Effect of Cortisone Administration on Intracellular Composition of Rat Liver.* (20544)

CHARLES UPTON LOWE AND W. LANE WILLIAMS.

From the Statler Research Laboratories, Children's Hospital, Department of Pediatrics, University of Buffalo School of Medicine, Buffalo, N. Y., and Department of Anatomy, University of Minnesota, Minneapolis.

Profound alteration in the histology of rabbit(1,2) and rat(3) hepatic parenchyma and changes in nucleic acid content of such livers have been reported(1,3,4) following cortisone administration. These changes are characterized by increase in cell size, accumulation of glycogen and marked decrease in cytoplasmic basophilia. Since the major contribution to cellular basophilia (toluidine blue and gallocyanin chromalum staining) is the pentose nucleic acid (PNA) of the particulate material of the liver cell, it seemed important to investigate the effect of cortisone administration upon the PNA content and the total nucleic acid contents of the liver.

Methods. Three groups of Wistar strain rats, 100-200 g in weight, were maintained *ad libitum* on a Rockland rat diet. Group I: Normal controls. Group II: Cortisone-treated for 5 days and sacrificed 24 hours later. Group III: (Recovery group); cortisone-treated for 5 days but not killed until 8 days after the last dose of cortisone. Animals in Groups II and III received 25 mg cortisone† by intramuscular injection daily for 5 days. All animals were killed after a 24-hour fast by exsanguination through the hepatic vein during light ether anesthesia. Control animals were similarly fasted and killed. Livers were removed rapidly, rinsed in isotonic saline and promptly expressed through the fine mesh of an iced tissue press. The tissue was then weighed, suspended in iced, slightly alkaline 0.85% saline, and further homogenized in a glass Potter-Elvehjem grinder. The brei was then diluted with additional alkaline saline so that each ml of solution was equivalent to 0.2 g of whole wet liver. The centrifugal separation was essentially that described by Huseby and Barnum(5,6), with the following fractions being obtained:

Fraction		Relative gravitational force	Time for sedimenting (min.)
Nuclear	(N)	1400	4
Mitochondrial	(Mit)	24000	5
Microsomal	(Mi)	24000	90
Ultracentrifugal	(U)	110000	90
Nonsedimentable	(S)		

The crude fractions were treated as outlined by Barnum and Huseby(7); chemical separation of the phosphorus-containing compounds was carried out essentially as described by Schneider(8), and subsequent analyses for pentose, desoxypentose, phosphorus, nitrogen, glucose and dry weight performed as previously described(1). Histochemical examination of sections was conducted as outlined by Williams, Lowe, and Thomas(2).

In each instance, the phosphorus as well as the carbohydrate content of every particulate nucleic acid fraction was determined in duplicate. The theoretical carbohydrate content based on phosphorus was calculated, assuming a statistical tetranucleotide residue with a pentose-to-phosphorus ratio of 4.84 for pentose nucleic acid(5). If the theoretical and actual values were not within 5% of each other, the results were discarded, except in the case of nuclear PNA, when discrepancies tended to be as great as 10-15%.

Results. Table I‡ summarizes the data ob-

* Supported in part by Contract with the Atomic Energy Commission, through Research Grants, National Institutes of Health, U.S.P.H.S., and by the Medical Research Fund, Graduate School, University of Minnesota.

† Cortisone acetate in a water suspension containing also 0.9% benzyl alcohol, 0.9% sodium chloride, 0.4% polyoxyethylene sorbitan monooleate and 0.5% sodium carboxymethylcellulose was used. This was generously supplied by Dr. Elmer Alpert of Merck and Co., Rahway, N. J.

‡ While the text uses the notation PNA and DNA, all tables indicate values for ribose and desoxyribose, since these were the substances actually measured.

TABLE I. Effect of Cortisone Administration upon Rat Liver Composition.*

Group	Type of exp.	μg/g wet liver — Rib†	DRib‡	Avg Rib/DRib	mg/g wet liver — Glucose	Nitrogen	% dry wt
I	Normal	4409 ± 75 (21)	955 ± 31 (21)	4.70 ± .01	5.21 (5)	35.3 ± .04 (6)	28.8 ± .01 (5)
II	Cortisone	3449 ± 87 (15)	751 ± 15 (15)	4.61 ± .13	56 ± 5.8 (13)	32.1 ± .7 (9)	30.5 ± .03 (10)
III	Recovery§	4786 ± 226 (5)	930 ± 31.7 (5)	4.98 ± .22 (5)	5.8 ± .71 (5)	36.2 ± .11 (5)	30.2 (2)

* Avg and stand. error of mean.
† Ribose × 2.
‡ Desoxyribose.
§ Animals killed 8 days after last dose of cortisone.
Figures in parentheses refer to No. of exp.

TABLE II. Effect of Cortisone Administration upon Composition of Rat Liver Cell Cytoplasm.*

Group	Type of exp.	Rib† — Mit‡	Mi§	U‖	S¶
I	Normal	158 ± 14 (7)	1029 ± 35 (8)	235 ± 21 (5)	197 ± 9 (7)
II	Cortisone	0 (15)	0 (15)	1003 ± 56 (13)	276 ± 10 (17)
III	Recovery**	392 ± 43 (4)	1168 ± 159 (5)	229 ± 28 (5)	296 ± 3 (5)

* Avg and stand. error of mean. † Ribose × 2, μg/g wet liver. ‡ Mitochondria. § Microsomes. ‖ Ultracentrifugable. ¶ Non-sedimentable. ** Animals killed 8 days after last dose of cortisone.
Figures in parentheses refer to No. of exp.

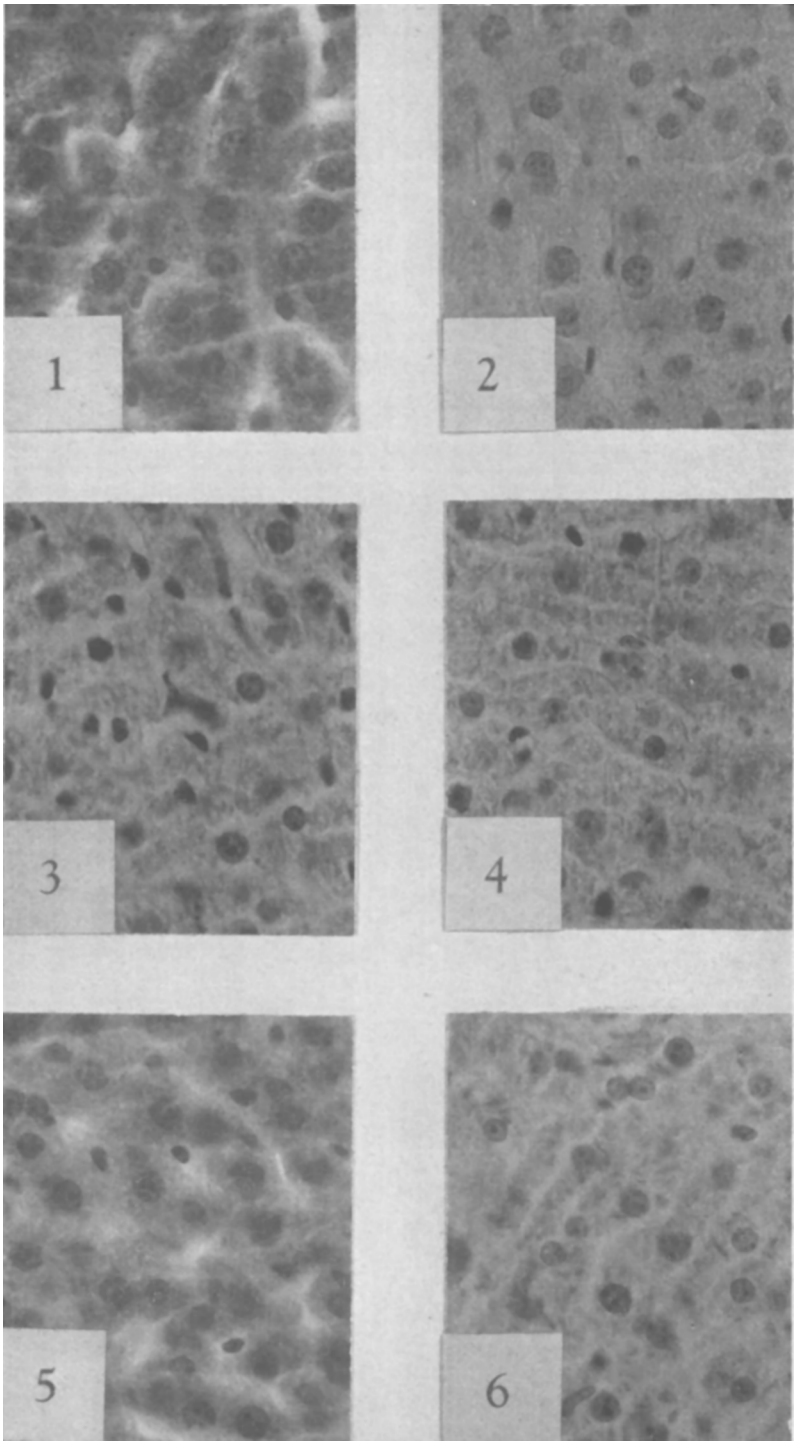
tained on the effects of cortisone upon the composition of rat liver. Following cortisone administration for 5 days (Group II) the PNA and DNA per g of liver decreased as compared to controls (Group I), while the ratio PNA/DNA remained essentially unchanged. Hepatic glucose content increased 10-fold while the nitrogen and water content were quite similar to that found in the normal animals. Eight days after cortisone administration (Group III), the liver composition had returned to that of untreated controls.

Table II shows the results of cytoplasmic fractionation. Mitochondria and microsomes from normal liver were rich in PNA. The most striking finding following cortisone administration (Group II) was the inability to demonstrate mitochondrial and microsomal PNA, although in each instance a small pellet was obtained by centrifugation. The figure for mitochondria of Group II given in Table II is zero, although in 2 out of 15 experiments significant material was obtained. Appreciable PNA was obtained in the microsomal fraction on only one occasion. The U fraction increased almost 5-fold, while the nonsedimentable nucleic acid increased about 30%. While the possibility exists that the increase in the U fraction represented mitochondria and microsomes that sedimented under increased G, this possibility was discarded because of two considerations. Studies (unpublished) using P_{32} indicate the normal U relative specific activity (RSA) to be 4 times that of mitochondria or microsomes. The RSA of U in livers of cortisone-treated animals is es-

TABLE III. Effect of Cortisone upon Composition of Rat Liver Cell Nuclei.*

Group	Type of exp.	DRib†	Rib‡	Avg Rib/DRib
I	Normal	955 (17)	57.1 ± 2.6 (7)	.06
II	Cortisone	751 (15)	43.7 (4)	.058

* Avg and stand. error of mean.
† Whole liver desoxyribose, μg/g wet liver.
‡ Nuclear ribose × 2, μg/g wet liver.
Figures in parentheses refer to No. of exp.



All figures show rat liver, ×750.

Fig. 1-4, sections stained with aqueous toluidine blue.

FIG. 1. Normal rat, buffer (pH 6.8) control section. Abundant basophilic granulation in cytoplasm.

FIG. 2. Section of same liver placed in ribonuclease-buffer solution (pH 6.8) for 5 hr. Basophilia greatly reduced by enzymic hydrolysis.

FIG. 3. Cortisone-treated rat, buffer-control section. Very small amount of cytoplasmic basophilia.

FIG. 4. Same liver as in Fig. 3, but section was subjected to ribonuclease. No significant reduction of basophilia by action of enzyme.

Fig. 5 and 6, sections stained by gallocyanin-chromalum at pH 2.5.

FIG. 5. Normal rat. Large amount of cytoplasmic basophilia.

FIG. 6. Cortisone-treated animal. Very sparse cytoplasmic basophilia.

sentially the same as that of the normals. Dilution of an active fraction with an inactive one should produce significant decrease in RSA of the active one. This was not observed. The second consideration arises from microscopic examination of films made of the U fraction using osmic acid fixation. No significant mitochondrial material was seen.§ Table II further shows that in the "recovery" animals (Group III) a return to almost normal values was noted except for what appears to be over production of Mit and S PNA.

Table III summarizes the studies of nuclei. Following cortisone treatment (Group II), nuclear PNA was reduced on a per g basis as compared to control animals (Group I), but the amount of PNA per unit of DNA was almost identical in the two groups.

The sum of isolated cytoplasmic and nuclear PNA does not equal the amount found in the total liver brei in either the control or experimental animals. This is probably due to the fact that wash waters were not analyzed. However, the loss of PNA was of the same order of magnitude (approximately 50%) in each group of animals.

The histologic findings are illustrated in Fig. 1-6. In contrast to normal animals, following administration of cortisone for 5 days, essentially no cytoplasmic basophilia could be demonstrated by staining with aqueous solutions of toluidine blue or with gallocyanin-chromalum at pH 2.5. The small amount of residual basophilia was not significantly decreased by ribonuclease hydrolysis. While the demonstration of cytoplasmic basophilia by these methods is considered to be specific for PNA(2,9), such methods are not specific for mitochondria. However, it is generally believed that the major contributor to cytoplasmic basophilia of liver cells is the PNA normally associated with mitochondria and microsomes. In no sections was there evidence of necrosis.

Eight days after cessation of injection of cortisone, cytoplasmic basophilia and acidophilia had increased, and returned to levels observed in normal rats.

Discussion. The findings demonstrate that no mitochondrial or microsomal PNA is isolated by centrifugation from livers of rats that had previously received cortisone, when isotonic alkaline saline is used as the suspending medium. Furthermore, the hepatic cells are altered by this hormone to the extent that the cytoplasm no longer responds characteristically to staining methods that demonstrate PNA. From these results, however, it cannot be concluded that mitochondria or microsomes as organized particulate units are not present in the livers of cortisone-treated rats.

Since the ratio of total PNA to DNA in the liver was not significantly altered by cortisone, one may conclude that on the basis of considerations previously presented(1) there was probably no overall loss of PNA; however, the location of PNA in the cytoplasm seemed altered. Since the particles making up the U fraction are not visible in the light microscope, an increase in PNA in that fraction should not affect cellular basophilia. The increase of PNA in U and S was approximately equal to the amount normally present in mitochondria and microsomes. These two findings suggest that one effect of cortisone is to cause a translocation of PNA in liver cells from mitochondria and microsomes to U and S. If mitochondria and microsomes are not removed from liver cells by cortisone, both these particulates are nevertheless grossly altered, for they no longer seem to be associated with significant amounts of PNA.

§ We are indebted to Dr. Daljit Sarkaria for the preparation and interpretation of these films.

It is doubtful that all mitochondria are removed from liver by cortisone, since these particles are the locus of several enzymes, without whose function metabolism seems unlikely(10). The observation of alterations in mitochondrial material in liver cells was an unexpected finding in these experiments. However, several carcinogenic dyes have been reported to change not only the PNA content of mitochondria but also the number per liver cell(11,12). Accompanying these changes there was an alteration in the amount of succinoxidase activity per cell(13,14).

The essentiality of microsomes and microsomal PNA for cellular metabolism is not definite. It is interesting in this connection that Barnum and Huseby(15) have recently shown that PNA can be removed *in vitro* from microsomes of mouse mammary gland infected by the milk agent virus without any loss of infectivity of such microsomes.

Elucidation of the problem of whether or not mitochondria and microsomes are truly removed from liver cells by cortisone may be accomplished by studies using staining methods specific for mitochondria and electron microscope examination for microsomes.

Summary and conclusions. (1) PNA was not demonstrated in the mitochondria or microsomes obtained by differential centrifugation from liver cells of cortisone-treated rats. (2) The characteristic hepatic parenchymal basophilia usually demonstrated by staining with toluidine blue and by gallocyanin-chromalum at pH 2.5 was also lacking. (3) Eight days after cessation of cortisone administration, liver composition had returned to normal.

1. Lowe, C. U., Williams, W. L., and Thomas, L., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 818.
2. Williams, W. L., Lowe, C. U., and Thomas, L., *Anat. Rec.*, 1953, v115, 247.
3. Timiras, P. S., and Koch, P., *Anat. Rec.*, 1952, v113, 349.
4. Gros, F., Bonfils, S., and Macheboeuf, M., *C. Rend. Soc. Biol., Par.*, 1951, v233, 990.
5. Huseby, R. A., and Barnum, C. P., *Arch. Biochem.*, 1950, v26, 187.
6. Barnum, C. P., Nash, C. W., Jennings, E., Nygaard, O., and Vermund, H., *Arch. Biochem.*, 1950, v25, 376.
7. Barnum, C. P., and Huseby, R. A., *Arch. Biochem.*, 1950, v28, 7.
8. Schneider, W. C., *J. Biol. Chem.*, 1945, v161, 293.
9. Lagerstedt, S., *Acta Anat.*, 1949, Supp. IX, 1-116.
10. Schneider, W. C., *J. Biol. Chem.*, 1946, v165, 585.
11. Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., *Cancer Res.*, 1950, v10, 18.
12. Striebich, M. J., Shelton, E., and Schneider, W. C., *Cancer Res.*, 1953, v13, 279.
13. Potter, V. R., Price, J. M., Miller, E. C., and Miller, J. A., *Cancer Res.*, 1950, v10, 28.
14. Schneider, W. C., Hogeboom, G. H., Shelton, E., and Striebich, M. J., *Cancer Res.*, 1953, v13, 285.
15. Barnum, C. P., and Huseby, R. A., *Cancer Res.*, 1950, v10, 523.

Received July 17, 1953. P.S.E.B.M., 1953, v84.

Calorimetric Estimation of Catalase Activity.*† (20545)

HERBERT D. LANDAHL. (Introduced by J. M. Coon.)
From the U. S. Air Force Radiation Laboratory, University of Chicago.

The estimation of catalase activity is generally carried out by measuring the amount of oxygen evolved or the amount of peroxide decomposed(1-5). Though hydrogen peroxide is generally used, perborate has also been

* This study was supported by funds provided under contract with USAF School of Aviation Medicine, Randolph Field, Texas.

† The author is indebted to Mr. T. Tracewell and to Miss J. Taubman for technical assistance in some parts of this work. The human blood samples were supplied through the courtesy of Dr. Evelyn Adams. The author is indebted to Dr. J. M. Coon for reading and discussing the manuscript.