Nuclear Size Changes During Autolysis in Normal Mouse Liver, Kidney, and Adrenal Gland.* (24014)

FREDERICK H. KASTEN (Introduced by H. O. Kunkel)
Department of Biology, A. and M. College of Texas, College Station, Texas

The term, autolysis, was first used by Jacoby(1) to describe the process of cellular self-digestion. Since then, many workers studied the phenomenon from the standpoint of morphological and chemical changes. In recent years, a cytochemical study(2) and some biochemical studies(3,4) were reported dealing with aspects of this process. The present paper is a continuation in a series dealing with nuclear size changes in mouse tissues(5, 6) and reports the results on autolyzing mouse liver, kidney, and adrenal gland.

Materials and methods. Nuclear size measurements were obtained from adult mouse tissues. Two to 3 mm of tissue were cut from liver and renal cortex and removed along with whole adrenal glands. They were placed in a dish and kept moist with 0.9% saline in 37°C oven for varying periods of time. The dish was kept partly covered to minimize bacterial contamination. Subsequent staining showed that this potential complication was of no importance here. Tissues were removed at definite times and were fixed in 50% formalin. Control samples of tissues from the same animal (or litter in the case of adrenal gland) were fixed immediately upon removal from the animal(s). Paraffin sections were made and all tissues to be compared were mounted on the same slide and stained by Feulgen technic(7). Nuclei were observed under oil immersion and their sizes were measured from camera lucida drawings. The usual precaution was taken to standardize all drawings by keeping the camera lucida at a 45° angle. Measurements of nuclear diameters were made to nearest 0.1 cm (0.1 cm = 0.37 μ). In some cases, slightly aspherical nuclei were measured. Here, the major and minor axes were averaged to obtain mean diameter. Nuclear volumes were computed by considering the round or slightly aspherical nuclei to be spheres and letting their volumes be a function of the diameter cubed. About 200 interphase nuclei were measured in each sample from renal cortex and adrenal cortex (zona fasciculata). About 500 interphase parenchymal nuclei were measured from each liver sample. Standard statistical analyses were made on data from kidney and adrenal gland utilizing the IBM 650 at the Texas A. and M. Computing Center. Because of the complication of polymodal classes in liver statistical analyses were not made. Approximately 6000 nuclei were measured and reported here.

Results. Fig. 1 summarizes nuclear size changes during autolysis in renal and adrenal cortex. After 30 minutes incubation, the average nuclear size in adrenal cortex increased about 70% over the controls. This was followed by gradual decrease to control value after 2 hours' autolysis. In kidney tissue, nuclei increased in size over the control by 70% reaching a peak volume at one hour or soon afterward. This rise was followed by rapid decrease eventually leveling off to a volume of half that of the control in 28 hours. Statis-

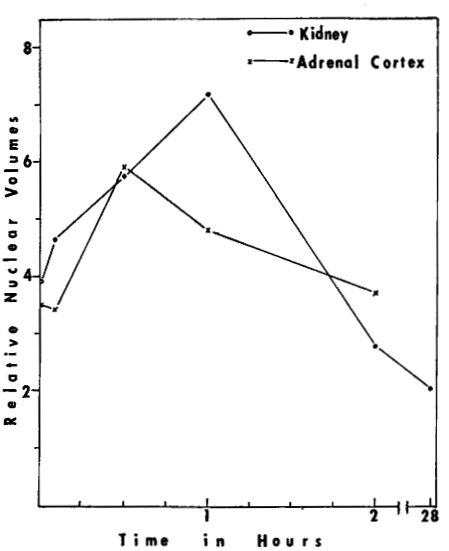

FIG. 1. Changes in avg nuclear sizes in mouse renal cortex and adrenal cortex during autolysis.

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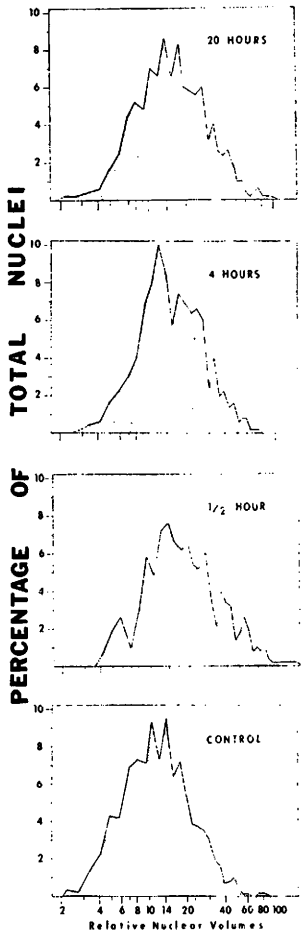

FIG. 2. Frequency polygons of nuclear size distributions from livers of control and autolyzed samples.

tical analyses of data from adrenal cortex revealed significant differences in nuclear size at the 1% level or less, between control tissue and tissue autolyzed for one-half and one hour. In the kidney, significant differences were found between control tissue and those autolyzed for one-half, one, 2, and 28 hours. Some results of autolysis of liver tissue are summarized in Fig. 2. The relative nuclear volumes are compared; no attempt was made to group original measurements into classes. The situation here is complicated by the large spread in nuclear volumes seen in all samples. There may be considerable overlap between different modal groups in each distribution to cause such continuous distributions. Relative nuclear volumes range from about 2 to 100 with the most frequent values at 8 to 20. Any obvious differences between control tissue and autolyzed samples are not apparent in these distributions. Grouping the raw data into classes only serves to reduce the spread and does not reveal anything significant.

Discussion. The term, autolysis, was coined by Jacoby in 1900(1) 10 years after Salkowski(8) showed that softening of dead tissues is brought about by intracellular enzyme action. The early studies on autolysis are summarized by Wells(9), especially from the chemical standpoint. According to Karsner (10), one of the striking changes observed in cells undergoing autolysis occurs in the nucleus. This structure often stains with neutral red or methylene blue, even before morphological alterations appear. Such alterations include paler staining followed sometimes by complete lysis. Lysis of nuclear membranes was not observed in any tissues studied here.

The nuclear size changes reported here in autolyzing kidney and adrenal gland may result from movement of water or other small inorganic molecules through the nuclear membrane. Changes found in autolyzed kidney and adrenal gland might also result from breakdown and displacement of organic constituents. The fact that nuclear volumes could still be measured in Feulgen preparations following extended autolysis indicates that some deoxyribonucleic acid (DNA) was still present. However, cytophotometric measurements would be required to evaluate this problem fully. Leuchtenberger(2) reported a progressive loss of DNA in pycnotic nuclei of autolyzing cells of mouse liver and Sarcoma 180. According to Roslansky and Alfert(11), autolyzing guinea pig kidney slices retain all their DNA after 24 hours.

Degradation of nuclear proteins could also account for nuclear size changes. Alfert(12) recently reported that pycnotic nuclei in autolyzing mouse kidney slices show increased histone stainability. This was also reported (12) in pycnotic nuclei of guinea pig ovary. Leuchtenberger(2) found that about 72-85% of Millon-protein was lost by final stages of pycnosis in mouse liver and Sarcoma 180.

Ordinarily, necrosis refers to changes in cells after death while in the body(13) but autolysis is usually used to describe specific intracellular effects of enzyme action on cells either in or removed from the body. These terms were used somewhat synonymously by Berenbom *et al.*(3,4) in recent studies. These studies dealt with chemical changes associated with mouse liver necrosis *in vivo* and *in vitro*. Ribonucleic acid (RNA) showed the most dramatic change, being reduced to 4% of its initial value 48 hours after pieces of liver were placed in the peritoneal cavity. DNA and protein nitrogen decreased much more gradually than did RNA. However, rate of disappearance of DNA and protein nitrogen was greater *in vitro* than *in vivo*. The fact that initial autolytic changes in kidney and adrenal gland in our study produce larger nuclei and are followed by decrease in nuclear size suggests that more than one cell constituent is undergoing change.

Summary. Small pieces of mouse kidney, liver, and whole adrenal glands were allowed to autolyze in incubator for varying times before fixation. Nuclear sizes were measured and compared with control tissues that had been mounted and stained together on the same slide. Nuclear volumes increased about 70% over controls during the first 30 to 60 minutes of autolysis in kidney and adrenal cortex. This was followed by a decrease in volumes eventually leveling off to about half that of the control in the kidney and the same as the control in adrenal cortex. A comparison of frequency distribution of nuclear volumes in liver revealed no differences between autolyzed samples and the control samples.

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Reaction of Brain Copper Proteins with Sodium Diethyldithiocarbamate in Normal and in Hepatolenticular Degeneration.* (24015)

HUNTINGTON PORTER AND SALLY AINSWORTH (Introduced by Byron H. Waksman)
Neurology Service, Mass. General Hospital and Dept. of Neurology, Harvard Medical School, Boston, Mass.

A previous report(1) described the separation from brain tissue under copper-free conditions of 3 different copper-containing fractions: fraction I, extracted from fresh tissue with 0.1 M acetate buffer pH 4.5 (or with water or with 0.1 M bicarbonate buffer pH 8.2); fraction II, obtained by subsequent extraction of tissue residue with water at pH 3.5 and vanishing ionic strength; and the residual fraction III. In brains from 2 cases of hepatolenticular degeneration, each with total copper content increased to more than 13-fold the normal, more than ⅔ of the pathological brain copper was extracted in fraction I in a form quantitatively undialyzable at pH

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