Changes in Desoxyribose Nucleic Acid per Nucleus in Renal Compensatory Hypertrophy in the Rat. By Norwin H. Becker* and Kazuo Ogawa. (*From the Henry and Lucy Moses Research Laboratories of the Laboratory Division, Montefiore Hospital, New York.*)‡

The mitotic activity of the compensatory hypertrophic kidney has been studied by several investigators (8, 12, 14). In general, the mitotic activity in the cortex, outer medulla, and inner medulla of the rat was largely restricted to the second day after nephrectomy. A second increase was noted in the outer medulla on the seventh day (8).

Under normal interphase conditions, the DNA content per nucleus is constant in the somatic cells of different tissues of the same species and there is a direct relation between its quantity and the degree of chromosome ploidy (2). However, in regenerating rat liver, an increased amount of DNA per nucleus has been noted by chemical methods (16, 17) and by cytophotometry (4). A similar tendency was noted in the compensatory hypertrophic kidney, by chemical methods (9) and by cytophotometry (3). These increases were ascribed to increases in DNA during mitotic activity (15) and/or polyploidy (4).

No work has been reported on the possible occurrence of polyploid nuclei in the compensatory hypertrophic kidney following uninephrectomy. In regenerating rat liver the ratio of polyploid to diploid nuclei increases (1, 4, 5). It occurred to us that the observed increase in DNA content per nucleus in the compensatory hypertrophic kidney might also be due, in part, to the occurrence of polyploid cells even if polyploidy does not exist in the normal kidney.

The present investigation was undertaken to determine the distribution pattern of DNA per nucleus during its increase in the compensatory hypertrophic kidney on various days following unilateral nephrectomy, using a cytophotometric method.

Materials and Methods

Sprague-Dawley male albino rats weighing approximately 180 grams were used. They were unilaterally nephrectomized on the left and maintained on a normal diet. On the 3rd, 10th, and 17th postoperative days, two, one, and one rat, respectively, and one normal control rat were killed; the kidneys were decapsulated, blotted gently, and weighed. Thin blocks were fixed in 10 per cent formalin, carried through alcohols and xylene, and embedded in paraffin. The paraffin blocks were cut at 12 microns and the sections mounted on glass slides. The preparation of the Feulgen reagent and the staining were carried out essentially as described by Stowell (13). Twelve minute hydrolysis at 60°C. in IN HCL was found to be optimal. All slides were hydrolyzed simultaneously in the same casette and stained in the Feulgen reagent for 1 hour. The stained tissue was mounted in oil having a refractive index ($N_D^{25°C.}$) of 1.560, which exactly matched the refractive index of the tubular cytoplasm. The relative amounts of DNA in the individual nuclei were measured by the two wave-length method of absorption microspectrophotometry as described by Pellister and Ornstein (10) and Patau (11). The two wave-lengths used were 575 mμ and 512 mμ isolated from a tungsten light source by means of a Bausch and Lomb grating monochrometer. The amount of DNA in arbitrary units was calculated according to the formula of Patau (11) using tables constructed by Mendelsohn (7). Fifty whole nuclei were selected at random from renal tubules in different areas of the kidney. The nuclei of the glomeruli and the stromal elements were not selected because of their extensive overlapping in thick sections.

RESULTS

On the 3rd, 10th and 17th days, the hypertrophied kidneys increased 11 per cent, 35 per cent, and 44 per cent respectively in wet weight over its nephrectomized mate. The distribution of DNA per nucleus is shown in relative units by the histograms in Fig. 1. It is noted that from specimen to specimen there are slight differences in the measured DNA content. This has been a common finding in cytophotometric studies and in many cases the measurement of a control standard (*e.g.*, a section of liver on the same slide) has shown that the variation is due to slight differences in the Feulgen nuclear reaction. Relative values, however, are valid within each specimen and specimens can be compared by a correction factor based on the difference between the mean of each groups' diploid nuclei.

It is evident from the histograms that occasional mitotic activity is present in the normal kidney of a young rat. This was confirmed by routine histo-

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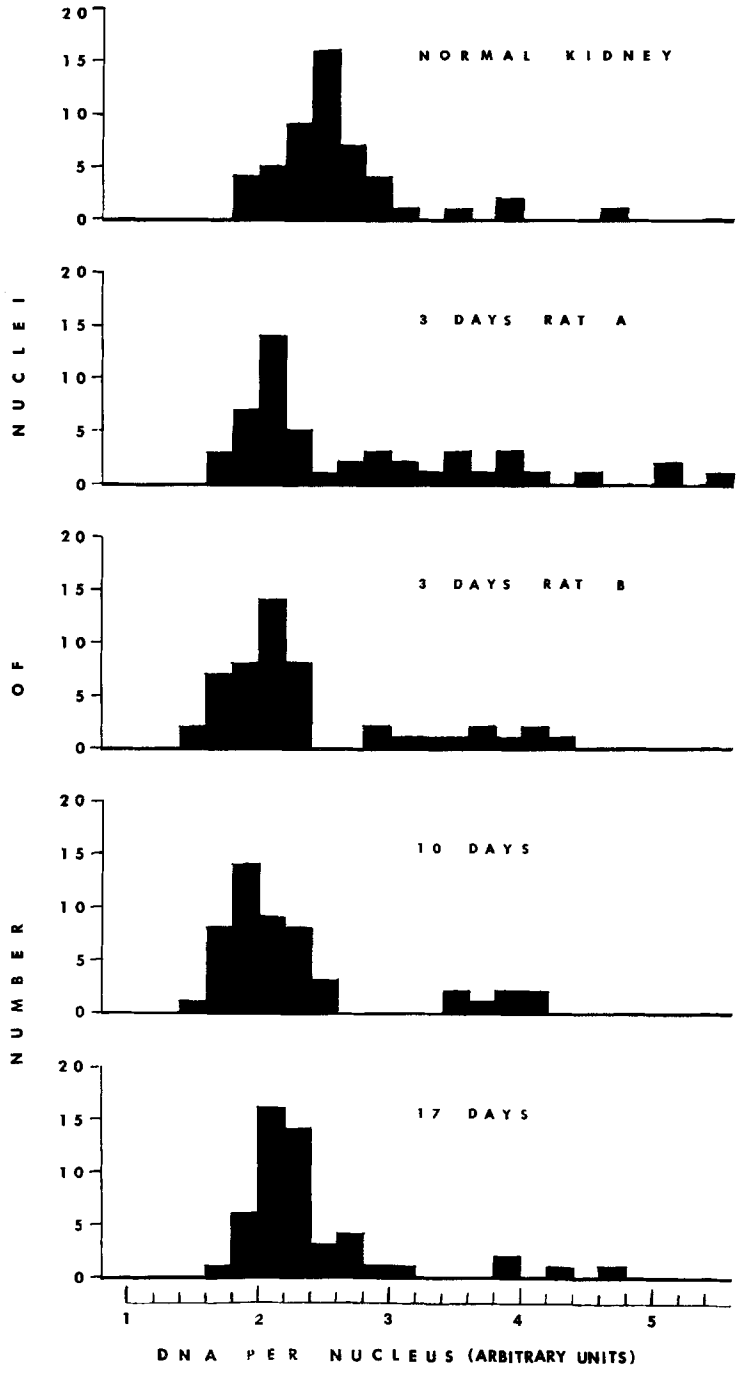

FIG. 1

logical preparations. During the 3rd postoperative day there is a striking increase in nuclei having hyperdiploid amounts of DNA, including some tetraploid nuclei. During the 10th and 17th postoperative days there is a decrease in the number of hyperdiploid forms and a return to the normal state. In no case is polyploidy greater than the tetraploid value of mitosis present.

DISCUSSION

The DNA per nucleus in the compensatory hypertrophic kidney has been studied previously by two other investigators who employed the cytophotometer. Fautrez, Cavalli, and Pisi (3) studied changes in five rats on the 7th postoperative day. Mitosis had been blocked in metaphase by colchicine. They noted that the mean values of the nephrectomized rats showed a tendency to increase without either the controls or the nephrectomized specimens reaching the theoretical diploid value. No data were provided to show the DNA distribution among the individual nuclei and polyploidy was not mentioned. Mangione (6) reported that the cells of the tubules showed a gradual decrease in ribonucleic acid and DNA in the compensatory hypertrophic kidney. Such a finding seems to be in error in view of the chemically demonstrated increases in DNA per nucleus as shown by Ogawa (9) in the kidney, and by Thomson *et al.* (16) and Tsuboi *et al.* (17) in regenerating rat liver.

The increased frequency of hyperdiploid and tetraploid nuclei noted on the 3rd day of the present study is correlated well with the known mitotic activity of the hypertrophic kidney (8, 12). The hyperdiploid forms represent interphase nuclei building up DNA prior to mitosis. It is known that the DNA for cell division builds up before the visible onset of prophase (15). Ogawa (9) studied the problem of the hypertrophic kidney using a chemical (diphenylamine) method. He found that the amount of DNA per nucleus increased within 3 days after operation and returned to normal within 10 days. Because of the return to normal he suggested that this increase was due to an increased rate of DNA synthesis rather than to the development of polyploidy.

If the average DNA per nucleus is calculated from the histograms (corrected for variations in the nuclear reaction), it is found that on the 10th and 17th days the average DNA per nucleus has returned to normal, thus confirming the findings obtained by Ogawa (9).

The return to low frequency of tetraploid nuclei on the 17th day, and the absence of hypertetraploid or octaploid nuclei in any of the histograms, confirms the impression based on chemical studies that polyploidy does not exist in either the normal or the compensatory hypertrophic kidney.

SUMMARY

The problem of whether the content of DNA per nucleus increases during the compensatory hypertrophy of the kidney after unilateral nephrectomy was reinvestigated using the cytophotometric method. It was found that the frequency of hyperdiploid and tetraploid forms increased within 3 days and returned to normal within 10 and 17 days. Polyploidy, except for the increase to the tetraploid value during mitosis, was absent in both the control and the hypertrophied kidneys.

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