An Electron Microscope Study of Isolated Nuclei of Liver Cells from Laboratory Animals[1]

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A study of normal cell components with the electron microscope is an essential preliminary to a study of cytological changes produced by disease. The results of an investigation of isolated nuclei from normal liver cells are given here.

On account of the thickness of whole cells, morphologic studies with the electron microscope can be performed with advantage on isolated cell constituents, although only isolated mitochondria (*5*) and chromosomes (*15*) have been studied so far. Morphologic constituents of the cell have been studied in a variety of other ways with the electron microscope. They have been observed *in situ* in tissue culture cells (*7, 19*), and in tissue sections (*6, 9, 16, 17*). Methods for the fractionation of the fresh cells into its unaltered morphological constituents have been devised and as an approach to the study of the chemical constitution of the cell, Bensley and Hoerr (*2*) have isolated mitochondria and other "large granules." Submicroscopic particulates or "microsomes" were discovered by Claude (*3*) and particulate glycogen was separated by Lazarow (*11*). Nuclei have been segregated by Dounce (*8*), and chromatin threads have been separated by Claude and Potter (*4*). Mirsky and Ris (*13*) isolated chromosomes.

Four mammals—the monkey (*Macaca mulatta*), guinea pig, rat, and mouse—and the 16-day chick embryo of fowl were selected for study and healthy specimens were used. The mammals were all adult. Animals were anesthetized and allowed to bleed in order to eliminate as much blood as possible from the liver. Livers were placed immediately in ice-cooled Petri dishes and were transferred to a cold room (4° C), where the remainder of the process was carried out.

The livers were rinsed with isotonic saline solution and minced with scissors. Buffered hypotonic saline solution, which was 0.6% NaCl buffered to pH 6.2 with phosphates, was used throughout the separation and purification of the nuclei. The centrifugation technique of Dounce (*8*), as modified by Hoerr (*10*), was used for isolating the liver cell nuclei. When the final preparation was obtained, the sediment containing the nuclei was resuspended in a small amount of distilled water and deposited on electron microscope screens, where the drop meniscus was aspirated after 1 min, and the sediment was allowed to dry.

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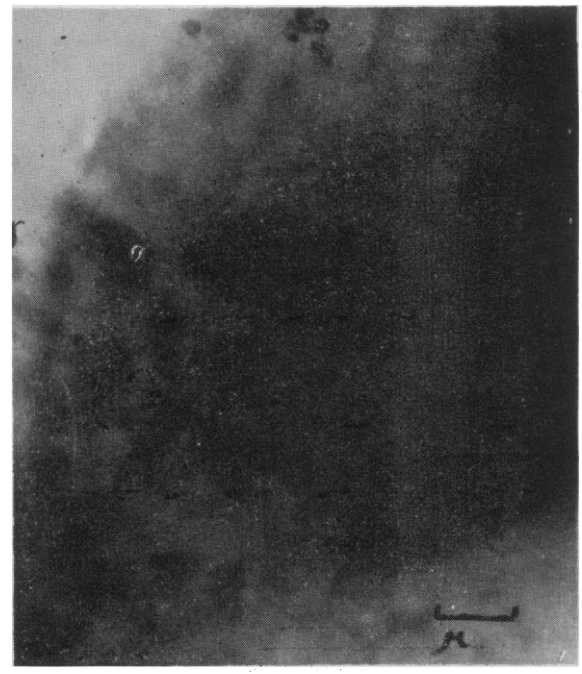

FIG. 1. Isolated nucleus from a monkey liver cell. Unfixed, unstained. Magnification, × 10,000.

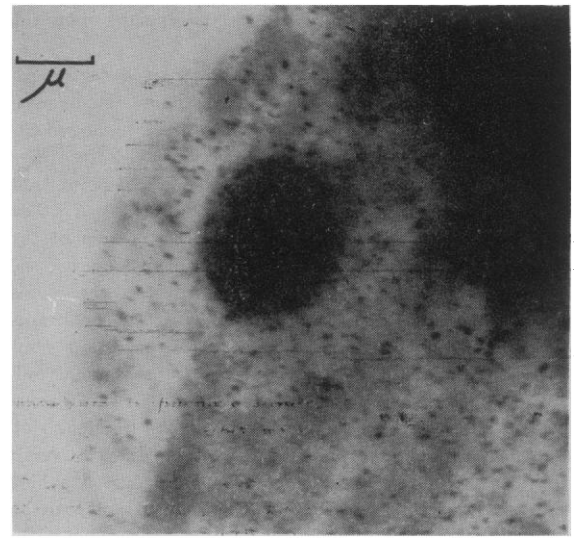

FIG. 2. Isolated nucleus from a monkey liver cell. Unfixed, unstained. Magnification, × 10,000. The nucleus is partly covered by debris.

A few specimens of liver cell nuclei from guinea pig, mouse, and chick embryo were shadow-cast with chromium metal. Image contrast was in general sufficient through the nuclei without shadow-casting, but this process did show up interesting surface morphology not otherwise visible. Original magnifications of a little less than 4,000 times were employed, but in a few cases lower magnifications, around 800 times, were used so that the whole outline of the nuclei would be more readily

observable. An optical microscope was employed for examining control wet preparations with and without staining with 1% methyl green. Smears of the preparation were fixed with methanol and stained with Giemsa's stain for further controls.

In more than twenty isolations of liver cell nuclei from laboratory animals, the shape of the nucleus was preserved and often was almost perfectly oval (Figs. 1, 2), but in most nuclei the nuclear contour was not sharply defined. Many nuclei revealed a coarse network (Figs. 1 and 3), which was indistinct in a few chick embryo nuclei, but easily visible in most of the adult animal nuclei specimens. An identity of this network with the chromatin network observed in the Giemsa-stained con-

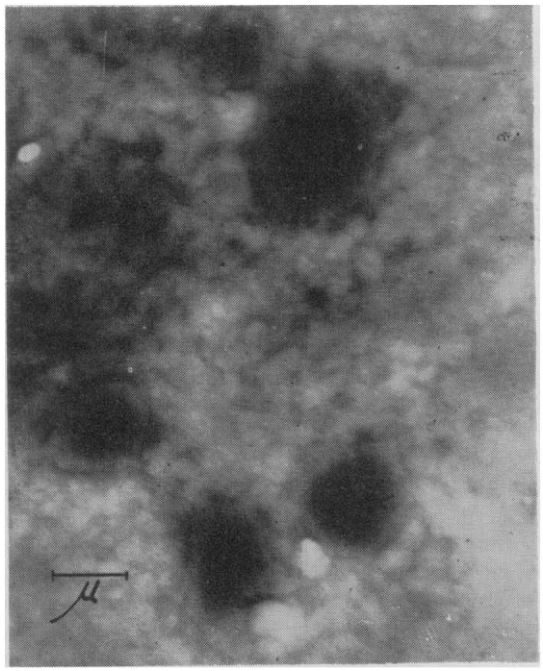

FIG. 3. A portion of an isolated nucleus from a mouse liver cell. Unfixed, unstained. Magnification, × 10,000. To show certain structure in what seem to be nucleoli.

trol smears has not been definitely established, but it is suggested by the close morphologic similarity between them. The nuclei studied here probably have been dehydrated to some extent in the electron microscope, and according to Martens (*12*) and Pischinger (*18*), dehydration reveals the chromatin framework of the nucleus.

Certain dense zones which correspond to structures with enhanced electron-scattering power are seen in all of the nuclei in sharp contrast with the nuclear background. There is no absolute proof that these dense zones correspond to nucleoli, but several observations indicate this. Whenever the word "zone" is used hereafter, it refers to these "dense zones." The number of zones seems to be a function of the animal species from which the nuclei are taken, although in a given species the number may fluctuate slightly at times. Thus, only one zone is noticed in every monkey liver

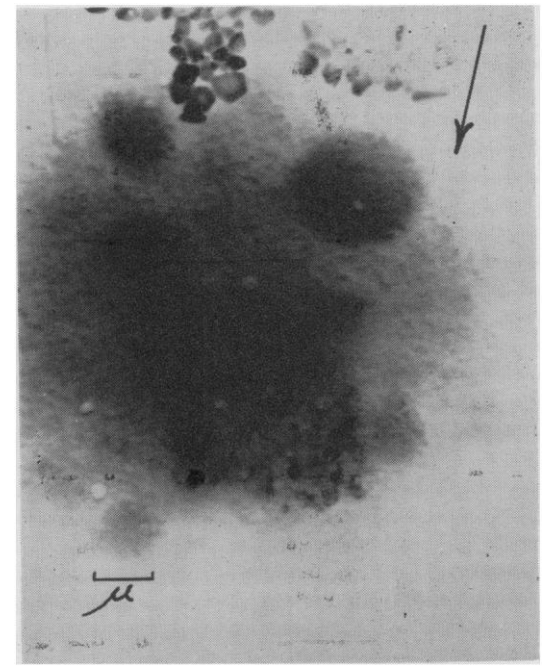

FIG. 4. Isolated nucleus from a guinea pig liver cell. Unfixed, unstained. Shadowed with chromium at an angle whose tangent was 0.24. Magnification, × 8,000.

cell nucleus, two or rarely one in a chick embryo nucleus, and from three to seven in a guinea pig nucleus, although a majority of guinea pig nuclei show four or five each. A number similar to that in a guinea pig nucleus is observed in rat or mouse nuclei.

For a given species the size of the zones varies in the different nuclei, and this difference in size is sometimes quite marked (Figs. 3, 4). However, the zones show an equal density despite their difference in size, so that they must be quite thick, and consequently their shapes are probably either ellipsoidal or spheroidal. No tendency is observed for the zones to occur in definite positions or to be grouped in any repetitive manner within the nucleus, and where there is only one, as in the monkey, it was not seen in a centric position—an observation which points to the identity of zones with nucleoli.

Most of the zones do not show distinct outlines, and although several of them appear homogeneous over their surfaces, others do not. Some have areas of lower density, as well as indefinite and even irregular contours (Fig. 5); the irregular contours are also observed more readily in the shadowed micrographs, as in Fig. 4. This micrograph is a shadow-cast guinea pig nucleus where the shadowing has shown up the fact that the whole nuclear surface is rough, the roughnesses being about 0.1 μ–0.2 μ across. This structure proceeds right across the complete area of the nucleus, including that of the zones, and is apparently a function of the surface of the nucleus as a whole. This, however, is an observation based upon a limited sample. Proceeding as it does over the whole nuclear area, the structure indicates that

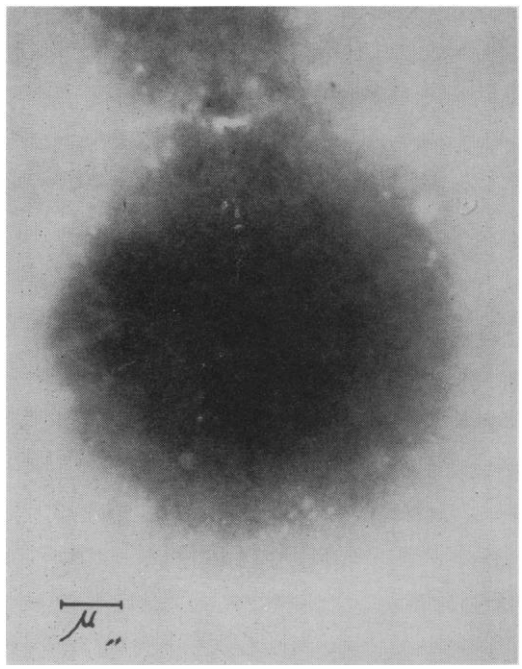

FIG. 5. Isolated liver cell nucleus from a 16-day chicken embryo. Unfixed, unstained. Magnification, × 8,000. This nucleus has apparently suffered plasmolytic changes.

the zones are within the nucleus, not on its surface.

Some of the zones are composed of a dense, oval mass surrounded by two layers: a transparent inner layer and a dense outer layer somewhat thinner than the first (Figs. 2 and 3). This structure has been seen both in monkey nuclei, which show only one zone, and in mouse and guinea pig nuclei, which show several zones per nucleus; it may be revealed in only one zone or in several in a nucleus. The layers are not always visible, and hence this structure may be a function of the preparation. Osmotic action or plasmolysis, or drying during the electron microscope handling may account for the presence of the layers, just as the cell wall of a bacterium is often made visible by these same processes (*14, 20*). In Fig. 3 the outermost layer has a variable thickness ranging from about 600 A to 1200 A. These values are maxima for the observed cases and are either below the resolution of an optical microscope or so close to it that observation of the layers optically would be rather difficult at best.

In the control preparations stained with methyl green, which according to Semmens and Badhuri (*21*) is a specific stain for the nucleolus, deeply stained nucleoli similar in size, shape, position, and number to the zones in the corresponding specimens examined with the electron microscope were seen clearly. Thus, in the optical microscope, only one such nucleolus was observed in preparations obtained from monkey liver; two, or infrequently one, were seen in chick embryo liver specimens, and two to five or even seven corpuscles were noticed in nuclei from guinea pig, rat, and mouse livers. In rat nuclei, there were usually one or two round nucleoli, the rest being either elongated or rather angular. The refringence and affinity for methyl green were the same for all, regardless of their shape or size. In the mouse, the nucleoli in large nuclei were usually larger, but some instances of small ones in large nuclei were noted.

The absence of chemical fixation has not provoked any important distortion in the isolated nuclei examined; rather, all of the morphologic characteristics seem to have been maintained without fixative. This remarkable result is in accordance with the same observation made during a study of chick embryo erythrocytes and reported by us (*1*).

The network of the nucleus observed in unfixed, isolated liver cell nuclei studied here by electron microscopy is morphologically similar to the chromatin framework of Giemsa-stained control nuclei examined with the optical microscope, but it is not similar to the nuclear framework observed in osmic acid-fixed, ultrafine tissue sections examined by electron microscope (*6, 9, 16*).

It may be that the network seen in these electron micrographs of unshadowed nuclei is a manifestation of the pitted nuclear surface visible in shadow-cast specimens. The rims of the pits would indeed appear as a network in the images of unshadowed specimens. Since the observation has been made on a limited sample, it should be confirmed.

The shape, size, and position of the dense zones observed in all nuclei examined, their different electron-scattering power as compared with that of the nuclear background, and the presence of a characteristic number of zones in each animal species examined, all point to an identification of the zones as nucleoli. The nucleoli are known to have a chemical constitution different from that of the remainder of the nucleus. Such differences often result in a difference in electron-scattering power, but since all elements of the nucleus are likely to be light elements, the probability is that the increased scattering power of the zones, as compared with the main body of the nucleus, is due more to an increased physical density. The occurrence of a dense external layer around some of the zones is evidence that some sort of a nucleolar wall may exist, although no nucleolar or nuclear membrane has been detected.

A comparison of the electron micrographs shown here with those obtained from liver sections (*6, 9, 16*) shows that for morphologic studies of the liver cell nucleus unfixed, isolated liver cell nuclei are comparable in electron microscopy with ultrafine, fixed tissue sections, for the present development of both techniques. The isolated nucleus, of course, can be seen in its entirety, whereas the ultrafine tissue sections cannot.

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Blood Findings in Men on a Diet Devoid of Meat and Low in Animal Protein

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During the course of a nutritional survey to be published elsewhere, we became interested in a community of men who do not consume meat and whose animal protein intake is low. Since there is a paucity of data upon the blood findings of humans on prolonged low animal protein intake, it was decided to conduct a blood study of the men in this community.

TABLE 1

PERSONAL HISTORY

Case No.	Age	Age when placed on diet	No. of years on diet	Occupation
1	73	26	47	Woodman
2	62	25	37	Log hauling
3	65	33	32	Cook
4	42	19	23	Gardener
5	44	24	20	Baker
6	36	19	17	Construction
7	37	21	16	Teacher
8	32	17	15	Mechanic
9	48	34	14	Plumber
10	35	23	12	Chauffeur
11	31	19	12	Painter

The 11 men selected for the study had been on the diet from 12 to 47 years, as shown in Table 1. The subjects were never allowed meat, poultry, or fish. The daily animal protein ingested was the milk added to coffee. Since the 1.5 pt of coffee served contained ¼ pt of fresh skim milk, the men consumed ⅝ pt of milk per day. In addition, 5 months of the year, 3 times a week, extra animal protein was consumed in the form of fresh skim milk and American Cheddar cheese; during the remaining 7 months the extra animal protein was consumed once a week for only 17 weeks. During the year, on 28 occasions, the men were served 2 oz of butter at break-

TABLE 2

DIETARY HISTORY

		April 1st–September 13th	September 14th–March 30th
Breakfast*		6 oz whole wheat bread 1.5 pt coffee†	2 oz whole wheat bread 1.5 pt coffee
Noon meal		1.5 pt vegetable soup‡ a vegetable serving§ a fresh fruit serving or 2 oz preserve‖ whole wheat bread ad lib. coffee	1.5 pt vegetable soup a vegetable serving a fresh fruit serving or 2 oz preserve whole wheat bread ad lib. coffee
Evening meal	Sat. Mon. Wed. Fri.	1.5 pt vegetable soup a fresh fruit serving or 2 oz preserve whole wheat bread ad lib. coffee	6 oz whole wheat bread 2 oz preserve 1.5 pt coffee
			17 Sundays
	Sun. Tue. Thurs.	1.5 pt milk 3 in. × 2 in. × ⅝ in. American Cheddar cheese whole wheat bread ad lib. coffee	1.5 pt milk 3 in. × 2 in. × ⅝ in. American Cheddar cheese whole wheat bread ad lib. coffee

* On 28 occasions 2 oz of butter was served.

† The coffee was prepared from barley grounds or a mixture of equal parts of barley and soybean grounds. One and one-half pints of coffee contained ¼ pint of fresh skim milk.

‡ No meat stock was added to the soup and it was thickened with flour.

§ The vegetable was cooked in water, salt being the only condiment added.

‖ On 28 occasions the fresh fruit or preserve was replaced by a 5-oz piece of plain cake.

TABLE 3

DAILY ANIMAL PROTEIN INTAKE DURING YEAR

No. of days	Foods	Amount in g	Protein* in g
277	Skim milk	177.3	5.7
57	Skim milk	827.5	26.4
	American Cheddar cheese	100.0	24.0
		Total	50.4
28	Skim milk	827.5	26.4
	American Cheddar cheese	100.0	24.0
	Butter	56.2	0.56
	Egg	14.3	1.85
		Total	52.81

* The values for the protein content of the foods were taken from the Tables in *Nutrition in health and diseases* by Cooper, Barber, and Mitchell (*1*).