Published April 1, 1968

A QUANTITATIVE DESCRIPTION OF CORTISONE-INDUCED ALTERATIONS IN THE ULTRASTRUCTURE OF RAT LIVER PARENCHYMAL CELLS

JOSEPH WIENER, ALDEN V. LOUD, DANIEL V. KIMBERG, and DAVID SPIRO

From the Departments of Pathology and Medicine, College of Physicians and Surgeons, Columbia University, New York 10032

ABSTRACT

A stereological comparison of the hepatic parenchymal cells from 125-g male rats given a daily injection for 6 days of either 5 mg of cortisone acetate or saline (controls) was carried out with both light and electron microscopy. Cortisone treatment results in an increase in average parenchymal cell cytoplasmic volume from 5100 to 5800 μ^3 and a decrease in average nuclear diameter from 7.1 to 6.5 μ. The volume of the average mitochondrion is increased fourfold in midzonal and peripheral regions of hepatic lobules, and there is a decrease in the number of mitochondria per cell such that the total mitochondrial volume per cell remains approximately unchanged. The numbers of peroxisomes are reduced, while the numbers of lysosomes and lipid droplets are increased in all parts of the lobules. The average volume of glycogen is doubled in all cells. The areas of membranes of the smooth- and rough-surfaced endoplasmic reticulum are decreased to one-half and two-thirds of their control values, respectively. The effects of cortisone on these various structural elements is discussed with respect to steroid-related alterations in biochemical processes.

A number of biochemical processes in the liver, such as protein synthesis (1), glycogen formation (2), lipogenesis (3, 4), certain mitochondrial functions (5, 6), and the release of hydrolytic enzymes (7), are known to be affected by cortisone treatment. Many of these processes can be related to specific ultrastructural elements of the cytoplasm. However, no systematic examination of the effects of cortisone on these various cytoplasmic organelles has been made.

Previous studies indicated that cortisone treatment in the rat produces a decrease in the number (8) as well as an increase in the size of mitochondria (8, 9) in hepatic parenchymal cells. These observations suggested that the morphometric method recently used for the quantitative description of normal liver parenchymal cells (10) would be particularly applicable to the study of livers from cortisone-treated rats. Consequenily, the present stereological investigation of the livers from such animals was undertaken. The results of this study show that cortisone treatment produces a generalized decrease in the area of membranes that limit nuclei, mitochondria, and peroxisomes, as well as the membranes of the rough- and smooth-surfaced endoplasmic reticulum. A detailed description of cortisone-related alterations in intramitochondrial structure and function is given in another report (11).

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

MATERIALS AND METHODS

Albino male rats of the Columbia-Sherman strain, weighing about 125 g and fed a stock laboratory diet ad libitum, were used in these experiments. Nine of these animals received daily subcutaneous injections of 5 mg cortisone acetate (Cortone acetate from Merck, Sharp, and Dohme, West Point, Pa.; 25 mg per ml in saline suspension) for 6 days. Five control animals received injections of saline. All the animals were sacrificed 24 hr after the last injection, i.e. between 10:00–11:00 a.m. of the 7th day.

The median lobe of the liver was quickly excised from lightly anesthetized animals, and immediately immersed in 6.25% glutaraldehyde in 0.075 M phosphate buffer at pH 7.4. The marginal and surface cells were trimmed away, and the remaining tissue minced into several hundred blocks. These blocks were then fixed for an additional 3 hr in fresh buffered glutaraldehyde, washed thoroughly with buffer, post-fixed in veronal-acetate buffered (pH 7.4) 2% osmium tetroxide with added sucrose for 4 hr, dehydrated in acetone, and embedded in Araldite. 1-μ-thick sections of the plastic-embedded tissues from the control and from the cortisone-treated animals were examined under the phase-contrast microscope. Thin sections cut from representative blocks of all animals were stained with lead-citrate and uranyl acetate and examined under a Siemens-Elmiskop I electron microscope.

The tissues from three typical cortisone-treated rats and two control animals were subjected to quantitative morphometric analysis by light and electron microscopy. The procedures were identical with those previously used for the stereological description of normal rat liver parenchymal cells (10) and will only be summarized here. The following cytoplasmic structural characteristics were measured: average diameter of parenchymal cell nuclei; average cytoplasmic volume per cell (nucleus); volume fraction of cytoplasm occupied by mitochondria, peroxisomes, lysosomes, lipid, and glycogen; surface density of smooth- and rough-surfaced endoplasmic reticulum and of mitochondrial envelope; and the number of transections of mitochondria, peroxisomes, and lysosomes per unit area of cytoplasmic cross-section. These measurements also provided the basis for calculation of the average dimensions of mitochondria and peroxisomes. Finally, the average volumes, areas, and numbers of cytoplasmic constituents were expressed per cell (nucleus).

RESULTS

Qualitative Observations

A survey of the plastic-embedded sections under the phase-contrast microscope revealed similar changes in hepatic parenchymal cells of all the steroid-treated animals. The nuclei are somewhat smaller and more compact than those in the controls (Figs. 1 *a* and *b*), but the cells do not appear enlarged. The cells from cortisone-treated animals contain more extensive lakes of glycogen and more numerous lipid droplets. Many oval elongated particles, measuring up to 6 μ in length and subsequently identified by electron microscopy as enlarged mitochondria, are seen around the periphery of the parenchymal cells, but not in the cells of control animals.

There are marked changes in the ultrastructure of the hepatic parenchymal cells from all steroid-treated animals under the electron microscope. Many mitochondria are enlarged (Fig. 2). Some have irregular profiles, exhibiting annular and branched shapes (Figs. 3 and 4). The largest mitochondria are found in the midzonal and peripheral portions of hepatic lobules, while smaller ones are localized in centrilobular cells (Figs. 7–9). Matrix density and the typical number and distribution of cristae in the enlarged mitochondria seem to be unaltered. In these respects, they retain the characteristic appearance of normal hepatic parenchymal cell mitochondria and show no evidence of "swelling" or degeneration. Lipid droplets are increased in number (Fig. 5) and are often partially or completely surrounded by a mitochondrion (Fig. 5; inset). There is considerably more glycogen in the parenchymal cells following cortisone treatment. This appears primarily as discrete beta particles (12) (Figs. 2, and 7–9), in contrast to the predominance of clustered alpha particles in the controls. Although cortisone-related changes in lysosomes, peroxisomes, and both smooth- and rough-surfaced endoplasmic reticulum are not evident by inspection, specific alterations in these organelles are revealed by the quantitative methods in the following section.

Cortisone administration produces no recognizable cytological effects in Kupffer cells (9). In particular, the mitochondria of Kupffer cells exhibit no enlargement.

Quantitative Observations

The interpretation of stereological measurements of cellular structure and the statistical variation in these methods have been discussed in a preceding paper (10). Therefore, only those aspects of morphometric analysis that are necessary for

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

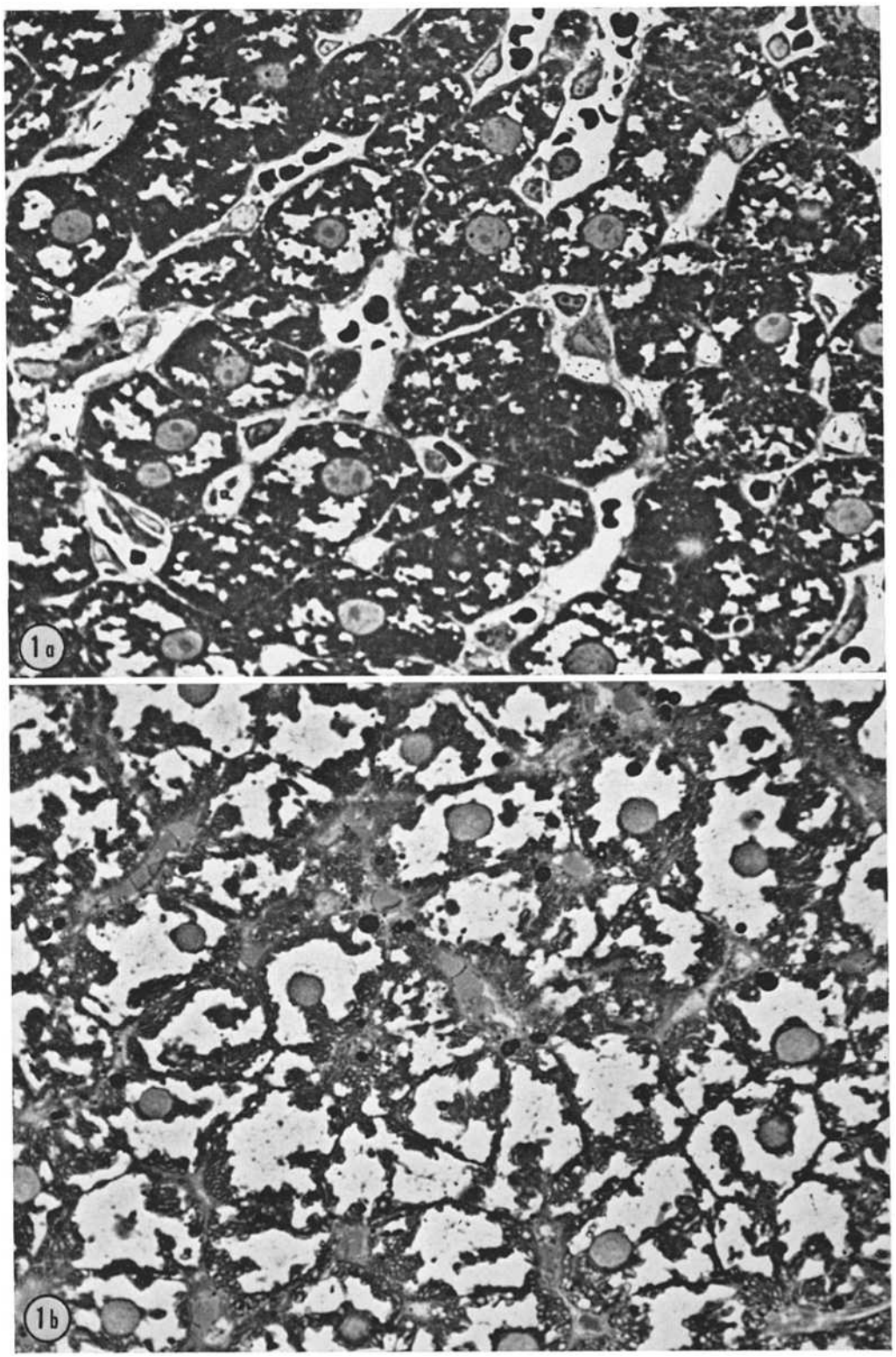


FIGURE 1 These are phase-contrast micrographs of 1-μ-thick Araldite-embedded sections of livers from control (1 *a*) and cortisone-treated (1 *b*) rats. The parenchymal cells of the treated animals have smaller and more compact nuclei, extensive lakes of glycogen, increased numbers of lipid droplets, and prominent enlarged mitochondria. × 800.

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

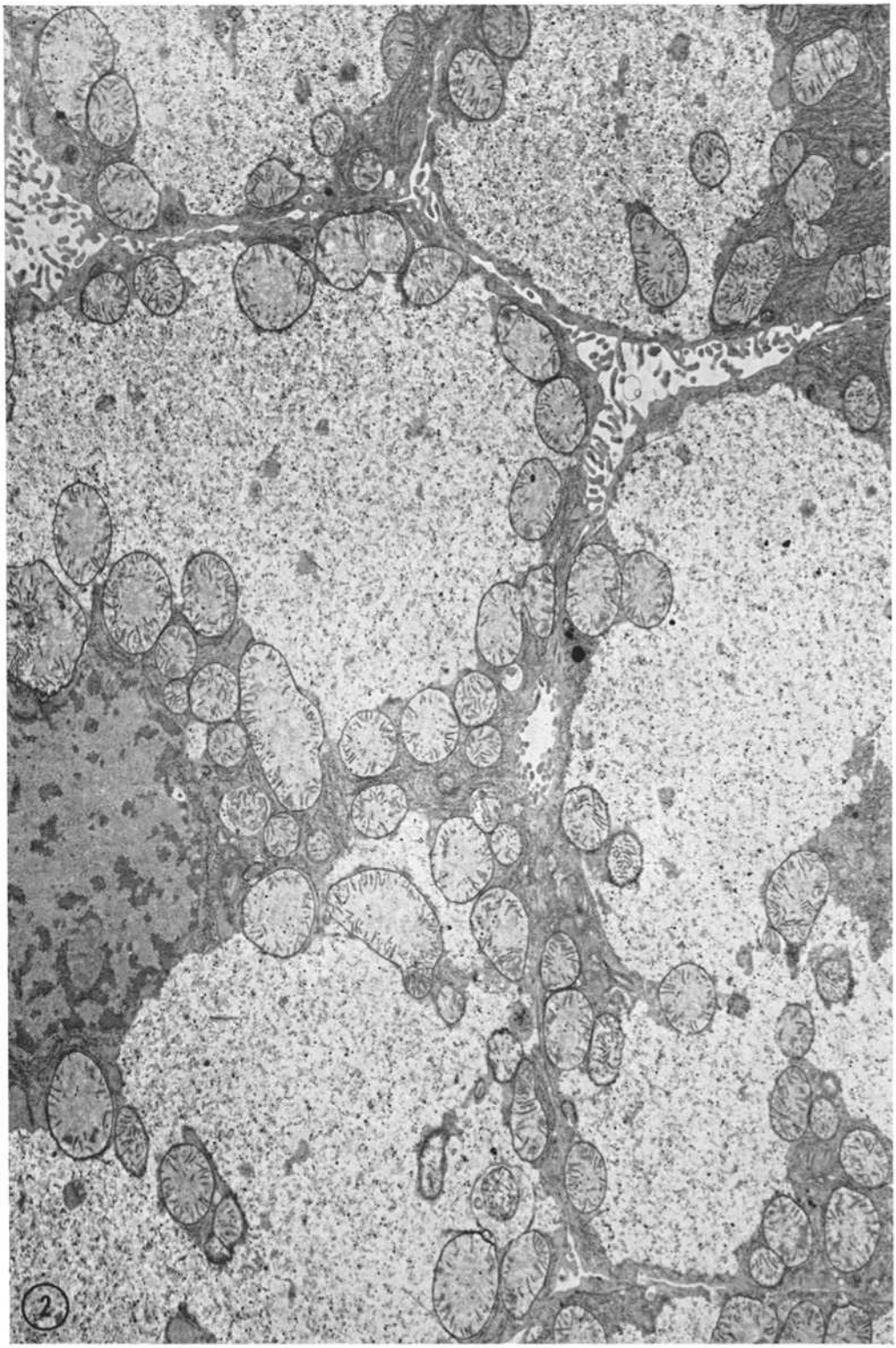


FIGURE 2 This electron micrograph from the liver of a cortisone-treated animal shows portions of several hepatic parenchymal cells with extensive glycogen deposits surrounded by enlarged mitochondria. × 9,000.

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

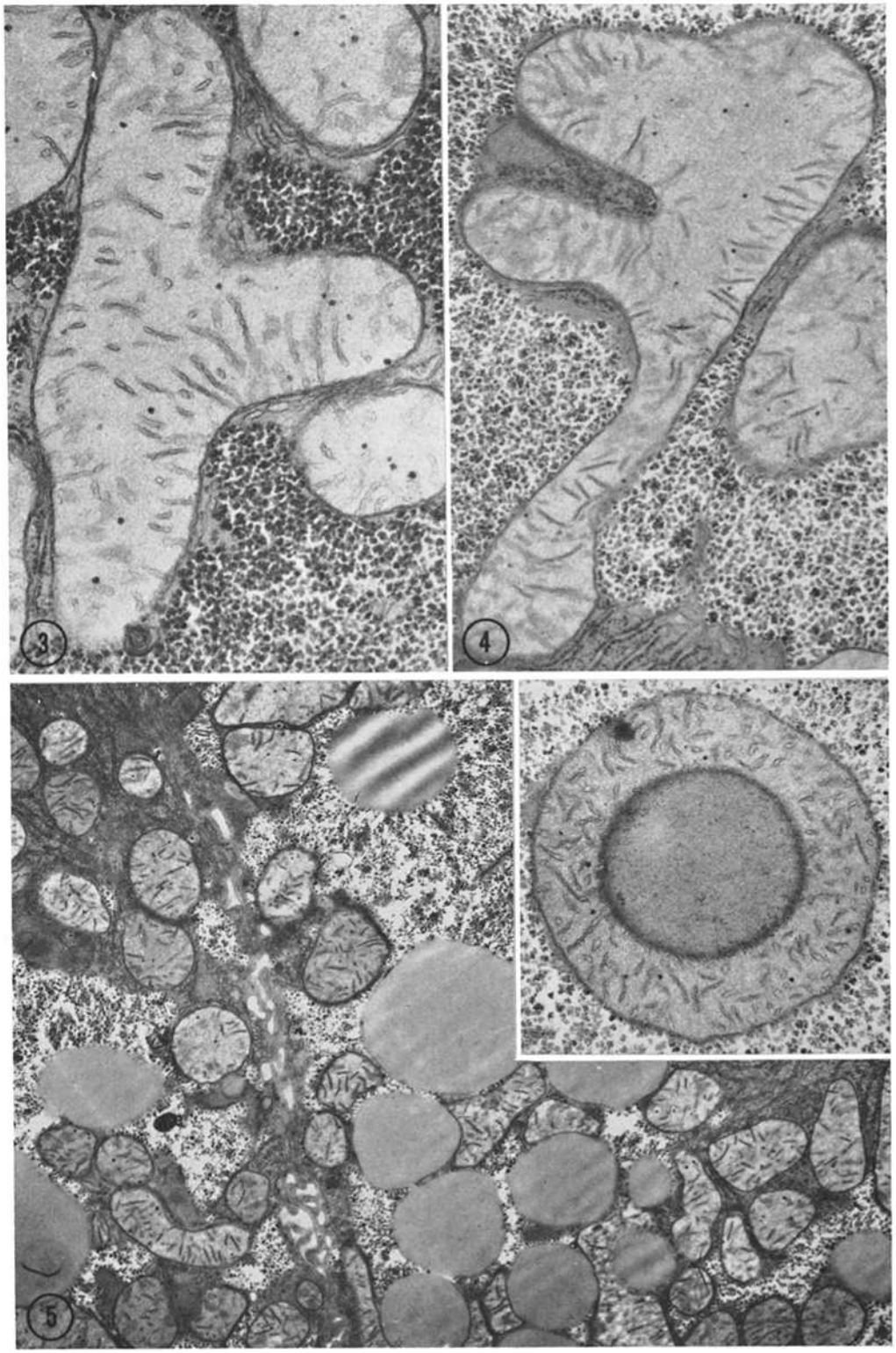


FIGURE 3 Irregularly shaped mitochondrion in liver of animal treated with cortisone. × 35,00

FIGURE 4 Similar to Fig. 3. × 25,000.

FIGURE 5 Numerous lipid droplets are seen in this micrograph from the liver of a steroid-treated animal. × 13,000. *Inset:* An example of a mitochondrion completely surrounding a lipid droplet. × 22,000.

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

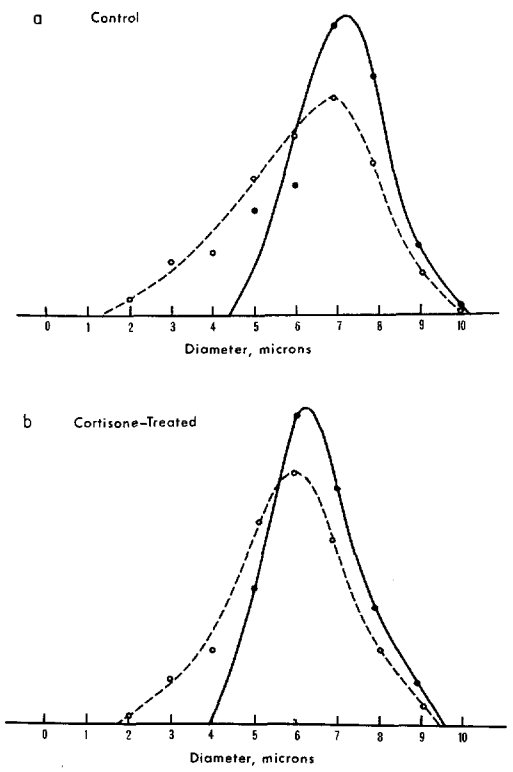


FIGURE 6 *a* and *b* These graphs show the distribution curves of the diameters of nuclear sections (○—○) measured in light micrographs of plastic-embedded tissues from the livers of control (6 *a*) and cortisone treated (6 *b*) rats. The corresponding distributions of spherical nuclei (●—●) were calculated according to the method of Bach (13). These calculations are based on measurements of 698 and 628 nuclei from control and cortisone-treated rats, respectively.

understanding the present results will be mentioned here.

The distribution curves of the diameters of parenchymal cell nuclei determined from light micrographs at × 1000 are plotted together with the data from which they are derived in Fig. 6. The mean diameters and standard deviations are 7.09 ± 1.18 μ and 6.52 ± 1.05 μ for the nuclei in the tissues of control (Fig. 6 *a*) and steroid-treated animals (Fig. 6 *b*), respectively. The average volume of parenchymal cell cytoplasm per nucleus determined from measurements of approximately 2,400 cellular cross-sections is 5100 μ^3 for the controls and 5800 μ^3 for the treated animals. The average diameters of cells in the various zones of the lobules were also measured in light micrographs, and no significant differences were found other than the consistently larger size (22.3 μ) in steroid-treated animals as compared to those in the controls (20.1 μ).

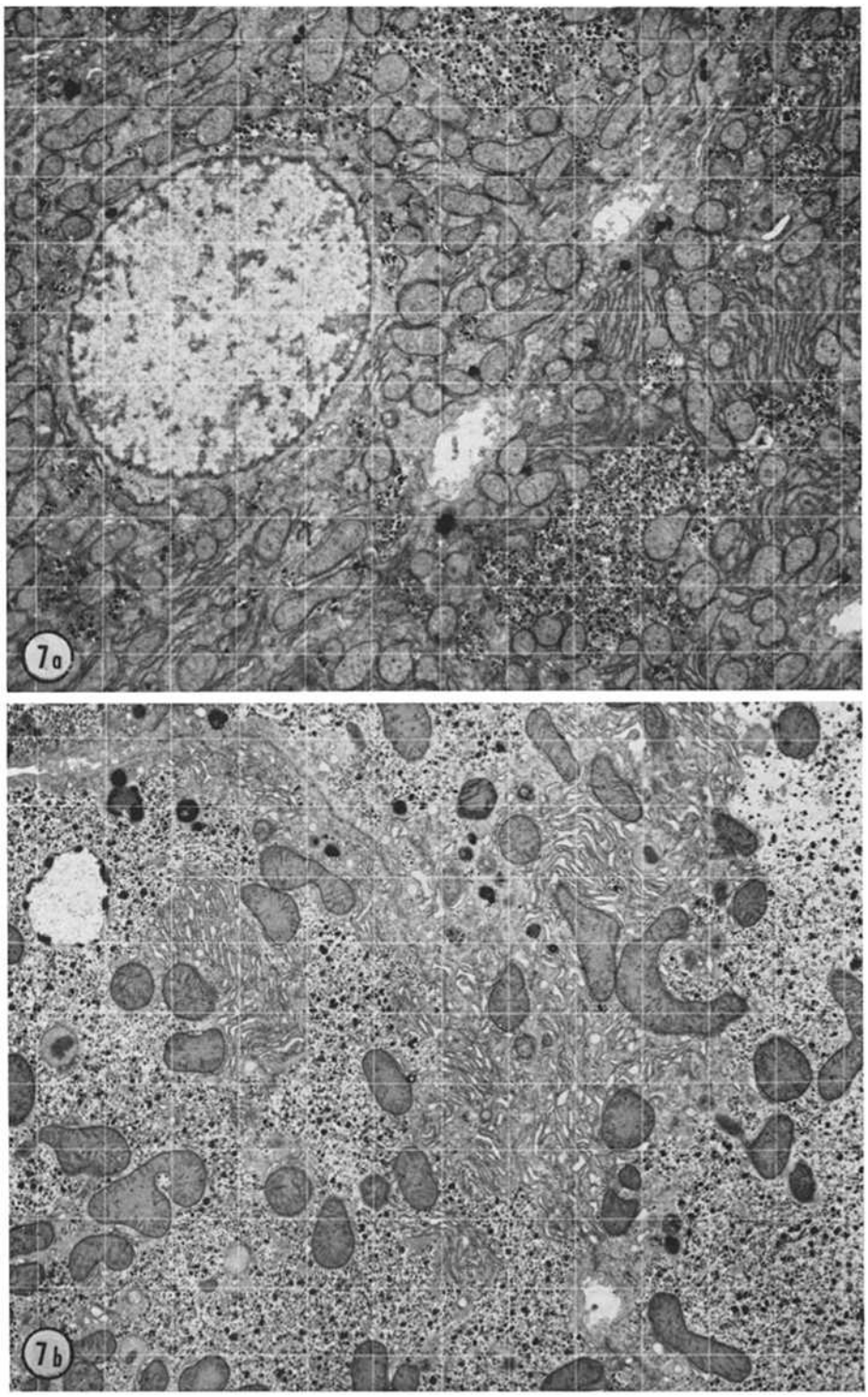
Data derived from electron micrographs are given in Tables I–IV. The values from control and steroid-treated animals are compared for each sublobular zone. These measurements were derived from 90 electron micrographs (× 12,500) distributed among the different zones, as shown at the top of Table I and illustrated in Figs. 7–9. The volume fractions of various cytoplasmic components noted in Table I indicate a small, but consistent increase in mitochondria, lysosomes, and lipid in cortisone-treated animals. The volume of glycogen is nearly doubled in all zones. In addition, there is a decrease in the volume of peroxisomes in the midzonal and peripheral portions of the lobules ($p < 0.05$).

The areas of the membranes of mitochondrial envelope and endoplasmic reticulum for both groups of animals are shown in Table II. This type of measurement is made by counting the number of intersections between the membrane profiles and a set of sampling lines superimposed on the electron micrographs. The total number of intersections counted are listed in the lower portion of Table II. The area of mitochondrial envelope per unit volume of mitochondrion is significantly reduced in all sublobular zones ($p < 0.001$), a geometrical consequence of the enlargement of the mitochondria. Cortisone administration also results in a reduction of both the smooth- and rough-surfaced endoplasmic reticulum, especially in the midzonal ($p < 0.001$) and peripheral regions ($p < 0.001$).

The numbers of mitochondria, peroxisomes, and lysosomes counted in the measured areas of cytoplasm are listed at the top of Table III. In the case of mitochondria, these figures were combined with corresponding data in Tables I and II, to yield average values for their lengths, diameters, and numbers. This calculation is based on the assumption that the mitochondria can be represented as a population of right circular cylinders (14). The quantitative data confirm the morphological observations that the mitochondria are enlarged in all zones and that this change is most pronounced in the midzonal region ($p < 0.001$). The standard errors for the several mitochondrial dimensions listed in Table III are larger in the steroid-treated animals. These indicate a greater heterogeneity in the mitochondrial population, a consequence of

Downloaded from jcb.rupress.org on April 11, 2014

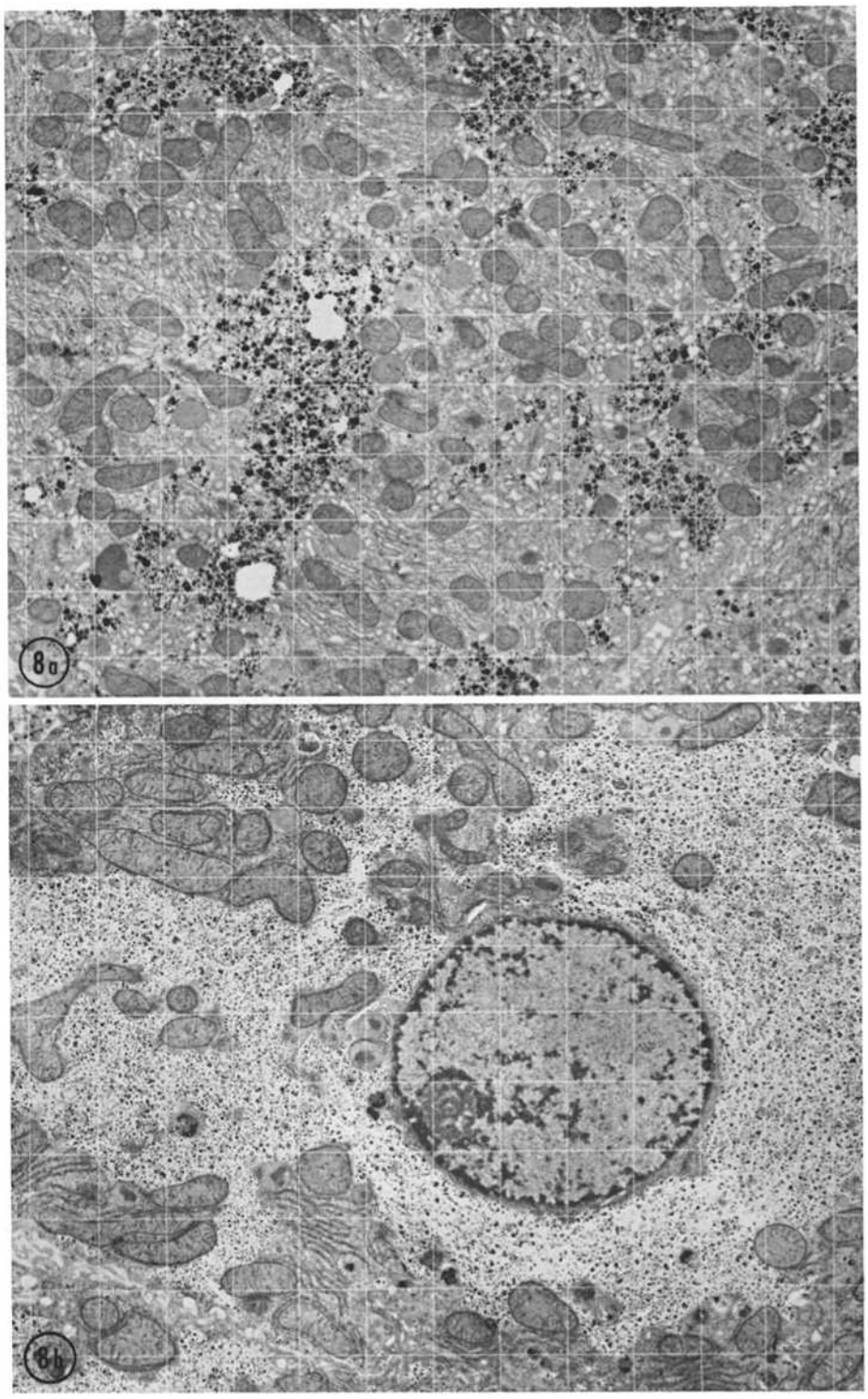
Published April 1, 1968



FIGURES 7–9 These figures are × ½ reproductions of electron micrographs printed with the superimposed grid used for stereological structural analysis, at an original magnification of 12,500 ×. The micrographs labeled *a* were taken from control animals, and those labeled *b* from steroid-treated animals. Typical cells from peripheral lobular, midzonal, and centrilobular regions of hepatic lobules are shown in Figs. 7–9, respectively.

Downloaded from jcb.rupress.org on April 11, 2014

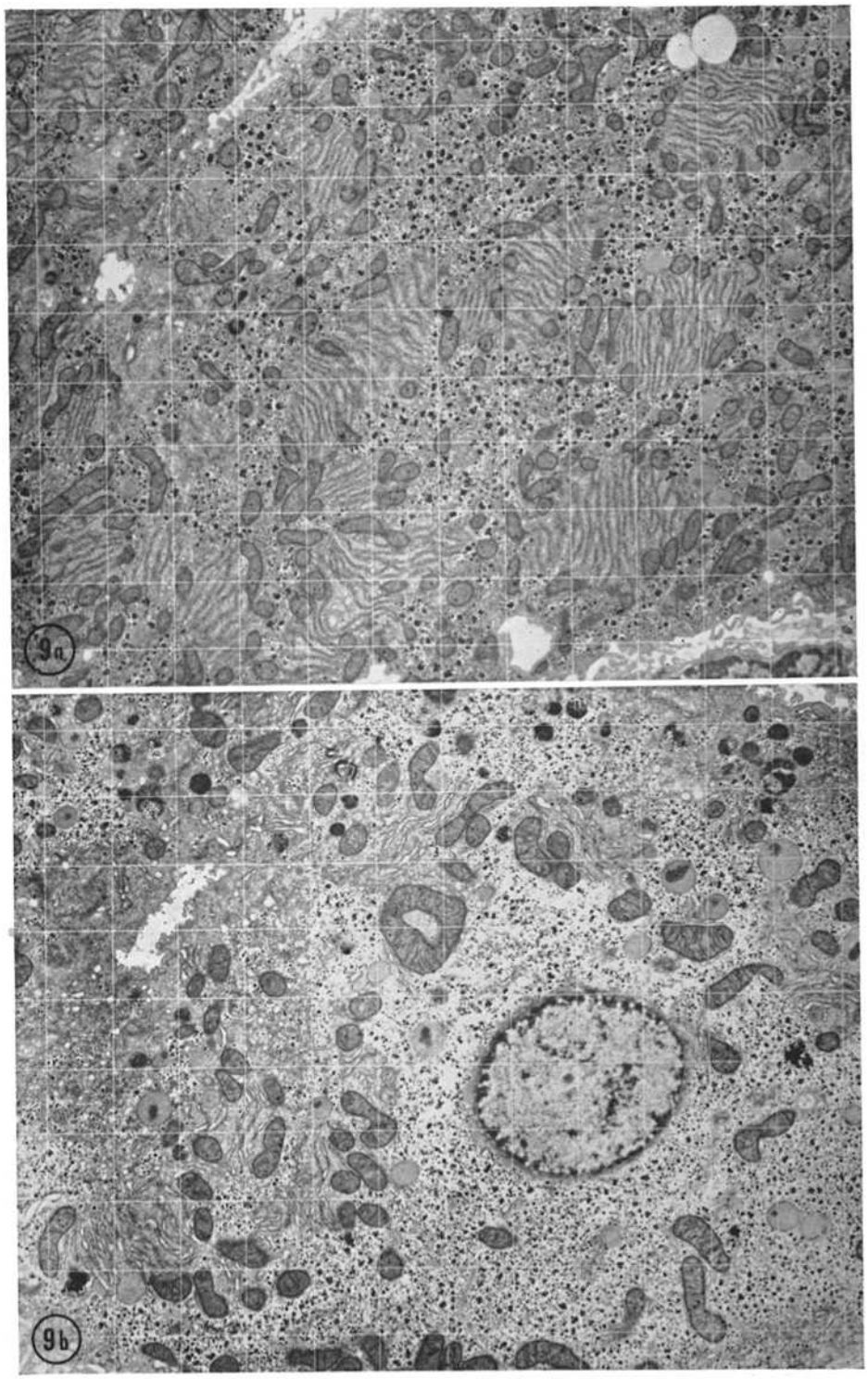
Published April 1, 1968



(For legend, see Fig. 7)

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968



(*For legend, see Fig. 7*)

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

TABLE I

Volume Fraction of Cytoplasmic Components

	Peripheral		Midzonal		Central	
	control	treated	control	treated	control	treated
No. of micrographs	12	17	12	20	12	17
Total area of cytoplasm (μ^2)	3147	4721	3293	5580	3306	4660
% Cytoplasmic volume						
Mitochondria	19.5	22.1	20.5	24.4	15.0	15.3
(Standard error)	(1.4)	(1.6)	(1.6)	(1.4)	(0.5)	(1.0)
Peroxisome	1.7	1.1	1.8	1.0	1.7	1.7
(Standard error)	(0.3)	(0.2)	(0.2)	(0.1)	(0.2)	(0.2)
Lysosomes	0.2	0.8	0.2	0.6	0.4	0.8
(Standard error)	(0.05)	(0.1)	(0.02)	(0.1)	(0.05)	(0.2)
Lipid	0.1	0.6	0.2	0.6	0.2	0.3
(Standard error)	(0.1)	(0.2)	(0.06)	(0.2)	(0.1)	(0.1)
Glycogen	20.6	37.5	21.6	39.4	17.0	30.3
(Standard error)	(1.8)	(2.3)	(2.3)	(1.2)	(1.6)	(2.4)
Other	57.9	38.2	55.7	34.1	65.7	51.6
(Standard error)	(1.2)	(1.9)	(2.6)	(3.0)	(1.9)	(2.3)

TABLE II

Areas of Mitochondrial Envelope and Endoplasmic Reticulum Membrane

	Peripheral		Midzonal		Central	
	control	treated	control	treated	control	treated
μ^2 Mitochondrial envelope / μ^3 Mitochondria	7.51	5.03	7.45	4.27	10.9	7.73
(Standard error)	(0.49)	(0.19)	(0.43)	(0.31)	(0.3)	(0.55)
μ^2 Endoplasmic reticulum / μ^3 Cytoplasm						
Smooth-surfaced	2.21	0.94	1.39	0.57	3.76	2.26
(Standard error)	(0.27)	(0.07)	(0.07)	(0.02)	(0.36)	(0.24)
Rough-surfaced	3.98	2.18	3.82	2.38	2.96	2.46
(Standard error)	(0.17)	(0.14)	(0.18)	(0.19)	(0.21)	(0.16)
Total intersections counted						
Mitochondrial envelope	2997	3407	3275	3783	3504	3590
Smooth endoplasmic reticulum	4522	2875	2984	2051	8094	6846
Rough endoplasmic reticulum	8164	6688	8186	8632	6362	7453

the presence of some irregularly shaped mitochondria within each sublobular zone of the livers from treated animals. Of the 3,958 mitochondrial profiles counted in the liver cells of treated animals, 27 (0.7%) had annular shapes and 38 (1.0%) showed branched profiles. These figures are in contrast to the 5,135 mitochondrial profiles counted in the control animals, in which there were no annular forms and 18 (0.3%) branched shapes.

The average dimensions of peroxisomes were calculated on the assumption that they have a spherical shape. Although cortisone induces no significant change in the diameter of individual peroxisomes, it does produce a significant decrease in the number of these organelles per unit volume of cytoplasm in the peripheral and midzonal regions ($p < 0.01$). No values for the dimensions of lysosomes are presented because of their size, variation, and small number. Nevertheless, the

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

TABLE III

Number and Dimensions of Mitochondria and Peroxisomes (Microbodies)

	Peripheral		Midzonal		Central	
	control	treated	control	treated	control	treated
No. particles counted						
Mitochondria	1424	1032	1623	1103	2088	1823
Peroxisomes	206	175	240	121	226	255
Lysosomes	86	200	57	165	103	198
Mitochondrial dimensions						
Average diameter (μ)	0.58	0.85	0.60	1.08	0.39	0.58
(Standard error)	(0.04)	(0.05)	(0.05)	(0.08)	(0.02)	(0.04)
Average length (μ)	3.23	6.12	2.48	3.62	4.28	2.59
(Standard error)	(0.83)	(0.60)	(0.26)	(0.52)	(0.56)	(1.46)
Average volume (μ^3)	0.85	3.48	0.71	3.29	0.50	0.67
(Standard error)	(0.16)	(0.24)	(0.14)	(0.55)	(0.06)	(0.30)
Average number/100 μ^3 cytoplasm	23	6	29	7	30	23
(Standard error)	(3)	(1)	(4)	(1)	(4)	(4)
Peroxisome dimensions						
Average diameter (μ)	0.69	0.77	0.68	0.64	0.70	0.74
(Standard error)	(0.02)	(0.12)	(0.04)	(0.09)	(0.03)	(0.04)
Average volume (μ^3)	0.17	0.23	0.16	0.14	0.18	0.21
(Standard error)	(0.02)	(0.06)	(0.02)	(0.10)	(0.03)	(0.04)
Average number/100 μ^3 cytoplasm	10	5	11	7	10	8
(Standard error)	(1)	(1)	(1)	(1)	(1)	(1)

data accumulated for lysosomes in Tables I and III suggest an approximate doubling of their number as a consequence of cortisone administration.

The results in Tables I–III are expressed per unit volume of cytoplasm. When these values are multiplied by the average volume of cytoplasm per cell (nucleus), the figures listed in Table IV are obtained. The upper portion of this table shows the actual volume of cytoplasm occupied by various components. The membrane areas of endoplasmic reticulum per average cell (nucleus) have been corrected to compensate for the lack of visibility of obliquely sectioned membranes in electron micrographs. This correction factor is not applicable to mitochondrial envelope measurements, but is appropriately applied to the estimation of intramitochondrial cristae. The administration of cortisone causes a decrease in the area of smooth-surfaced endoplasmic reticulum to about one-half, rough-surfaced endoplasmic reticulum to about two-thirds, and mitochondrial envelope to about four-fifths of the control values. On the other hand, the surface area of mitochondrial cristae appears to be essentially unaltered. From the table (Table IV), it can be seen that the mitochondrial cristae have the greatest area among all classes of membranes in each lobular zone from both control and steroid-treated animals.

The numbers of mitochondria and peroxisomes per average cell (nucleus) are given in the lower portion of Table IV. Steroid treatment reduces the number of mitochondria to less than one-third of the control values in the midzonal and peripheral regions of the lobules. The number of peroxisomes is also decreased in all lobular zones, but the ratio of mitochondria to peroxisomes changes from a control value of more than two to a ratio of one in the midzonal region of cortisone-treated animals.

DISCUSSION

The net result of cortisone administration on the ultrastructure of rat liver parenchymal cells is a decrease in the area of membranes in contact with the cytoplasmic ground substance. Nuclear membrane is reduced as a result of smaller nuclear diameters; peroxisome membrane is reduced by a loss of peroxisomes without a change in size; mitochondrial envelope is reduced as a consequence of mitochondrial enlargement; and the membranes of smooth- and rough-surfaced endo-

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

TABLE IV

Ultrastructural Composition of Rat Liver Parenchymal Cells Calculated Per Average Cell (Nucleus)

	Peripheral		Midzonal		Central	
	control	treated	control	treated	control	treated
Volume (μ^3)						
Total cytoplasm*	5100	5800	5100	5800	5100	5800
Mitochondria	995	1280	1046	1415	765	886
Peroxisomes	87	64	92	58	87	99
Lysosomes	10	35	10	35	20	46
Lipid	5	35	10	35	10	17
Glycogen	1050	2176	1100	2285	867	1757
Other	2953	2210	2842	1972	3351	2995
Membrane area (μ^2)						
Smooth endoplasmic reticulum‡	17000	8170	10600	4960	28800	19600
Rough endoplasmic reticulum‡	30400	18900	29200	20600	22600	21300
Mitochondrial envelope	7470	6440	7790	6040	8300	6860
Mitochondrial cristae§	39600	40800	32800	36500	31400	29400
No. of						
Mitochondria	1160	370	1480	430	1530	1320
Peroxisomes	490	280	560	420	490	460

* Cytoplasmic volume was measured per nucleus because of the significant number of large binucleate parenchymal cells and is approximately equivalent to that of an average mononucleate cell (10).

‡ These figures, which were obtained by multiplying the measured membrane area per unit volume by the average volume per cell, have been further increased by 50% to correct for the effect of oblique sectioning on the observability of membrane profiles (15).

§ These numbers are based on higher magnification measurements of intramitochondrial structure reported elsewhere (11).

plasmic reticulum are reduced by "disappearance." The alteration in mitochondria is equivalent to a repackaging of mitochondrial substance into larger, but fewer units with relatively little change in their volume, composition, or area of cristae membrane per cell.

The quantitative pattern of cytoplasmic ultrastructure in hepatic parenchymal cells has recently been described from samples of the left lateral lobes of normal adult female rats (10). The corresponding values for the control animals in the present study were drawn from the median lobes of young male rats. The agreement between these two sets of measurements is almost exact and indicates a uniformity of hepatic ultrastructure in different lobes, sexes, and ages.

Although no increase in the size of hepatic parenchymal cells was observed qualitatively in this study, the quantitative data indicate a slight increase in their volume. The same course of treatment produces a small (10–15%) increase in total liver weight that is commensurate with the increase in average cell volume, and this accounts for the fact that the amount of DNA in the whole liver is unchanged.[1] Thus, the total number of liver cells would appear to be unaltered. Other workers, using larger doses of cortisone or more prolonged treatment have reported larger increases in total liver weight (16, 17) and observable increases in hepatic parenchymal cell size by light microscopy (9). The increase in cytoplasmic volume is accentuated in appearance by the decrease in the average nuclear diameters of these cells. While it appears unlikely that the nuclear change is the result of a decrease in the amount of DNA per cell nucleus, it may be related in some manner to the inhibitory effects of cortisone on cell division (18–20).

Cortisone administration also results in a marked decrease in the membrane areas of both the rough- and smooth-surfaced endoplasmic reticulum. While some studies on the turnover of endoplasmic

[1] J. N. Loeb. Personal communication.

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

reticulum membrane have been performed (21), there is no information available regarding the turnover of these membranes in the livers of cortisone-treated animals. Recent work has shown that liver RNA content is not diminished after an identical course of cortisone treatment in rats[1]. The decrease in rough-surfaced endoplasmic reticulum and the absence of significant alterations in total RNA suggest, assuming there is no change in ribosomal RNA, that the number of free ribosomes may be correspondingly increased. Alternatively, the number of ribosomes bound per unit area of membrane may be increased following cortisone treatment. Alterations in the distribution of free and membrane-bound ribosomes have been observed in other experimental situations, e.g. fasting (22), following partial hepatectomy (23), in certain liver cell neoplasms (22, 24) and in dorsal root ganglion cells undergoing chromatolysis (25). Although recent studies have failed to demonstrate intrinsic differences in the properties of free and membrane-associated ribosomes (26), the possibility does exist that such alterations in ribosomal distribution may be related to the synthesis of different types of proteins (27).

Following cortisone treatment, a two- to threefold increase is noted in the number of lysosomes per unit area of cytoplasm. This may represent decreased lysosomal degradation in vivo, since it is well established that cortisone administration stabilizes the membranes of lysosomes when subsequently tested in vitro (7). On the other hand, the number of peroxisomes in the midzonal and peripheral regions of the hepatic lobule is decreased following cortisone administration, roughly in parallel to the decrease in the endoplasmic reticulum in these regions. This is of interest in view of both biochemical (28) and morphological (29–33) evidence that implicates the participation of endoplasmic reticulum in the formation of peroxisomes. The twofold increase in the volume of glycogen following cortisone treatment results from an increase in both the formation and activity of several enzymes participating in amino-acid degradation and gluconeogenesis (1, 17, 34–36), as well as from an increase in glycogen synthetase activity (37, 38) and a decrease in peripheral glucose utilization (39, 40).

The well known increase in hepatic lipid that follows cortisone administration appears to result from the mobilization of lipid from peripheral stores (41–43). The intimate structural association between lipid droplets and mitochondria in the hepatic parenchymal cells of cortisone-treated animals has been previously interpreted as a morphological representation of lipid utilization for energy purposes (44).

The most striking effect of cortisone treatment on hepatic parenchymal cells is the appearance of greatly enlarged mitochondria. The quantitative data indicate an increase as great as fourfold in the average volume of mitochondria in midzonal regions. Such calculations of the volume and other average dimensions of mitochondria have been based on the assumption that mitochondria can be represented as a population of right circular cylinders (14). With the presence of irregularly shaped mitochondria in the liver, this assumption may lead to an underestimation of the average particle volume (10). However, since irregular shapes constitute only 1.7% of the mitochondrial profiles in the cells of treated animals, it seems unlikely that their presence will introduce serious errors into the calculation of mitochondrial characteristics or invalidate the usefulness of the cylindrical model as a device for expressing mitochondrial changes.

Structural data also confirm the morphological observations that the mitochondria are not simply "swollen," by showing that the ratio of cristae membranes to matrix substance remains relatively constant, as does the total mitochondrial volume per cell. The enlarged mitochondria could result from either fusion of smaller mitochondria or from a defect in mitochondrial genesis, with continued growth of some of these organelles. This matter could not be resolved by the morphological observations.

The influence of cortisone administration on parenchymal cell cytoplasm occurs proportionately in all sublobular zones, preserving the normal pattern of relative sizes and amounts of structural elements. Since mitochondria in centrilobular cells are normally smaller, the enlarged mitochondria in the central cells of cortisone-treated rats are still only about the same size as those in the peripheral cells of control animals. Because of this similarity in size, random sections of liver from treated animals may appear to show that cortisone affects only some, but not all, of the parenchymal cells. The fact that cortisone affects all cells becomes apparent, however, when proper sublobular sampling is used in conjunction with an understanding of the normal distribution of the sizes of mitochon-

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

dria. Finally, the application of quantitative stereology to a variety of experimental situations may be expected to result in a better understanding of structure-function relationships (11).

Presented in part at the Fifty-First Annual Meeting of the American Society for Experimental Pathology, on April 19, 1967 in Chicago, Illinois (45).

This study was supported in part by grants HE-05906, AM-07625, HE-05741, TI-AM-5397, and the General Research Support Grant of the National Institutes of Health of the United States Public Health Service.

The authors wish to acknowledge the helpful suggestions of Dr. John N. Loeb and the technical assistance of Mr. M. Rosen, Mr. L. W. Koster, Mrs. M. Demeri, Miss B. Shiner, Miss C. Anderson, Miss D. Botyrius, and Mr. L. Borgenicht.

Received for publication 28 July 1967, and in revised form 4 December 1967.

REFERENCES

1. FEIGELSON, P., and M. FEIGELSON. Studies on the mechanism of cortisone action. 1964. *In* Actions of Hormones on Molecular Processes. G. Litwack and D. Kritchevsky, editors. John Wiley and Sons, Inc. New York. 218.
2. LONG, C. H. N., O. K. SMITH, and E. G. FRY. 1960. Actions of cortisol and related compounds on carbohydrate and protein metabolism. *In* Metabolic Effects of Adrenal Hormones. Ciba Foundation Study, Group No. 6. G. E. W. Wolstenholme and M. O'Connor, editors. Little, Brown and Co. Boston. 4.
3. BRADY, R. O., F. D. W. LUKENS, and S. GURIN. 1951. Synthesis of radioactive fatty acids in vitro and its hormonal control. *J. Biol. Chem.* **193**:459.
4. ASHMORE, J., G. F. CAHILL, JR., R. HILLMAN, and A. E. RENOLD. 1958. Adrenal cortical regulation of hepatic glucose metabolism. *Endocrinology*. **62**:621.
5. KERPPOLA, W. 1960. Uncoupling of the oxidative phosphorylation with cortisone in liver mitochondria. *Endocrinology*. **67**:252.
6. KIMBERG, D. V., and S. A. GOLDSTEIN. 1967. Binding of calcium by liver mitochondria of rats treated with steroid hormones. *J. Biol. Chem.* **241**:95.
7. WEISSMANN, G., and L. THOMAS. 1963. Studies on lysosomes. II. The effect of cortisone on the release of acid hydrolases from a large granule fraction of rabbit liver induced by an excess of vitamin A. *J. Clin. Invest.* **42**:661.
8. LOWE, C. U., D. MACKINNEY, and D. SARKARIA. 1955. Effects of cortisone on rat liver mitochondria. *J. Biophys. Biochem. Cytol.* **1**:237.
9. WIENER, J., T. S. COTTRELL, W. MARGARETTEN, and D. SPIRO. 1967. An electron microscopic study of steroid induced reticuloendothelial blockade. *Am. J. Pathol.* **50**:187.
10. LOUD, A. V. 1968. A quantitative description of the ultrastructure of normal rat liver parenchymal cells. *J. Cell Biol.* **37**:27.
11. KIMBERG, D. V., A. V. LOUD, and J. WIENER. 1968. Cortisone-induced alterations in mitochondrial function and structure. *J. Cell Biol.* **37**:63.
12. REVEL, J. P. 1964. Electron microscopy of glycogen. *J. Histochem. Cytochem.* **12**:104.
13. BACH, G. 1964. Bestimmung der Häufigkeitsverteilung der Radien Kugelförmiger Partikel aus den Häufigkeiten ihrer Schnittkreise in zufälligen Schnitten der Dicke δ. *Z. wissensch. Mikroskop.* **66**:193.
14. LOUD, A. V., W. C. BARANY, and B. A. PACK. 1965. Quantitative evaluation of cytoplasmic structures in electron micrographs. *Lab. Invest.* **14**:996.
15. LOUD, A. V. 1967. Quantitative estimation of the loss of membrane images resulting from oblique sectioning of biological membranes. *In* Proceedings 25th Anniversary Meeting Electron Microscopy Society of America. C. J. Arceneau, editor. Claitor's Book Store. Baton Rouge. 144.
16. LOWE, C. U., and R. N. RAND. 1956. Alterations in biochemical composition and ribonucleic acid metabolism induced in rat liver by cortisone. *J. Biophys. Biochem. Cytol.* **2**:331.
17. WEBER, G., and R. L. SINGHAL. 1964. Role of enzymes in homeostasis. V. Actinomycin and puromycin inhibition of cortisone-induced synthesis of hepatic glucose-6-phosphatase and 1,6-diphosphatase. *J. Biol. Chem.* **239**:521.
18. RAGAN, C., E. L. HOWES, C. M. PLOTZ, K. MEYER, and J. W. BLUNT. 1949. Effect of cortisone on production of granulation tissue in the rabbit. *Proc. Soc. Exptl. Biol. Med.* **72**:718.
19. GROSSFELD, H., and C. RAGAN. 1954. Action of hydrocortisone on cells in tissue culture. *Proc. Soc. Exptl. Biol. Med.* **86**:63.
20. BULLOUGH, W. S. 1955. Hormones and mitotic activity. *Vitamins and Hormones.* **13**:261.
21. OMURA, T., P. SIEKEVITZ, and G. E. PALADE. 1967. Turnover of constituents of endoplasmic

Downloaded from jcb.rupress.org on April 11, 2014

Published April 1, 1968

reticulum membranes of rat hepatocytes. *J. Biol. Chem.* **242**:2389.

22. WEBB, T. E., G. BLOBEL, and V. R. POTTER. 1964. I. A study of *in vivo* patterns in liver and hepatomas. *Cancer Res.* **24**:1229.
23. CAMMARANO, P., G. GIUDICE, and B. LUKES. 1965. Polyribosomes in regenerating liver. *Biochem. Biophys. Res. Commun.* **19**:487.
24. WEBB, T. E., G. BLOBEL, V. R. POTTER, and H. P. MORRIS. 1965. Polyribosomes in rat tissues. II. The polyribosome distribution in the minimal deviation hepatomas. *Cancer Res.* **25**:1219.
25. MACKEY, E. A., D. SPIRO, and J. WIENER. 1964. A study of chromatolysis in dorsal root ganglia at the cellular level. *J. Neuropathol. Exptl. Neurol.* **23**:508.
26. LOEB, J. N., R. R. HOWELL, and G. M. TOMKINS. 1967. Free and membrane-bound ribosomes in rat liver. *J. Biol. Chem.* **242**:2069.
27. BIRBECK, M. S. C., and E. H. MERCER. 1961. Cytology of cells which synthesize protein. *Nature.* **189**:558.
28. HIGASHI, T., and T. PETERS, JR. 1963. Studies on rat liver catalase. II. Incorporation of ^{14}C-leucine into catalase of liver cell fractions *in vivo*. *J. Biol. Chem.* **238**:3952.
29. HAGIWARA, A., T. SUZUKI, and F. TAKAKI. 1961. Electron microscopic cytohistopathology. VIII. Electron microscopic studies of the liver. 2. Studies on the origin of so-called "microbodies" as a precursor of rat liver mitochondria, and their relations to the secretion granules of the bile. *Jikeikai Med. J.* **8**:51.
30. HRUBAN, Z., H. SWIFT, and R. W. WISSLER. 1963. Alterations in the fine structure of hepatocytes produced by β-3-thienylalanine. *J. Ultrastruct. Res.* **8**:236.
31. DALTON, A. J. 1964. An electron microscopical study of a series of chemically induced hepatomas. *In* Cellular Control Mechanisms and Cancer. P. Emmelot and O. Muhlbock, editors. Elsevier Publishing Co., New York. 211.
32. ERICSSON, J. L. E. 1964. Absorption and decomposition of homologous hemoglobin in renal proximal tubular cells. *Acta Pathol. Microbiol. Scand.* Suppl. **168**:1.
33. NOVIKOFF, A. B., and W. H. SHIN. 1964. The endoplasmic reticulum in the Golgi zone and its relations to microbodies, Golgi apparatus and autophagic vacuoles in rat liver cells. *J. Microscop.* **3**:187.
34. WEBER, G., G. BANERJEE, and S. B. BRONSTEIN. 1961. Role of enzymes in homeostasis. III. Selective induction of increases of liver enzymes involved in carbohydrate metabolism. *J. Biol. Chem.* **236**:3106.
35. NICHOL, C. A., and F. Rosen. 1964. Adaptive changes in enzymatic activity induced by glucocorticoids. *In* Actions of Hormones on Molecular Processes. G. Litwack and D. Kritchevsky, editors. John Wiley and Sons, Inc., New York. 234.
36. RAY, P. D., D. O. FOSTER, and H. A. LARDY. 1964. Mode of action of glucocorticoids. I. Stimulation of gluconeogenesis independent of synthesis *de novo* of enzymes. *J. Biol. Chem.* **239**:3396.
37. HILZ, H., W. TARNOWSKI, and P. AREND. 1963. Glucose polymerisation and cortisol. *Biochem. Biophys. Res. Commun.* **10**:492.
38. SIE, H.-S., and W. H. FISHMAN. 1964. Glycogen synthetase: its response to cortisol. *Science.* **143**:816.
39. MUNCK, A., and S. B. KORITZ. 1962. Studies on the mode of action of glucocorticoids in rats. I. Early effects of cortisol on blood glucose and on glucose entry into muscle, liver and adipose tissue. *Biochim. Biophys. Acta.* **57**:310.
40. LECOCQ, F. R., D. MEBANE, and L. L. MADISON. 1964. The acute effect of hydrocortisone on hepatic glucose output and peripheral glucose utilization. *J. Clin. Invest.* **43**:237.
41. GILLMAN, J., C. GILBERT, E. EPSTEIN, and J. C. ALLAN. 1958. Endocrine control of blood sugar, lipaemia, and ketonaemia in diabetic baboons. *Brit. Med. J.* **2**:1260.
42. SCOW, R. O., S. C. CHERNICK, and B. A. GUARCO. 1959. Ketogenic action of pituitary and adrenal hormones in pancreatectomized rats. *Diabetes.* **8**:132.
43. HILL, R. B., JR., W. E. DROKE, and A. P. HAYS. 1965. Hepatic lipid metabolism in the cortisone-treated rat. *Exptl. Molec. Pathol.* **4**:320.
44. PALADE, G. E., and G. SCHLIDOWSKY. 1958. Functional association of mitochondria and lipid inclusions. *Anat. Record.* **130**:352.
45. LOUD, A. V., J. WIENER, and D. V. KIMBERG. 1967. Cortisone-induced alterations in rat liver fine structure. *Federation Proc.* **26**:575.

Downloaded from jcb.rupress.org on April 11, 2014