, I am greatly indebted to Dr. M. Kunitz, of the Rockefeller Institute for Medical Research, Princeton.
citric acid disodium phosphate buffer solution of pH 7.0, since Kunitz has found that the optimum pH range for ribonuclease is 7.0 to 8.3 (28). The incubation was allowed to proceed for 2 hours and 20 minutes at 50°C ± 2°C in a water bath. Control slides were treated just as the experimental except that no ribonuclease was added to the buffer solution. After incubation, the slides were passed through 2 changes of distilled water, and experimental and control slides were placed together in the same container and stained with Unna’s carbol pyronin methyl green stain at 40°C for 10 minutes. Later another set of control and experimental slides was incubated and stained. Incubation lasted only 2 hours, but otherwise the procedure was the same.

Pyronin methyl green stains chromatin of the resting nucleus, as well as the chromosomes, a blue-green or purple, while the red stain of the pyronin is located in the cytoplasm and the plasmosomes. Brachet (9) found that the basic dye, pyronin, often failed to stain tissue sections after ribonuclease digestion.

The control and experimental slides from the ribonuclease digestions were compared side by side with a dissecting microscope, and slides of the various organs were likewise compared for relative degree of cytoplasmic basophilia. The best that could be done by this method was to arrange the organs in a series of intensities of enzyme-preventable stain.

RESULTS

The results of the determinations of chromosome volume are summarized in Table I. The range of average chromosome volume that characterizes each organ is well maintained from one animal to another in the adult group, as can be seen in the kidney and liver, even in regeneration of the latter (3). Chromosome size in a given organ may be different for different stages of development. The liver and kidney chromosomes increase about one-half in average volume from the 2-day male to the adult male rat, but the chromosomes of the small intestine stay about as small in the adult as they were in the young animal. Chromosome size is more uniform in the young animal than in the adult; this suggests a still greater uniformity in the embryo. Bimodality in the frequency distribution of average chromosome volumes in the adult liver, attributed previously (3) to several cell types, is again found. The following seriation of chromosome sizes is evident in the adult animals: beginning with the

### Table 1: Distribution of Metaphase Figures According to Average Chromosome Volume

<table>
<thead>
<tr>
<th>Average volume of chromosomes (μm²)</th>
<th>Sm. Int.</th>
<th>Testis</th>
<th>Skin</th>
<th>Kidney</th>
<th>Liver</th>
<th>Spleen</th>
<th>Sm. Int.</th>
<th>Lung</th>
<th>Kidney</th>
<th>Liver</th>
<th>Rat B</th>
<th>Liver</th>
<th>Rat C</th>
<th>Kidney</th>
<th>Adrenal</th>
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<td>0.2-0.3</td>
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<tr>
<td>Av. Vol. (μm³)</td>
<td>0.55</td>
<td>0.61</td>
<td>0.63</td>
<td>0.64</td>
<td>0.72</td>
<td>0.46</td>
<td>0.50</td>
<td>0.51</td>
<td>0.93</td>
<td>1.12</td>
<td>0.93</td>
<td>0.96</td>
<td>0.85</td>
<td>0.72</td>
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<tr>
<td>St. Dev.</td>
<td>±0.12</td>
<td>0.07</td>
<td>0.08</td>
<td>0.09</td>
<td>0.16</td>
<td>0.10</td>
<td>0.12</td>
<td>0.11</td>
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<td>0.27</td>
<td>0.31</td>
<td>0.16</td>
<td>0.13</td>
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Biele—Chromosome Size in Normal Rat Organs

Fig. 1.—From small intestine of 2 day old Rat A; 42 chromosomes, average volume 0.6 cubic micron; diploid.

Fig. 2.—From testis of Rat A; 41 chromosomes, average volume 0.6 cubic micron.

Fig. 3.—From skin of Rat A; 43 chromosomes, average volume 0.7 cubic micron.

Fig. 4.—From kidney of Rat A; 44 chromosomes, average volume 0.6 cubic micron.

Fig. 5.—From lung of 84 day old Rat D; 44 chromosomes, average volume 0.5 cubic micron.

Fig. 6.—From spleen of Rat D; 42 chromosomes, average volume 0.4 cubic micron.

Fig. 7.—From small intestine of Rat D; 40 chromosomes, average volume 0.5 cubic micron.

Fig. 8.—From adrenal of adult Rat C; 42 chromosomes, average volume 0.7 cubic micron.

Fig. 9.—From kidney of Rat C; 42 chromosomes, average volume 0.7 cubic micron.

Fig. 10.—From kidney of Rat D; 41 chromosomes, average volume 0.9 cubic micron.

Fig. 11.—From liver of Rat A; 37 chromosomes, average volume 0.9 cubic micron.

Fig. 12.—From liver of Rat A; 174 chromosomes, average volume 0.7 cubic micron; octoploid.

Fig. 13.—From liver of Rat C; 42 chromosomes, average volume 0.7 cubic micron.

Fig. 14.—From liver of Rat B; 40 chromosomes, average volume 0.8 cubic micron.

Fig. 15.—From liver of Rat D; 42 chromosomes, average volume 1.2 cubic micron.

Fig. 16.—From liver of Rat C; 81 chromosomes, average volume 1.3 cubic micron.

Fig. 17.—From liver of Rat D; 41 chromosomes, average volume 1.3 cubic micron.

Fig. 18.—From liver of Rat D; 84 chromosomes, average volume 3.5 cubic micron; tetraploid.

The seriation of the rat organs with respect to average chromosome volume is maintained in the B vitamin assays of organs of Wistar rats made by Mitchell and Isbell (33) and Taylor, Pollack, and Williams (37); approximately similar seriations of organs were found for all the individual B vitamins studied except inositol and folic acid. It is known, or thought probable, that nicotinic acid, thiamin, riboflavin, pantothenic acid, pyridoxin, and biotin are closely related to enzymes; inositol, on the other hand, seems to be concerned structurally in phospholipid formation (8). Since chromosomes have been looked on as enzyme factories (43), inositol was not considered in drawing up the series of organs according to total B vitamin content. If the weights of thiamin, riboflavin, nicotinic acid, pantothenic acid, pyridoxin, biotin, and folic acid given by Mitchell and Isbell (33) be added together for the individual organs, the following series in descending order of vitamin concentration is obtained: liver, kidney, heart, muscle, spleen, brain, lung, in approximately the proportion of 10:6:6:3:3:3:2 respectively. From the graphs of Taylor, Pollack, and Williams (37) the descending series of vitamin concentration, again excluding inositol, may be read off: liver, kidney, heart, adrenal gland, brain, spleen, lung, muscle. Combining the 2 series and omitting the organs in which no mitoses were found, we have the master series of liver, kidney, adrenal, spleen, and lung for comparison with the chromosomal series. The total quantities of vitamins are reasonably different from one another, with the exception of the spleen and the lung. The series of organs with respect to decreasing average chromosome volume is liver, kidney, adrenal, lung, and spleen, but the values of the last 2 are not significantly different. The total B vitamin content has also been determined for fetal rat liver (40). It is about half that of the adult liver. Since the liver chromosomes of the newborn rat are considerably smaller than those of other organs,
the adult, it may be assumed that chromosomes of the embryonic liver are likewise small and that here again there is a direct relation between chromosome size and B vitamin content of the organ.

In both incubations with ribonuclease the enzyme was effective in removing material with affinity for the basic dye, pyronin. In some tissues of which the experimental slides had more than a modicum of stain there must have been acid material other than ribonucleic acid that stained with pyronin. Ribonuclease digestion had no effect on the pyronin-staining properties of the mucin of goblet cells or of the granules in the large mast cells of the heart and lung and in the small mast cells of the villi. The stained materials in these cases are sulfuric acid esters of complex polysaccharides (14). It was found that the ribonuclease digestion had left very little of the other material, presumably ribonucleic acid, that stained with pyronin in the pancreas, the epithelium of the small intestine, the lung, and the spleen. Ribonucleic acid seemed to be concentrated in the pancreas in the cytoplasm near the nuclei of the acinar cells, and there was a little in the zymogen granules of the distal cytoplasm. In the lung ribonucleic acid was distributed in good quantity through the cytoplasm of the cells of the interalveolar septa and the epithelium of the bronchioles. In the small intestine ribonucleic acid was most concentrated in the 2 mitochondrial zones of the cells of the intestinal epithelium. In the spleen the concentration of the acid was low and variable; the cell types corresponding to the r-cells of Bryson (10), with relatively large, pale nuclei and visible plasmosomes (especially the megakaryocytes) contained more cytoplasmic ribonucleic acid than did the cell types, such as the lymphocytes, in which the nucleic acid was chiefly intranuclear and of the desoxyribose type. The liver control slides were stained about 4 times as intensely as the experimental; ribonucleic acid was found in the nucleoli and in rough masses in the cytoplasm of the hepatic cells, especially near the nuclei. The kidney sections stained perhaps one-third as heavily with pyronin after ribonuclease digestion as without the enzyme treatment; ribonucleic acid seemed to be diffusely distributed through the cytoplasm of the renal tubule cells, but the pyronin stain was very weak in the glomeruli and the endothelium of the blood vessels. In the heart ribonuclease digestion eradicated only a small part of the moderate staining capacity of the muscle cells.

The following series of organs was found in both digestion experiments in order of decreasing concentration of ribonucleic acid, i.e., of stainable material removable by ribonuclease: pancreas, lung, small intestine, liver, kidney, spleen, and heart. The liver and kidney seemed to have about the same concentration of ribonucleic acid; although the total intensity of stain may have been slightly greater in the kidney, a greater proportion of stainable material was removed by ribonuclease from the liver. In view of the different species used, this series is not made improbable by the fact that Davidson and Waymouth (15) found adult sheep organs to have the following order of decreasing concentration of ribonucleic acid: testis, gut, kidney cortex, lung, spleen, liver, brain, heart, muscle, and thyroid.

The series of organs in order of decreasing average chromosome volume: liver, kidney, lung, small intestine, and spleen, is unlike that for ribonucleic acid concentration: lung, small intestine, liver, kidney, and spleen. Therefore the concentration of polynucleotides in the cytoplasm does not determine the size of chromosomes in mitosis, nor does the size of chromosomes determine the concentration of ribonucleic acid.

The data on nuclear volumes, their frequency, and the number of plasmosomes in nuclei of different volume-groups are included in Figs. 19 to 24.

The organs studied in 2 day old Rat A have essentially unimodal distributions of nuclear volumes. The average nuclear volumes are 390 cubic microns for the small intestine; 401 for the kidney; 411 for the testis; 440 for 44 epidermal nuclei and 477 for 6 dermal nuclei of the skin; and 501 for the liver, exclusive of the one nucleus of 1,220 cubic microns found. Since this large nucleus contained 12 plasmosomes, a number double the maximum found in the others, it was probably made up of a tetraploid number of chromosomes instead of the diploid number presumably in the others. Besides 24 approximately diploid metaphase figures drawn from Rat A liver, one metaphase (Fig. 12) was found with 174 chromosomes, nearly the octoploid number, 168. Evidently a small degree of polyploidy is already present in the liver of the rat soon after birth.

All 5 organs of the very young rat show 6 as the maximum number of plasmosomes, with the exception of the single presumably tetraploid nucleus of the liver. Apparently, then, there are 3 plasmosome-bearing chromosomes in the haploid set of the rat.

Like the chromosomes, the nuclei in the 2 day rat have a restricted range of size. The liver shows the largest chromosomes and also has the largest nuclei. The small intestine has not only the smallest average nuclear volume, but also the smallest average chromosome volume. To a certain degree, then, we see upheld here Jacoby's assumption (26) of a direct proportionality between nuclear and chromosomal size in different tissues of the same animal. The nuclei of these 5 organs differ with respect to amount of stainable chromatin and development of plasmosomes. The liver nuclei have the best development of chromatin and nucleoli. The nuclei of the small intestine have a plasmosomal development about equal to that of the
basal cells of the epidermis. The testis of the 2 day rat does not show the good differentiation between heavily heteropycnotic small spermatogonial nuclei and lightly-staining r-nuclei with big plasmosomes noted by Bryson (10) in the 5 day mouse testis. The smallness and frequent apparent lack of plasmosomes and heterochromatic granules in the nuclei of the 2 day rat kidney probably indicate an absence of correspondence between size of normal mitotic chromosomes and concentration of histone or ribonucleic acid, as enzymatic digestion of adult tissues also demonstrated.

Let us examine the adult organs with respect to plasmosomes and nuclear volumes. The kidney and adrenal gland of Rat C, the small intestine, kidney, lung, and spleen of Rat D show little difference from the organs of the 2 day rat in the range of volumes over which the nuclei are distributed or in the number of plasmosomes carried by the nuclei. We note that among the 50 nuclei studied in the adrenal of Rat C, one was twice as large as the others and had 12 plasmosomes, twice the maximum number exhibited by the smaller nuclei. This nucleus was probably tetraploid in number of chromosomes.

In all the adult livers, however, a peculiar situation is apparent. The mode of nuclear volumes has shifted to about twice that in the 2 day liver, but the number of plasmosomes has not increased in the nuclei of the modal volume-group. Only in the largest nuclei do the plasmosomes give evidence of chromosomal polyploidy. In the liver of Rat B the percentage of resting nuclei with more than 6 plasmosomes is 3, and the percentage of polyploid metaphase figures is 28. In Rat C these percentages are 7 and 8 per cent respectively; in Rat D, 12 and 28 per cent. In a regenerating 50 per cent from the newborn to the adult rat kidney. The nuclei in the adult kidneys give the impression, however, of having bigger plasmosomes and a slightly greater amount of stainable chromatin than do the nuclei of the young rat kidney. The adrenal nuclei of Rat C are slightly larger than the kidney nuclei and have a somewhat better development of the nucleolar apparatus, yet the chromosomes of the kidney are larger by one-fourth.

The question marks in the table of plasmosome numbers in the spleen have reference chiefly to nucleoli in lymphocytes. In the spleen the nucleic acid balance is shifted far in favor of desoxyribonucleic acid (15), and this fact is seen cytologically in the heavily chromatic lymphocytes with questionable plasmosomes, if any. It is also difficult to make out plasmosome numbers in many of the nuclei of the lung, as evidenced by dash lines in the table.

The conclusions to be drawn from our study of nuclear volumes are that, although the supposed direct relation between chromosome size and nuclear volume, there are contradictory instances at hand. The size of the kidney nuclei seems not to increase from 2 days to adulthood. In the kidney of young Rat A, 50 nuclei averaged 401 cubic microns; in Rat C the average was 363 and in Rat D, 405 cubic microns. Nevertheless, the average chromosome volume increased about 50 per cent from the newborn to the adult rat kidney. The nuclei in the adult kidneys give the impression, however, of having bigger plasmosomes and a slightly greater amount of stainable chromatin than do the nuclei of the young rat kidney. The adrenal nuclei of Rat C are slightly larger than the kidney nuclei and have a somewhat better development of the nucleolar apparatus, yet the chromosomes of the kidney are larger by one-fourth.

To summarize the results, we note that in normal rat organs the average chromosome volume is (a) directly proportional to the total concentration of B vitamins with the exception of inositol, (b) not strictly proportional to nuclear volume, (c) not proportional
### Figure 19: Nucleolar Volume Apparent Numbers of Plasmodesmata in Cubic Microns in 50 Resting Nuclei

**Rat A Liver**:  
- 500-600: 4 6  
- 600-700: 3  
- 700-800: 4 6  
- 800-900: 5  
- 900-1000: 12  

**Rat A Kidney**:  
- 500-600: 3 4 4 6  
- 600-700: 2 4 4 6  
- 700-800: 1  
- 800-900: 5  

**Rat A Brain**:  
- 500-600: 4 6  
- 600-700: 5  

**Rat A Thymus**:  
- 500-600: 4 6  
- 600-700: 6  

**Rat A Spleen**:  
- 500-600: 3 4 4 6  
- 600-700: 4 6  

**Rat B Liver**:  
- 500-600: 3 4 4 6  
- 600-700: 2 4 4 6  
- 700-800: 1  
- 800-900: 5  

**Rat B Kidney**:  
- 500-600: 3 4 4 6  
- 600-700: 6  

**Rat B Brain**:  
- 500-600: 4 6  
- 600-700: 5  

**Rat B Thymus**:  
- 500-600: 3 4 4 6  
- 600-700: 4 6  

**Rat B Spleen**:  
- 500-600: 3 4 4 6  

### Figure 20: Nucleou Volume Apparent Number of Plasmodesmata in Cubic Microns in 100 Resting Nuclei

**Rat C Liver**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  
- 400-500: 9 6  
- 500-600: 5 6  
- 600-700: 4 5  
- 700-800: 4 5  

**Rat C Kidney**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  
- 400-500: 9 6  
- 500-600: 5 6  

**Rat C Brain**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  

**Rat C Thymus**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  

**Rat C Spleen**:  
- 100-200: 3 4  
- 200-300: 7 4  

### Figure 21: Nucleolar Volume Apparent Numbers of Plasmodesmata in Cubic Microns in 100 Resting Nuclei

**Rat D Liver**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  
- 400-500: 9 6  

**Rat D Kidney**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  

**Rat D Brain**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  

**Rat D Thymus**:  
- 100-200: 3 4  
- 200-300: 7 4  
- 300-400: 8 4  

**Rat D Spleen**:  
- 100-200: 3 4  
- 200-300: 7 4  

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to the concentration of cytoplasmic ribonucleic acid, and (d) not strictly proportional to the relative development of heterochromatin and plasmosomes.

**DISCUSSION**

Several theories have related chromosomes and size of nucleus. In 1925 it was generally assumed (30) that the individual chromosome had a typical form and size, both of which could vary in each cell type of a given species, and that Boveri's law should state that nuclear or cellular size was determined not only by number of chromosomes, since that was usually constant in a given species, but also by the variable mass of each chromosome. Belar (1), in 1928, could say of chromosome size only that it varied slightly in each cell type of the same organism, and that larger cells ordinarily contained large chromosomes. Jacob (25, 26), too, was of the opinion that cell types with larger nuclei had larger chromosomes, and in his studies on nuclear volume found certain regularities that he applied to the chromosomes. Jacob and a number of others have presented evidence that the increase in nuclear volumes in many plant and animal tissues follows a discontinuous pattern such that the modes of nuclear volume make a geometric series of this type: 1:2:4:8. In 1935 Jacob transferred this concept from the single organ to the whole organism, in this instance the human body (26). Taking 1 as the volume of the typical cell nucleus, such as may be found in the pancreas, Jacob extended his geometric series downward to cover microlymphocytes and upward over spinal ganglion cells to make it 1/8:1/4:1/2:1:2:4:8:16:32. He concluded that the modal nuclear volumes of the various cell types were integral multiples of a fundamental quantity. The interpretation given was that differences in amount of chromosomal material, and certainly not hydration, accounted for the different nuclear classes. In transferring his ideas about the nucleus to the chromosomes, Jacob decided that the latter also underwent a rhythmic volume doubling by the process now called endomitosis. Chromosomes of larger cell types were called polymers. With reference to the monomeric chromosomes of a microlymphocyte, e.g., the chromosomes of the parotid gland, the nuclei of which were 4 times as large, were tetramers. Polymeric chromosomes either behaved as monomers or revealed their composite character by falling apart as separate monomeric chromosomes. Geitler (17), however, thought that endomitotic nuclear volume doubling always, except in a few special cases, involved a doubling of chromosome number, while to Hertwig (21) it always, but for special cases, involved a doubling of chromosome set. To Geitler a polyploid chromosome set was entirely equivalent to a diploid set of endopolyploid chromosomes.

**ADULT RAT CONTROLS FOR REGENERATING LIVERS**

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**BIESLEY—CHROMOSOME SIZE IN NORMAL RABBIT ORGANS**

**DISCUSSION**

Several theories have related chromosomes and size of nucleus. In 1925 it was generally assumed (30) that the individual chromosome had a typical form and size, both of which could vary in each cell type of a given species, and that Boveri's law should state that nuclear or cellular size was determined not only by number of chromosomes, since that was usually constant in a given species, but also by the variable mass of each chromosome. Belar (1), in 1928, could say of chromosome size only that it varied slightly in different cells of the same organism, and that larger cells ordinarily contained large chromosomes. Jacob (25, 26), too, was of the opinion that cell types with larger nuclei had larger chromosomes, and in his studies on nuclear volume found certain regularities that he applied to the chromosomes. Jacob and a number of others have presented evidence that the increase in nuclear volumes in many plant and animal tissues follows a discontinuous pattern such that the modes of nuclear volume make a geometric series of this type: 1\(\times\)2\(\times\)4:8. In 1935 Jacob transferred this concept from the single organ to the whole organism, in this instance the human body (26). Taking 1 as the volume of the typical cell nucleus, such as may be found in the pancreas, Jacob extended his geometric series downward to cover microlymphocytes and upward over spinal ganglion cells to make it 1/8:1/4:1/2:1:2:4:8:16:32. He concluded that the modal nuclear volumes of the various cell types were integral multiples of a fundamental quantity. The interpretation given was that differences in amount of chromosomal material, and certainly not hydration, accounted for the different nuclear classes. In transferring his ideas about the nucleus to the chromosomes, Jacob decided that the latter also underwent a rhythmic volume doubling by the process now called endomitosis. Chromosomes of larger cell types were called polymers. With reference to the monomeric chromosomes of a microlymphocyte, e.g., the chromosomes of the parotid gland, the nuclei of which were 4 times as large, were tetramers. Polymeric chromosomes either behaved as monomers or revealed their composite character by falling apart as separate monomeric chromosomes. Geitler (17), however, thought that endomitotic nuclear volume doubling always, except in a few special cases, involved a doubling of chromosome number, while to Hertwig (21) it always, but for certain exceptions, resulted from the formation of
polymeric chromosomes, that could, however, be broken down by multiple successive divisions into sets of monomeric chromosomes in the daughter cells.

Our data on chromosome volumes are not in good agreement with the opinions of Jacoby and his school. For instance, we have roughly 3 groups of organs that in average nuclear volume bear to one another a 1:2:4 relationship well within Jacoby's standards. The lung, which has an average nuclear volume of 203 cubic microns, and the spleen, with 277, form the first group. The second group is made up of the adult kidney, small intestine, and adrenal, which have mean nuclear volumes of about 400 cubic microns. Finally, many adult liver nuclei are about 1,000 cubic microns in volume. Since most of the nuclei are diploid, Jacoby's theories call for a 1:2:4 volume relationship of the chromosomes in these 3 groups. However, the lung and spleen chromosomes average about 0.5 cubic micron; the kidney chromosomes average 0.9, those of the small intestine 0.5, and those of the adrenal 0.7 cubic microns; while the chromosomes of the larger sort in the liver are about 1.2 cubic microns. Jacoby's views are not accurately validated. Instead of a discontinuous increase of chromosome volume by progressive doublings, there is a gradual and nearly continuous change of average chromosome volume when all organs studied are considered. Since these average volumes are not in a 1:2:4 relation, the chromosomes cannot be considered members of a polymeric group; that is, the larger chromosomes have not been derived by progressive doublings of all ultimate protomeres, as Jacoby (26) believed. This is true not only of Clara's chromosome strands "in der Anlage" (13), but also of definitive chromosome strands that are separated from one another by interfaces and additional space, whether visibly or not. The larger chromosomes cannot be composed of more strands than the smaller ones in the sense that cancer chromosomes are, because all sizes of diploid nuclei in the normal rat tissues seem to have a maximum of 6 plasmosomes, while the diploid set of cancer chromosomes often carries 12 (4).

Even within the same organ, the increase in average chromosome volume from 2 day to adult rat is not by 100 per cent, which would be required for any increase by Jacoby's theory of rhythmic volume doubling, but is more of the order of 50 per cent. Examples are furnished by the kidney and the liver.

In summary, then, let us say that at least in normal tissues of the rat the theory that nuclear growth by rhythmic doublings in volume is underlain by a similar doubling in chromosome volume that takes place by exact duplication of protomeres (protein molecules) and chromosome strands is inaccurate, except in the case of polyploidy, and there the doubling is in total chromosomal material, not in the volume of the individual chromosomes. Even the statement that the chromosomes present in large diploid nuclei are large and those in small diploid nuclei small is true only with exceptions. Degree of dispersion of chromosomal material in the resting nucleus must vary more with function than Jacoby (26) assumed. The larger normal rat chromosomes, it must be emphasized, are not larger by virtue of an increased number of discrete strands.

It may be conjectured that at least part of the explanation for differences in size of normal chromosomes lies in the quantity of nucleic acid in the cell, if this determines the amount of cytoplasmic ribonucleic acid that enters the nucleus to be transformed into thymonucleic acid and attached to the mitotic chromosomes. Perhaps the nucleic acid on the chromosomes could influence their apparent size by its own bulk. Mitchell (34) found that after x-ray or gamma radiation, when mitosis was inhibited, the concentration of nucleotides within the nucleus apparently failed to increase, although there was considerable increase of ribose nucleotides in the cytoplasm, probably because the synthesis of desoxyribose nucleic acid from cytoplasmic nucleotides was inhibited in the nuclei of irritated cells. Crepis fuligiosa chromosomes have about one-fourth the volume of chromosomes of C. neglecta, even within the hybrid of the 2 species, and this is attributed by Tobgy (38) in part to an increased amount of heterochromatin and consequently greater ability to manufacture nucleic acid displayed by the chromosomes of C. neglecta. Caspersson and Santesson (12), noting that cancer cells possess an unusually high development of the heterochromatin and nucleoli, have found by ultraviolet absorption studies that those cells lying favorably situated with respect to nutriment have high concentrations of ribonucleic acid. Koller (27) has elaborated a theory of carcinogenesis built on increased nucleic acid metabolism. The writer and his associates have pointed out that degree of malignancy, frequency of repeated endomitosis as evidenced by greatly enlarged chromosomes, and concentration of ribonucleic acid seem to go hand in hand (7). In normal organs of the rat, however, the cytoplasmic concentration of ribonucleic acid indicated by the ribonuclease-pyronin method is not at all paralleled by average chromosome volume. Therefore not in normal tissues, and probably not in cancers, can it be assumed that the larger chromosomes are large because they carry disproportionately great amounts of nucleic acid on the same protein skeleton.

Likewise, in spite of the fact that not only are the chromosomes large in cancers but the system of heterochromatin and nucleoli is also well-developed, we find that in normal tissues the relative development of
heterochromatin and nucleoli is not a trustworthy indicator of mitotic chromosome size. The kidney furnishes the best example of big chromosomes accompanying small nucleoli and little heterochromatin in the resting nucleus, while the intestinal epithelium illustrates the converse. The size of the mitotic chromosomes in normal organs seems more likely to be governed by, and to be a reflection of, the development of the euchromatin.

The fairly normal distribution of average chromosome volume per metaphase about a mean distinctive for each cell type indicates a differentiation of the chromosomes that either causes or accompanies the differentiation of the rest of the cell. However, the variation of chromosome size around a mean for a given cell type may, so far as it is not a result of random errors in measurement, suggest that differences in chromosome size from one cell type to another are not absolute but are derivatives of a functional differentiation of the chromosome subject to certain conditions like the availability of proper substrate for its own autopsynthesis or for synthesis of gene-products.

But how are the B vitamins concerned in this chromosomal differentiation that expresses itself morphologically in size of chromosomes?

First we may consider the possibility that the difference in size of chromosomes from one cell type to another is solely the result of a different amount of vitamins, or perhaps of nucleotides containing vitamins, held in or on the chromosomes. Such a concept as this, however, is similar to, and less likely than, the suggestion we have previously discarded, namely, that the polynucleotide ribonucleic acid, some of which may be changed to deoxyribonucleic acid and attached to the chromosomes in mitosis, determines through differences in its concentration the size of mitotic chromosomes. Furthermore, in view of the low concentrations of the known vitamins in tissues, it would seem to require the assumption of considerable quantities of additional unknown vitamins or nucleotides notably to affect the size of the chromosomes by their own volume. It is, moreover, probably true that in most tissues the greater amount of the vitamins of a cell is in the cytoplasm. Data on this subject are admittedly meager, since among normal tissues only beef heart has been examined (24). Although here the nuclei hold greater concentrations of most vitamins than does the cytoplasm, it is probable that the nuclei make up a small fraction of the total mass and at least half of the total amount of B vitamin is in the cytoplasm. While we are not certain that all the cytoplasmic vitamins stay in the cytoplasm during mitosis, probably most of them remain attached to apoenzymes, since Peter (35) has lately modified his opinion on the mutual opposition of mitosis and cell work and has admitted that once materials have been taken up by the cell they are probably put through their normal metabolic course whether the cell is in mitosis or not. Therefore much less than the total B vitamin concentration of a given tissue is probably ever connected intimately with the chromosomes. It seems hardly likely that the greater size of some normal chromosomes, like those of the liver, is due solely to a greater amount of B vitamins on the mitotic chromosomes.

A second possibility is that the larger size of chromosomes in organs containing greater quantities of B vitamins is the result of increased amounts of primary or derived gene-products still remaining attached to the chromosomes in mitosis. These gene-products could well be proteins with a high affinity for certain vitamins. According to the differentiation of the cell the chromosomes would exhibit greater or less ability to manufacture these products, which would remain within or around the chromosomes for a time governed by the rate of their possible diffusion into the nuclear sap and cytoplasm. The recent finding of Mirsky and Pollister (32) that the nucleoprotein they have isolated from the nuclei of many cell types, including liver cells, is desoxyribonucleic acid combined with protamine or histone should not render this concept of higher protein gene-products on chromosomes unacceptable, because their method of isolation involves much washing with solutions in which higher proteins are readily soluble before the desoxyribonucleoprotein is extracted.

The third and most likely possibility is that the increased size in the larger chromosomes is the result of an increase in the chromosomal nucleoprotein. The additional chromosomal material, as we have seen, has been added in small, gradual steps, not by gross doublings, and has not been cut into more strands. It is proposed that the synthetic activity of the rat chromosomes parallels both their size and the cell's concentration of B vitamins. The chromosomes either (a) use vitamins in their own synthesis, or (b) use vitamins in the synthesis of gene-products, or (c) manufacture the protein parts of enzymes that are set free in the nuclear sap and ultimately the cytoplasm and that carry the vitamins in their prosthetic groups. While all three possibilities may be true, the last seems to be the predominant determiner of vitamin concentration for reasons set forth below.

Physiological geneticists have demonstrated that enzymes and antigens may be formed under the influence of one or a number of genes (23, 43). It has been proposed that antigens may be primary or secondary gene-products (20, 22), and that antigens and enzymes may be gene-replicas, at least in their active groups, the production of which is akin to genic reduplication (20, 43). Caspersson (11) has come to
the conclusion that the euchromatin synthesizes higher proteins, while the heterochromatin produces simpler proteins like histones, that pass from the nucleoli into the cytoplasm and stimulate there the formation of ribonucleic acid and cytoplasmic proteins.

The presence of a number of enzymes in fair concentrations can be demonstrated in the nuclei of rat liver. Thus Dounce (16) has found arginase, cytochrome oxidase, esterase, lactic acid dehydrogenase, and acid and alkaline phosphatases present in liver nuclei of Wistar rats in activities approaching or exceeding their activities in whole liver. To these Lan (29) has added d-amino acid oxidase, uricase, and choline oxidase. Mayer and Gulick (31) have reported the isolation from calf thymus nuclei of a fraction including a protein that resembles a globulin in solubility and isoelectric point and contains sulfur. Willmer (41) has found that Gomori's histochemical method for alkaline phosphatase indicates the presence of this enzyme on the chromosomes and in the nucleoli. It may be inferred from the data of Isbell and others (24) on the presence of quantities of the various B vitamins in isolated nuclei of beef heart and mouse cancer that enzymes containing these vitamins are normally present in the nuclei.

It seems reasonable to conclude that the euchromatin elaborates the protein portion of many enzymes, which may be given off rapidly to the cytoplasm. The euchromatin, according to its development as noted in size of chromosomes, synthesizes apoenzymes in variable quantity and thus determines the bound vitamin capacity of the organ. Here we have an explanation of the point raised by L. D. Wright and others (42), when they stated that the organ and species concerned are of more importance in determining the vitamin concentration than is the diet, within reasonable limits. It is apparent that the fact of differentiation of cell types demands that the euchromatin be functionally differentiated to synthesize not only different total concentrations can be demonstrated in the nuclei of rat liver. Thus Dounce (16) has found arginase, cytochrome oxidase, esterase, lactic acid dehydrogenase, and acid and alkaline phosphatases present in liver nuclei of Wistar rats in activities approaching or exceeding their activities in whole liver. To these Lan (29) has added d-amino acid oxidase, uricase, and choline oxidase. Mayer and Gulick (31) have reported the isolation from calf thymus nuclei of a fraction including a protein that resembles a globulin in solubility and isoelectric point and contains sulfur. Willmer (41) has found that Gomori's histochemical method for alkaline phosphatase indicates the presence of this enzyme on the chromosomes and in the nucleoli. It may be inferred from the data of Isbell and others (24) on the presence of quantities of the various B vitamins in isolated nuclei of beef heart and mouse cancer that enzymes containing these vitamins are normally present in the nuclei.

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Additional evidence that the relation between size of chromosome and concentration of B vitamins is perhaps mediated by the quantity of enzymes produced by the former and holding the latter is furnished by the work of Greenstein and Thompson (19) and of Shack (36). They have compared the activities of a number of enzymes in fetal and adult rat livers, and have found in general an increase from the fetal to the adult liver that corresponds well with our observation of a considerable difference in size of neonatal and adult liver chromosomes. Similarly, regenerating and control adult livers have much the same enzyme "spectrum" (19), and we have seen the very close chromosomal correspondence between these two tissues (3).

SUMMARY

1. Although chromosome sizes in normal rat organs, with some exceptions, vary in general with nuclear volume, they do not form a polymeric series because the change in average chromosome volume from one tissue to another does not progress by discontinuous doublings, and because the diploid chromosome set in all the organs examined carries only the same maximum number of 6 plasmosomes.

2. The average chromosome volume does not vary in accordance with the cytoplasmic concentration of ribonucleic acid, nor in accordance with the relative development of heterochromatin and plasmosomes. Hence it is likely that the size of the mitotic chromosome is not determined by the quantity of polynucleotides it carries.

3. The average chromosome volume in normal rat organs is closely paralleled by the total concentration of B vitamins, with the exception of inositol. The following series of adult rat organs is given in the order of decreasing average volume of chromosome: liver, kidney, adrenal, lung, small intestine, spleen. The last 3 do not differ significantly. In order of decreasing concentration of B vitamins, the literature gives the series: liver, kidney, adrenal, spleen, and lung. The same relation holds for embryonic and adult rat liver.

4. It is proposed that the difference in chromosome size from one normal cell type to another in rats depends on the development of the euchromatin. The greater the development of the euchromatin, i.e., the larger are the chromosomes, the greater is their synthesis of enzymes and therefore the greater is the bound vitamin capacity of the organ.

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Chromosome Size in Normal Rat Organs in Relation to B Vitamins, Ribonucleic Acid, and Nuclear Volume

John J. Biesele

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