

## EFFECTS OF CORTISONE ON REGENERATING RAT LIVER\*

By STANLEY L. EINHORN,† ERICH HIRSCHBERG, AND ALFRED GELLHORN

*(From the Institute of Cancer Research and the Departments of Biochemistry and Medicine, Columbia University College of Physicians and Surgeons, New York)*

PLATES 8 TO 10

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Many laboratory and clinical findings point to a definite inhibitory effect of cortisone on total body and organ growth (1-18). The central importance of the synthesis of nucleic acids in growth processes and in the general economy of the cell requires no elaboration. The effect of various growth inhibitors on nucleic acid metabolism has engaged the attention of a number of investigators (19-26); the studies of Skipper *et al.* (20), Lowe and collaborators (25), and Cavallero and associates (26) provide the background of the present investigation.

The effectiveness of cortisone, as of other growth-inhibiting agents, appears to be correlated with the rate of growth of the target tissue; in general, the most rapidly growing tissues, whether embryonic, neoplastic, or lymphoid, are affected most markedly. Regenerating rat liver was selected for the present study for a variety of reasons. The size and accessibility of the organ, the relative uniformity of its cell population, the fairly reproducible nature of the restorative process after partial hepatectomy, and the extensive literature available on different aspects of this biological system (27-41) may be cited. An evaluation of the advantages and disadvantages of regenerating rat liver for studies of this type has been made in a previous publication from this laboratory (19).

In the present investigation, the effect of cortisone on the rate of liver regeneration and on nucleic acid metabolism during the restorative process was examined from various points of view. In a paper which became available to us during the preparation of this manuscript, Roberts *et al.* (42) summarized their results on the effect of large doses of cortisone on the regeneration of mouse liver after partial hepatectomy. Their results are in substantial agreement with our findings in the rat.

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† Present address: University of Wisconsin Hospitals, Madison.

### *Materials and Methods*

1. *Preliminary Dosage Experiment.*—Nine female Wistar rats from our own colony, weighing between 161 and 192 gm., were divided into three groups and given daily subcutaneous injections of cortone acetate Merck. The first group received 3 mg./day, the second 5 mg./day, and the third 8 mg./day. After 5 days of treatment, the weight loss in the first group was not consistent, while that in the other two groups was 14 to 19 gm. The daily dosage level was kept at 5 mg./day in all experiments which followed.

2. *Effect of Cortisone on Liver Regeneration and Nucleic Acids.*—Three series of hepatectomies were performed in March, May, and September of 1952 under identical conditions. A total of 87 cortisone-treated rats (72 female and 15 male) and of 42 controls (33 female and 9 male) were included. These rats were derived from litters of the same approximate age and weighed between 140 and 199 gm. at the beginning of the experiment. All animals were kept in individual cages for 5 days before hepatectomy. The removal of two-thirds of the liver was carried out under ether anesthesia according to the method of Higgins and Anderson (38).

At the outset of each series, the animals were transferred from Purina laboratory chow to a special high protein synthetic diet (43). The animals were allowed food *ad libitum*. The basic mixture included crude casein 30 per cent, cerelose 54 per cent, commercial lard 10 per cent, Hawk-Oser salt mixture 4 per cent, ruffex 2 per cent, and a vitamin supplement. This synthetic diet was used in order to provide easily digestible nutrients to rats recovering from a major surgical procedure. For the same reason, a 20 per cent sucrose solution was substituted for the drinking water following hepatectomy. Accurate daily recordings of body weight, and fluid and food intakes were kept throughout the entire experiment, which lasted from 5 days pre-hepatectomy to 7 days (168 hours) posthepatectomy. Animals were sacrificed by cervical dislocation at eight time periods after hepatectomy (12, 24, 36, 48, 60, 84, 108, and 168 hours). The treated animals received 5 mg./day of cortisone in 1 ml. of saline suspension for the entire period from the 5th day before hepatectomy until the day of sacrifice.

The livers removed at hepatectomy and at sacrifice were blotted, weighed to the nearest 0.1 gm., and placed immediately in a deepfreeze unit at  $-20^{\circ}\text{C}$ . Sections of the livers were taken for hematoxylin and eosin and glycogen stains at the time of sacrifice in the first series. The thawed samples of liver were subsequently analyzed for water content by drying small portions in an  $80^{\circ}\text{C}$ . oven overnight. Other weighed portions were homogenized in Potter glass homogenizers in 0.77 M sucrose in 0.01 M phosphate buffer at pH 7.3. Aliquots of the homogenate were used for DNA and RNA determinations by the method of Schneider (44), total nitrogen was determined by the micro Kjeldahl technique, and enumeration of nuclei done according to the method of Price and Laird (27). The procedures used in a previous related investigation in this laboratory (19) were followed in all details. In the present paper, results are reported on the basis of tissue weight as well as per cell, since comparison of data calculated in these units proves helpful in the clarification of some relationships.

3. *Effect of Cortisone on Liver Weight.*—Eighteen female rats were treated for 5 days with cortisone in the manner outlined above. They were maintained on the

synthetic diet, weighed daily, and sacrificed on the 6th day. The weight of the whole livers was compared to that of 12 female rats which were given no drug.

4. *Effect of Cortisone on Mitotic Rate and Fat Content.*—Ten treated rats (6 females and 4 males) and ten control animals (all female) were hepatectomized in the usual manner and two animals from each group were sacrificed at 24, 36, 48, 60, and 108 hours. The rats were taken off the 20 per cent sucrose solution after 24 hours to permit blood sugar determinations to be carried out on the 108 hour group. All animals received a single intraperitoneal injection of 52  $\mu$ g. of colchicine in 1 ml., 8 hours before sacrifice. The animals in the 108 hour group were sacrificed with 0.5 ml. nembutal (60 mg./ml.), in order to keep the blood sugar level as normal as possible. Blood sugars were determined by the method of Folin and Wu on samples of heart blood in these animals.

## RESULTS AND DISCUSSION

### 1. *Effects of Cortisone before Hepatectomy*

(a) *Body Weight.*—The average weight loss of the cortisone-treated group was 3.4 gm. per animal in the 5 day period before hepatectomy. The control group, on the other hand, exhibited an average weight gain of 5.0 gm. per animal during the entire 5 day period. During this time, the average food intake of each animal in the treated group was 13.6 gm. of solid and 19.8 ml. of water daily, whereas the controls consumed 13.3 gm. of solid and 22.0 ml. of water per day. Any differences between the treated and control groups, therefore, cannot be attributed to significant discrepancies in food intake, but must be explained rather as a real metabolic action of cortisone.

(b) *Cellularity, Water Content, and Total Nitrogen.*—In this paper, as in a previous publication from this laboratory (19), "cellularity" is employed to denote the number of cells present in a unit weight of tissue. All pertinent data have been expressed as "number of cells or nuclei per milligram of wet weight of tissue." Table I summarizes the effect of the 5 day period of cortisone treatment before hepatectomy. It may be seen that there is a statistically significant decrease in the cellularity and water content of the liver. There is essentially no change in the total nitrogen content on a wet weight basis, and the decrease in cellularity is therefore mirrored in an apparently significant increase in the total nitrogen content on a cell basis. However, since nitrogen is not an exclusively intracellular component in contradistinction to the nucleic acids, the calculation of nitrogen per cell has little foundation in logic or fact.

(c) *Nucleic Acid Content.*—Table I also summarizes the effect of cortisone treatment before hepatectomy on the nucleic acid content of the resting livers. There is essentially no difference between the treated and control groups in DNA or RNA per cell. The drop in cellularity shown in Table I is sufficiently pronounced, however, to produce a significant decrease in DNA and RNA on a wet weight basis. This finding illustrates the importance of obtaining data

on a cell basis for any interpretation of biochemical mechanisms of action of pharmacological agents.

The studies of Boivin and the Vendrelys (45) and of Mirsky and Ris (46), which demonstrated that the DNA content per nucleus is a constant in various rat tissues, have been widely repeated and extended. Thomson and collaborators (47) have recently summarized these investigations. They also showed that the DNA content of liver nuclei of the adult rat does not vary with sex, strain, or body weight and is not affected by fasting, by a protein-free diet, by a thiamine-deficient diet, by a high fat diet, by thioacetamide, by alloxan, or by pregnancy. Ulmann *et al.* (19) found that the DNA content of the nuclei

TABLE I  
*Effect of Cortisone on the Cellularity, Water Content, Total Nitrogen, and Nucleic Acids of Resting Rat Liver*

	Control*	Treated*	D/SD
Cellularity, nuclei/mg. wet weight.....	177,000	163,000	2.6
Per cent of dry weight.....	30.6	32.3	6.1
Total nitrogen per cell, $\gamma \times 10^{-6}$ .....	16.5	18.5	4.0
Total nitrogen per mg. wet weight, $\gamma$ ...	29.4	29.9	1.4
DNA per cell, $\gamma \times 10^{-6}$ .....	11.0	10.0	2.2
DNA per mg. wet weight, $\gamma$ .....	1.9	1.6	5.4
RNA per cell, $\gamma \times 10^{-6}$ .....	31.1	32.0	0.9
RNA per mg. wet weight, $\gamma$ .....	5.4	5.1	3.1

$D/SD = \frac{M_1 - M_2}{\sqrt{\sigma_{m1}^2 + \sigma_{m2}^2}}$ . The difference between two values is considered significant if this figure is larger than 2.5 (*cf.* Hill, A. B., Principles of Medical Statistics, London, The Lancet, Ltd., 1942).

\* There were 42 rats in the control group and 87 rats in the treated group.

of resting liver was not altered by a single dose of nitrogen mustard, and the present experiments indicate that a series of five injections of cortisone before hepatectomy also fails to affect this biochemical entity to any significant degree.

## 2. Effects of Cortisone after Hepatectomy

(a) *Body Weight.*—After partial removal of the liver, the rats in the control group lost a daily average of 2.6 gm. of body weight, whereas the cortisone-treated animals exhibited a somewhat larger daily weight loss, an average of 3.7 gm. The average food intake of the controls was 4.2 gm. of solid and 16.8 ml. of 20 per cent sucrose per day, that of the treated animals 2.4 gm. of solid and 24.2 ml. of sucrose daily.

On the basis of these observations, it was thought possible that the effects of cortisone seen late in regeneration, which are to be described, might be

attributable simply to protein depletion. However, several earlier investigations yielded evidence in opposition to this possibility. Brues, Drury, and Brues (30) found that when rats were maintained on a high carbohydrate diet, similar to the conditions of the present experiments, the liver after hepatectomy showed weight gains only slightly smaller than in the animals on a normal diet. Drabkin (39) concluded that the amount of DNA restored after hepatectomy, while in close agreement with the degree of liver restoration, was essentially unaffected by changes in dietary protein and that the amount of RNA was completely independent of them. Muntwyler *et al.* (48) reported that maintenance of Wistar rats on a protein-free diet for 21 days did not decrease the RNA or DNA concentration in the livers, whereas the nitrogen concentration was significantly lower than that in control animals. It would appear, therefore, that the effects of cortisone to be described are not simply manifestations of significant dietary restriction in the treated rats.

(b) *Liver Weight*.—In order to determine the gain in liver weight, it was assumed that the median and left lateral lobes removed in partial hepatectomy constitute 68.4 per cent of the total weight of the liver. This value was established by Brues *et al.* (30) with a mean deviation of  $\pm 1.5$  per cent and has been confirmed by a number of other investigators (29, 30, 36, 39). In our series of 180 animals, this calculation was adequate in all but 4 rats. The latter, in which the liver removed at sacrifice weighed less than the amount calculated to have remained after hepatectomy although more than 12 hours had elapsed since the operation, were not included in the experiment.

This assumption appears to be more trustworthy than the alternate procedure of determining the weight of liver remaining after partial hepatectomy on the basis of a calculated ratio of liver weight to body weight (31, 38, 39); this ratio has been found to be more variable in untreated animals than the ratio of the weight of the median and left lateral lobes to the total weight of the liver; in addition, it may be subject to greater change under the influence of as biologically active a drug as cortisone. In our experiments, the average ratio of liver weight to body weight was 3.4 per cent (range 3.1 to 4.0 per cent) in 12 untreated female rats, and 3.8 per cent (range 3.4 to 5.0 per cent) in 18 female rats given five injections of 5 mg./day of cortisone. There is no reason to suppose that cortisone would affect the relationship of the weight of two lobes of the liver to the total weight of the liver in any way.

On this assumption, it is then possible to relate the weight of the liver at sacrifice to the weight of the portion of liver not removed at hepatectomy. The increase in liver weight is a measure of regeneration. Table II summarizes the average per cent gain in liver weight in the control and treated groups. On the basis of liver weight, cortisone has no significant effect on the course of liver regeneration in these experiments.

(c) *Total Gain in Number of Cells*.—This failure to demonstrate a cortisone

effect is predicated on the choice of units employed, in this instance wet weight of tissue. The very real effect of this drug on the course of regeneration is obscured by concomitant changes in the composition of the tissue under examination, but can be demonstrated easily by reference to the total number of cells present in the tissue with and without treatment.

For this determination, the weight of liver at hepatectomy and at sacrifice is multiplied by the corresponding cell counts (*cf.* Table IV) to yield the total number of cells per liver at various times after hepatectomy. Text-fig. 1 shows the pertinent data. The administration of cortisone leads to a significant depression in the restoration of liver in terms of the total number of cells in the later stages of regeneration.

TABLE II  
*Effect of Cortisone on Liver Regeneration in Terms of Liver Weight*

Time after hepatectomy	No. of animals		Average gain in liver weight		
	Control	Treated	Control	Treated	D/S <sub>D</sub>
<i>hrs.</i>			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
12	4*	7	4	9	—
24	2*	7	30	31	—
36	4*	10	32	48	—
48	7	13	67	70	0.3
60	7	13	95	72	1.5
84	3*	8	70	100	—
108	8	16	122	98	1.0
168	7	13	142	112	1.9

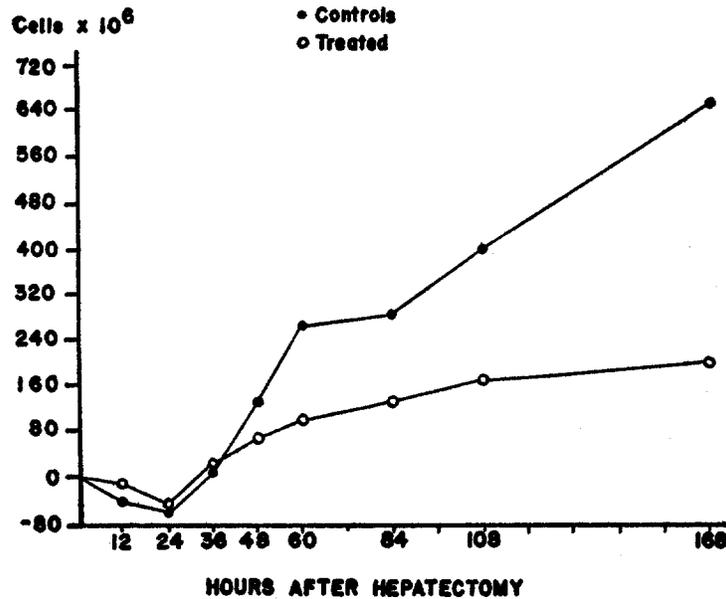
\* In this and in the succeeding two tables, statistical evaluation of the differences between control and treated groups was omitted at these time periods in view of the small number of animals in the control groups.

(*d*) *Mitotic Activity*.—The same effect is observed in a comparison of the mitotic activity after hepatectomy in the control and treated groups. Figs. 1 and 2 are typical photomicrographs of regenerating liver of colchicine-treated rats with and without cortisone treatment sacrificed 48 hours after hepatectomy. At this time, the mitotic activity of the untreated liver is at its peak, in agreement with the findings of Price and Laird (27), while mitoses are largely suppressed in the treated sample. An examination of sections taken at later time periods indicates that cortisone delays but does not permanently block mitosis in regenerating liver. Analogous findings have been made by Landing and collaborators (40) in experiments with nitrogen mustard.

(*e*) *Nucleic Acids*.—If the liver weights at hepatectomy and at sacrifice are multiplied by the corresponding values for nucleic acid content, and the difference is plotted, the extent of regeneration at any one time can be evaluated

in terms of new synthesis of the nucleic acids. Text-figs. 2 and 3 illustrate the effect of cortisone in these terms. These data clearly demonstrate that the administration of cortisone leads to a significant depression in the restoration of liver in terms of the total gains of nucleic acids in the later stages of regeneration.

An examination of these figures also indicates that cortisone exerts little demonstrable effect on the rate of restoration of the nucleic acids during the first 2 days of liver regeneration. The most attractive explanation of this



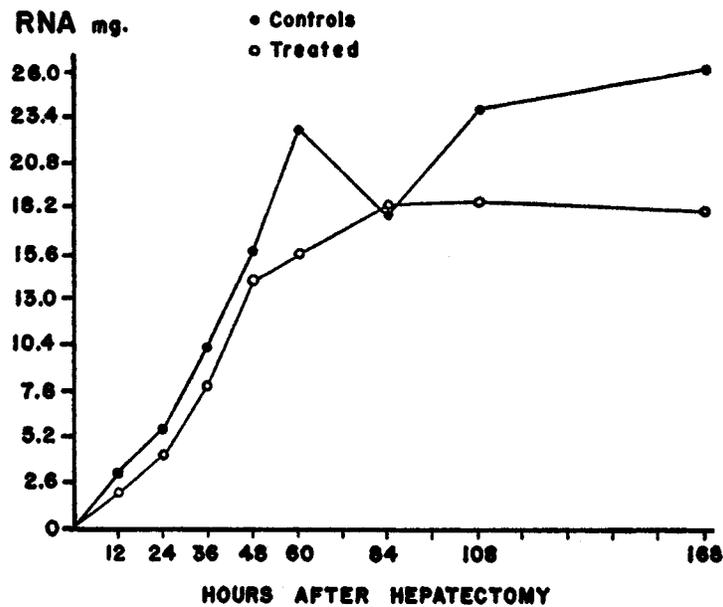
TEXT-FIG. 1. Average total gain in cells per liver (nuclei  $\times 10^6$ ) during the restorative process.

finding is based on the fact that nucleic acid synthesis in the organ as a whole does not begin immediately after hepatectomy, but only proceeds at an accelerating rate as more and more new cells are formed (*cf.* references 19 and 27); the absolute increases in nucleic acid levels during this period are too small to permit the demonstration of significant inhibition. Furthermore, the effect of cortisone may be cumulative rather than immediate. It may also be seen that although the restoration of DNA and RNA in untreated animals proceeds at essentially the same rate, cortisone has a more pronounced inhibitory effect on the restoration of DNA than of RNA.

In the absence of any evidence from the literature that corticosteroids or ACTH increases the breakdown of nucleic acids, the lower nucleic acid levels

in the livers exposed to cortisone must be attributed to an interference by this agent with synthetic processes. The finding of increased urinary levels of uric acid after cortisone or ACTH treatment in human subjects (49-51), which might raise some doubts on this point, appears to have been explained by the demonstration of an effect of ACTH on the renal tubule, to increase the rate of uric acid excretion (52).

The results which have been presented support the hypothesis that cortisone inhibits nucleic acid synthesis in this system. In this regard, the action of



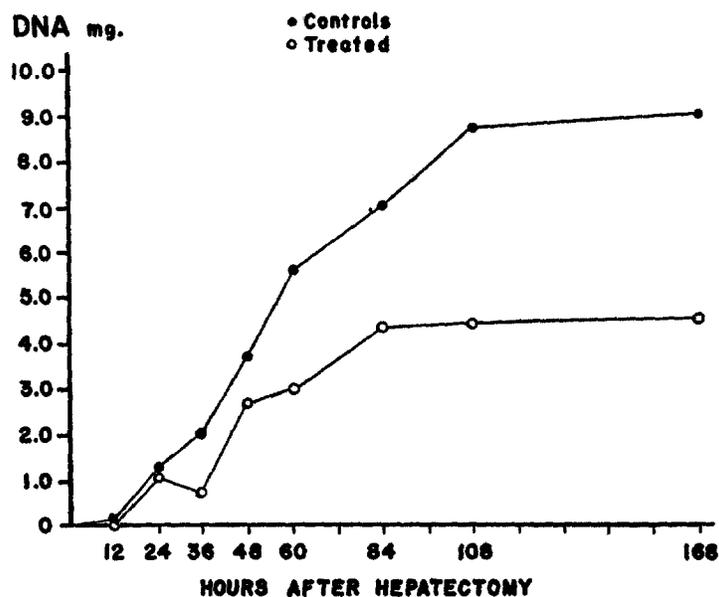
TEXT-FIG. 2. Average total gain in RNA per liver (milligrams) during the restorative process.

cortisone differs decisively from that of nitrogen mustard, since it was shown in an earlier study in this laboratory (19) that the average total content of nucleic acids per liver was actually slightly higher in treated than in untreated animals 36 hours after a single injection of nitrogen mustard.

A comparison of the effect of cortisone on the gain in number of cells (Text-fig. 1) and on the gain in nucleic acids (Text-figs. 2 and 3) shows that the degree of inhibition in both instances is almost identical. It was therefore of interest to determine the nucleic acid content of the average cell at the various times after hepatectomy in treated as well as in untreated livers. A direct approach to this question is the calculation of all nucleic acid levels on the cell basis (*cf.* references 19 and 27). For this purpose, the nucleic acid content

of a homogenate is divided by the number of cells in that homogenate obtained by direct enumeration of nuclei. Table III summarizes the nucleic acid data on this basis. Although there is a suggestion of an accumulation of RNA at 84, 108, and 168 hours, the difference in the nucleic acid per cell of the treated and untreated groups was not statistically significant at any time.

These data again indicate that the mechanism of action of cortisone in this biological system differs from that of nitrogen mustard (19). The subcutaneous injection of a single dose of nitrogen mustard was followed by a significantly



TEXT-FIG. 3. Average total gain in DNA per liver (milligrams) during the restorative process.

greater accumulation of both DNA and RNA in the liver cells at 36 hours after hepatectomy, and it was concluded that inhibition of mitosis by that agent was not mediated primarily through an inhibition of nucleic acid synthesis. The results obtained with cortisone in the present study, on the other hand, are entirely consistent with the interpretation that the inhibition of liver restoration is dependent on and keeps pace with the inhibition of nucleic acid synthesis by this drug.

It may be pointed out in passing that the average nucleic acid content per cell in untreated animals after hepatectomy is generally the same in the present investigation as in the related studies of Price and Laird (27) and Ultmann *et al.* (19).

(f) *Cellularity, Water Content, Total Protein, Non-Protein Nitrogen, Carbohydrate, and Fat.*—The characteristic decrease in cellularity of rat liver during the early stages of regeneration followed by a return to normal cellularity has been described (19, 27). Table IV shows that treatment with cortisone effectively prevents this return to normal cellularity. It was of interest to determine the cause of the marked differences in cellularity in the later stages of regeneration.

Histological studies with hematoxylin-eosin were a first step in this direction. To confirm the decreased cellularity in the treated livers, all parenchymal cell nuclei in 100 consecutive areas, marked off by a micrometer eyepiece, were counted under oil immersion. The comparative results in control and

TABLE III

*Effect of Cortisone on the Average Nucleic Acid Content per Cell after Hepatectomy, Expressed as Per Cent of the Prehepatectomy Value*

Time after hepatectomy	DNA per cell: $\frac{\text{Post-}}{\text{Pre-}} \times 100$			RNA per cell: $\frac{\text{Post-}}{\text{Pre-}} \times 100$		
	Control	Treated	D/S <sub>D</sub>	Control	Treated	D/S <sub>D</sub>
<i>hrs.</i>						
12	123	112	—	139	128	—
24	160	151	—	194	161	—
36	146	118	—	186	176	—
48	137	147	0.9	170	176	0.4
60	136	149	0.9	166	183	1.2
84	159	150	—	139	180	—
108	147	147	—	146	174	2.2
168	123	142	1.1	124	163	2.2

treated samples taken at the various time periods after hepatectomy were essentially the same as those obtained by direct enumeration of nuclei in the corresponding homogenates.

Histological examination further revealed (*cf.* Figs. 3 and 4) that the nucleocytoplasmic ratio is decreased in the treated animals. No change in vascularity is observed. An obvious feature is the extensive vacuolization of the cytoplasm in the treated animals.

The greater weight of the average liver cell under the influence of cortisone may be attributed, *a priori*, to increases in the water, protein, carbohydrate, or fat content of these cells. Data obtained on all four of these entities in the present study indicate that the greater weight of the average cell and the greater decrease in cellularity are caused by the infiltration of a lipid material into the cortisone-treated regenerating livers.

The second and third columns of Table V demonstrate that the water content

of the treated livers is consistently lower, not higher, than that of the controls. This is apparent from the consistent increase in the ratio "dry weight/wet weight" at almost all time periods.

The middle two columns of Table V summarize the effect of cortisone on liver restoration in terms of total nitrogen content. For this determination, the liver weights before and after regeneration are multiplied by the corresponding values for total nitrogen content, and the rise is tabulated. It is seen that, with the exception of a significantly lower value in the treated animals at 168 hours, cortisone does not exert any demonstrable effect on the restoration of liver in terms of total nitrogen. These data also show that the

TABLE IV  
*Effect of Cortisone on the Cellularity of Regenerating Rat Liver*

Time after hepatectomy <i>hrs.</i>	Cellularity, nuclei/mg. wet weight		
	Control	Treated	<i>D/S<sub>D</sub></i>
0*	177,000	163,000	2.6
12	153,000	162,000	—
24	146,000	115,000	—
36	146,000	111,000	—
48	143,000	106,000	3.4
60	131,000	115,000	2.1
84	184,000	111,000	—
108	166,000	118,000	4.7
168	186,000	114,000	4.1

\* From Table I.

greater weight of the treated liver cells cannot be attributed to any significant effect of cortisone on protein metabolism.

The production of negative nitrogen balance by this drug is well known (*cf.* references 53-56). In view of the finding that corticosteroids caused a marked rise in urinary non-protein nitrogen which ceased upon withdrawal of the steroid (57), it was of interest to determine the non-protein nitrogen content of representative homogenates of treated and untreated livers. These determinations did not bring to light any appreciable changes in liver non-protein nitrogen after cortisone treatment.

The ability of corticosteroids to cause an increased deposition of glycogen in the liver has long been recognized (58, 59). In the present study, histological sections reveal that glycogen is abundant at all time periods of regeneration in the cortisone-treated rats. Quantitative glycogen determinations by the method of Good, Kramer, and Somogyi (60) on livers taken at 108 and 168 hours, however, showed no consistent effect of cortisone on this biochemical

entity (*cf.* last two columns of Table V). In any event, the glycogen content never exceeded 6.0 per cent of the wet weight of liver.

Blood glucose determinations according to the method of Folin and Wu on the heart blood of 2 treated and 2 control rats at 108 hours showed no significant diabetogenic effect of cortisone under these conditions. The control animals had values of 131 to 133 mg. per cent, the value for the treated animals was 141 to 172 mg. per cent. In view of the tremendous variations in blood sugar levels in cortisone-induced diabetes (56), these values do not lend themselves to any meaningful interpretation.

TABLE V  
*Effect of Cortisone on the Water Content, Gain in Total Nitrogen, and Glycogen Content of Regenerating Liver*

Time after Hepatectomy	Average dry weight as per cent of wet weight		Average gain in total N		Glycogen as per cent of wet weight of liver	
	Control	Treated	Control	Treated	Control	Treated
<i>hrs.</i>			<i>mg.</i>	<i>mg.</i>		
12	27.7	30.5	5.1	10.0	—	—
24	34.0	33.4	10.0	6.7	—	—
36	27.7	35.5	17.9	20.5	—	—
48	32.1	36.1	37.5	43.3	—	—
60	27.6	36.1	64.5	43.2	—	—
84	30.8	39.0	52.5	58.1	—	—
108	29.3	35.7	72.4	63.5	4.3*	4.2*
168	32.0	35.4	98.3	78.6	4.4	2.5

\* On two animals chosen at random in each group. The glycogen content of the corresponding prehepatectomy samples ranged from 1.4 to 5.9 per cent of the wet weight of liver.

The absence of any significant increase in the water, protein, or carbohydrate content of regenerating rat liver focuses attention on the effect of cortisone on liver lipids. It has been shown that moderate doses of cortisone prevent a loss of body fat in both mice (61) and rats (62); however, the effect on liver fat differs in the two species. Kochakian and Robertson (63) found that cortisone caused a depletion of liver fat in mice, while Ingle reported that in rats the deposition of fat in the liver, under conditions which normally produce fatty livers, is decreased by adrenalectomy. In one previous study on regenerating rat liver (64), the tissue became very fatty after 24 hours of regeneration, but this fatty infiltration was decreased by adrenalectomy.

In our experiments, sections of livers from the last series of animals were stained with sudan black. Figs. 5 and 6 demonstrate that there is a pronounced increase in both intracellular and extracellular fat in the treated group as compared to the controls. Gross examination of the livers from treated ani-

mals in the fresh state showed a pronounced increase in the rubbery consistency, yellow color, and difficulty in homogenization which is characteristic of fatty livers. It is believed that this increased deposition of liver fat under the influence of cortisone adequately explains the greater weight of the average cells and the significantly lower cellularity of the treated livers.

#### SUMMARY

The effects of continuous administration of cortisone on the metabolism of regenerating rat liver have been studied. Whereas the restoration of the weight of the liver after partial hepatectomy was not markedly affected by cortisone, the multiplication of cells was reduced to a significant degree after the first 2 days of regeneration. Liver restoration in terms of nucleic acids was similarly inhibited by cortisone. The results are consistent with the interpretation that the inhibition of cell multiplication in this system is dependent on and keeps pace with the inhibition of nucleic acid synthesis by this drug. At almost any time after hepatectomy, the nucleic acid content of the liver cells was the same in treated and in untreated animals.

In ancillary studies, it was shown that cortisone caused the cells of regenerating liver to be increased in size and weight through the increased infiltration of lipids. Changes in water, protein, and carbohydrate content of the liver cells did not contribute to this increase in the weight of the cells.

Since all animals were treated with cortisone for 5 days before hepatectomy, data were also obtained on the effect of this agent on the resting liver. This course of treatment brought about a significant decrease in the number of cells per unit wet weight and in the water content of the livers. The nucleic acid content of the cells at hepatectomy, on the other hand, was unchanged.

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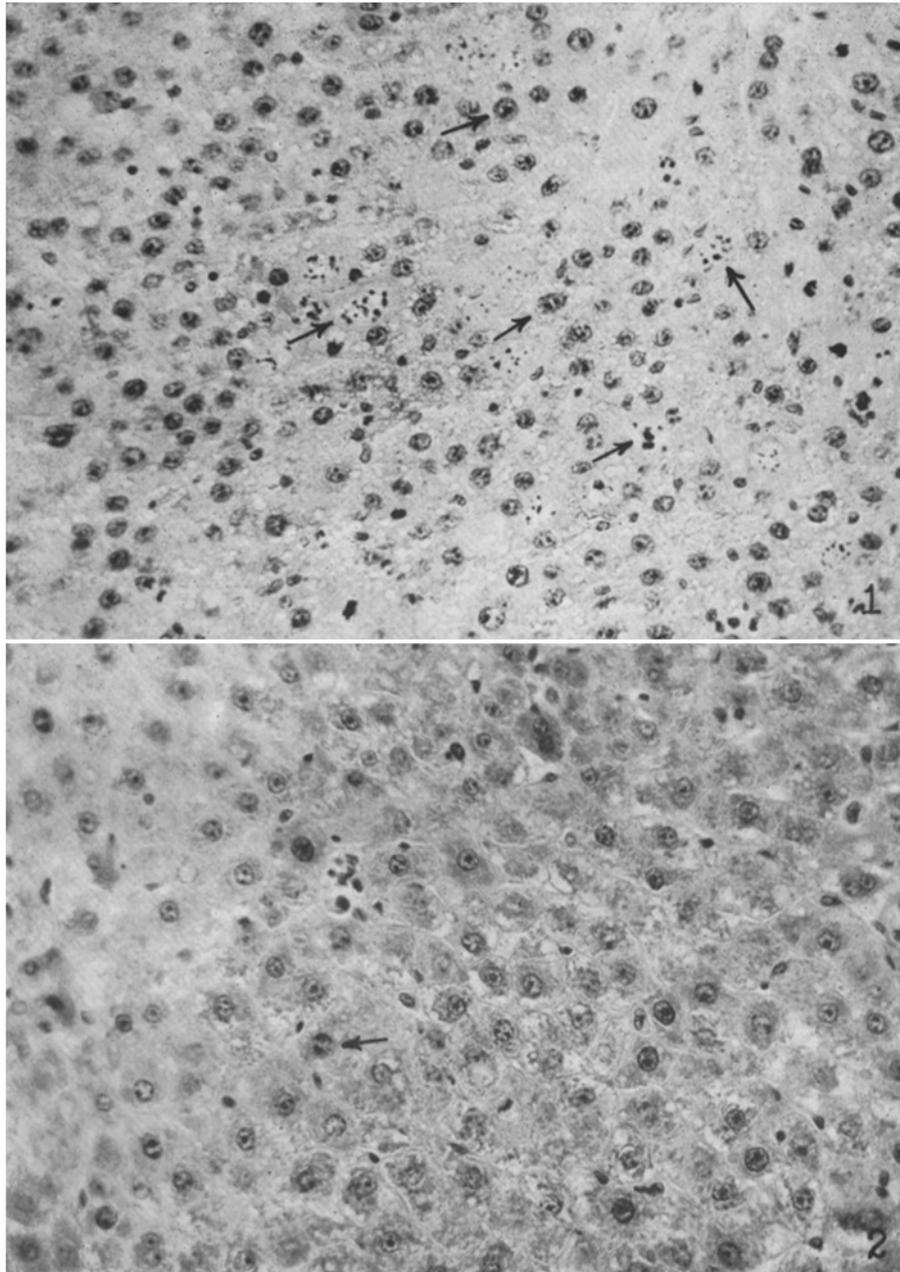
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## EXPLANATION OF PLATES

## PLATE 8

FIG. 1. Liver of control animal, following colchicine. This section, taken at 48 hours, shows the numerous mitotic figures in the liver, at the height of cell division activity. Arrows indicate some of the cells in mitosis. Hematoxylin and eosin.  $\times 320$ .

FIG. 2. Liver of treated animal, following colchicine. Note the decreased frequency of mitotic figures in this liver, also taken at 48 hours. The arrow indicates a cell in mitosis. Hematoxylin and eosin.  $\times 320$ .

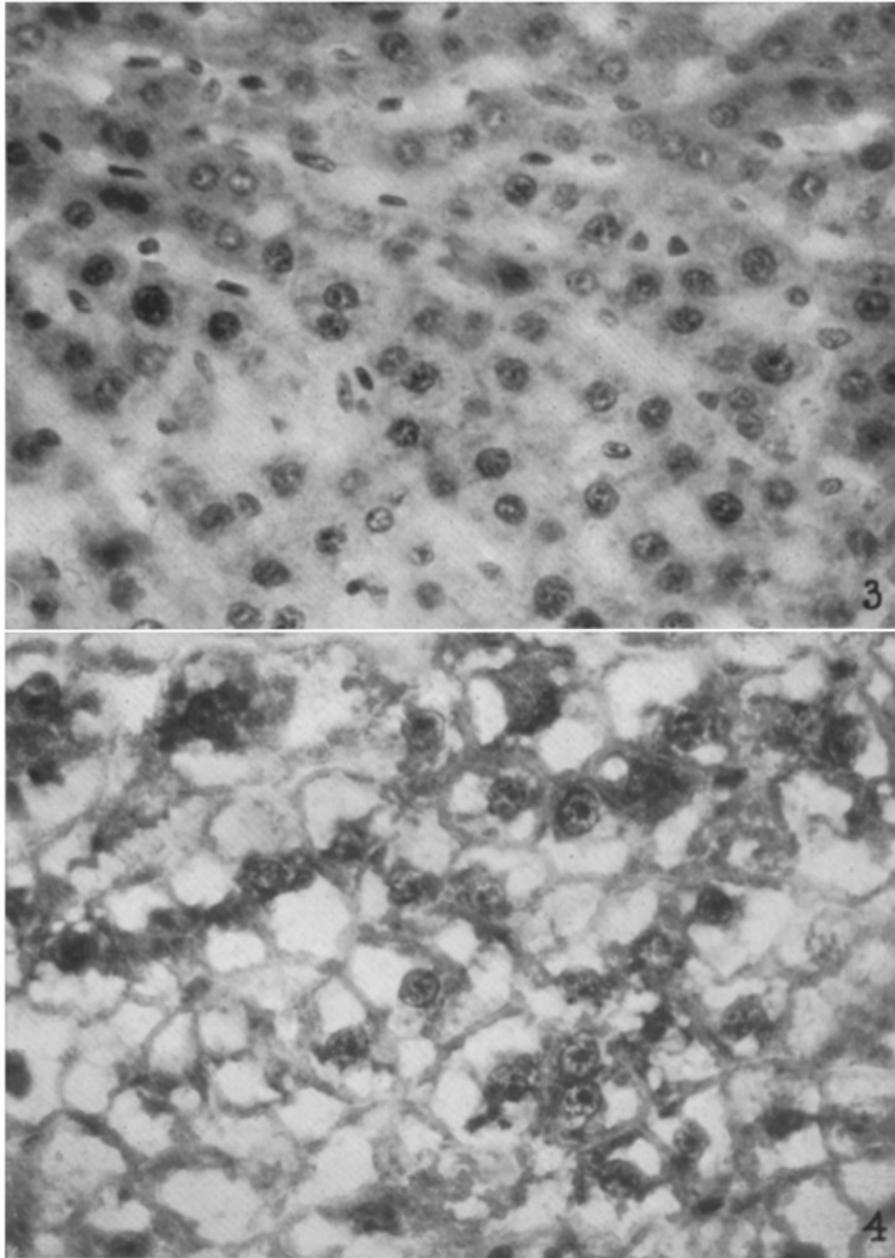


(Einhorn *et al.*: Effects of cortisone on regenerating rat liver)

PLATE 9

FIG. 3. Liver of control animal, 84 hours. Note the deep staining cytoplasm and the regularity of the parenchymal cells. The hepatic lobules appear uniform. Hematoxylin and eosin.  $\times 550$ .

FIG. 4. Liver of treated animal, 84 hours. Here the liver cells appear less compact and more irregular in size and staining properties. The cytoplasm is highly vacuolated. Hematoxylin and eosin.  $\times 550$ .

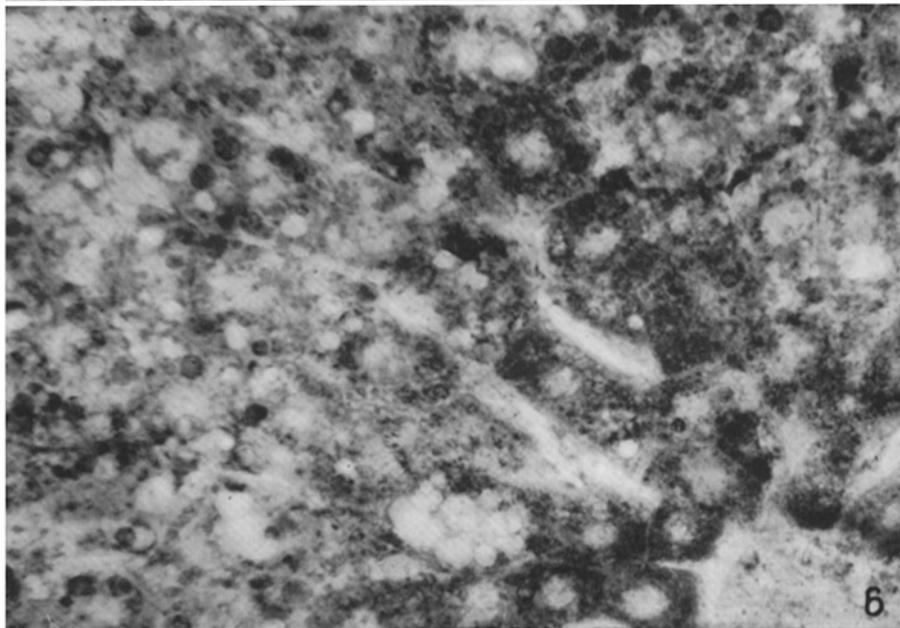
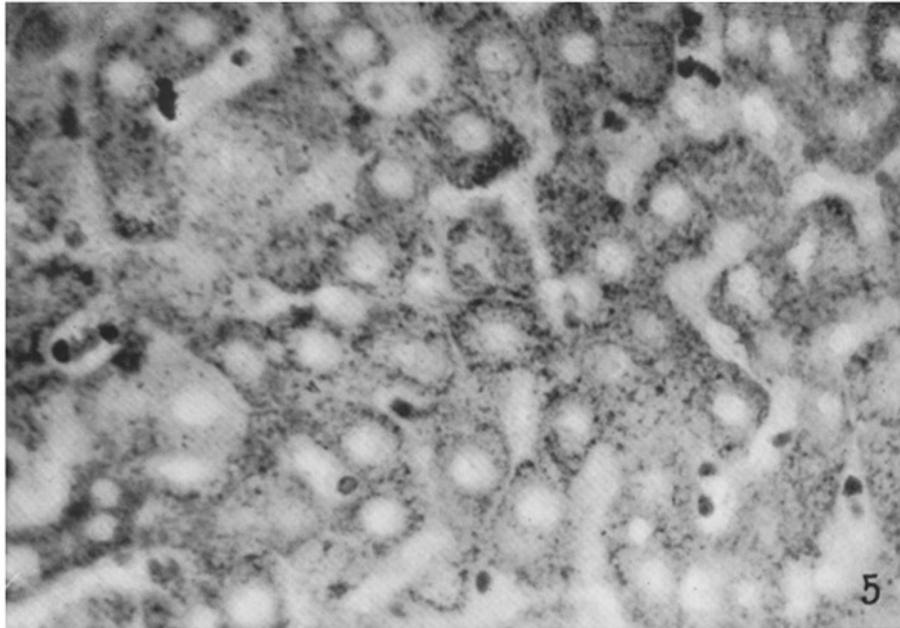


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PLATE 10

FIG. 5. Liver of control animal, 24 hours. Note the small, widely scattered particles of lipid material dispersed throughout the cytoplasm. No large globules are visible. Red blood corpuscles may be seen in the sinusoids. Sudan black.  $\times 550$ .

FIG. 6. Liver of treated animal, 24 hours. Note the large globules of fat as well as the fine particles which permeate the entire cytoplasmic space. Sudan black.  $\times 550$ .



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