Influence of Corticosteroids on the Hepatic Cell and Bile Secretion

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ABSTRACT

Daily administration of glucocorticoids for 10 days to dogs resulted in a significant increase in the hepatic bile secretion in response to secretory stimulants. The response of hepatic bile in testosterone-treated animals was not changed and the response was increased in DOCA-treated animals.

A significant increase of liver weight was induced by the animals receiving glucocorticoids. Other organ weight was not changed; however, a slight reduction of kidney weight was seen in prednisolone, dexamethasone, and DOCA treated animals and also in animals supplemented with cortisone following adrenalectomy. The presence of large areas of ballooning and vesicular changes of liver cells was seen in glucocorticoid treated animals, particularly in cases of dexamethasone and prednisolone. Both vesicular changes of liver cell and its glycogen content were increased by the repeated administration of prednisolone and reduced by the cessation of treatment. Special stain and liver glycogen determination demonstrated the material distending the liver cell was glycogen. These findings indicate that long term administration of glucocorticoids results in an increase of liver weight and hepatic glycogen content as well as increased bile secretion.

INTRODUCTION

It is evident by clinical observation that the jaundice of hepatitis often decreases rapidly following the administration of adrenocorticoids (Colbert et al, 1951; Evans et al, 1953; Sborov et al, 1954; Lee, 1966). Patterson et al (1954) studied this problem directly and concluded that cortisone possesses choleric and hydrocholeretic capacities. Against this interpretation are several studies which have not confirmed a choleric effect (Shay and Sun, 1957; Clifton et al, 1958). However, the observation of Gans and McEntee (1961) that repeated administration of large doses of prednisolone resulted in an increase in hepatic bile flow is of interest. They also observed a peculiar lesion characterized by the presence of large areas of vesicular degeneration in liver sections from the prednisolone treated animals. The present study was performed to explore the status of hepatic bile secretion as well as liver cell change during long term administration of glucocorticoids or other steroids, using secretory stimulants or histologic technique.

METHODS

Mongrel dogs of both sexes, weighing 8 to 14 kg were employed in this experiment and divided into following groups:
Group 1. Control; 10 dogs served as nontreated controls.
Group 2. Glucocorticoid treated; 17 dogs were used. Cortisone acetate, prednisolone, or dexamethasone was administered intramuscularly to

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each subgroup of three dogs for 10 days, at a daily dose of 8.0 mg/kg, 2.0 mg/kg and 0.4 mg/kg, respectively. In addition three adrenalectomized dogs and five prednisolone treated animals for successive liver biopsies were used.

Group 3. Desoxycorticosterone treated; Desoxycorticosterone acetate (DOCA) was administered intramuscularly to a group of three dogs for 10 days, at a dose of 4.0 mg/kg daily.

Group 4. Testosterone treated; Testosterone propionate was administered at a dose of 8.0 mg/kg daily as Group 3.

At the end of the experimental period, food was withheld from each dog for 15 hours. Each dog was then anesthetized with sodium pentobarbital and the trachea was cannulated. A laparotomy was performed. The cystic duct was ligated and a polyethylene tube of an appropriate size (PE 190, Clay-Adams Co) was passed into the common duct and ligated securely in place. Physiological saline solution, pH 8.0 was infused intravenously throughout the experiment. Hepatic bile collected for one hour and then bile samples were obtained following intravenous administration of secretory stimulants such as secretin 10 u, pancreozymin 10 u, and 100 mg of cholates, e.g., sodium taurocholate, sodium cholate or sodium desoxycholate. Samples of bile were taken during 2 consecutive 10 minute periods following the administration of the secretory stimulants.

Bile acid and bilirubin content were determined according to the methods of Irvin et al (1944) and Magee et al (1952), respectively. Total bile acid and bilirubin outputs in bile have been expressed in terms of mg per 10 kg of body weight per 10 min. Serum amylase and lipase were determined by the methods described previously (Hong et al, 1962). The methods for serum transaminases or alkaline phosphatase followed the technique described in the Sigma Bulletin. Glycogen content was determined by the Anthrone adapted method. The liver strips were dried in the oven at 80° C to constant weight. Ash was obtained following 24 hours incubation in a furnace at the temperature of 800 -1,000° C.

A part of the liver was fixed in 10 % neutral formalin and a part of the liver in absolute alcohol.

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Both tissues were embedded in paraffin after sufficient fixation, and were cut in 6 μ thickness for microscopic sections. All sections from the tissue fixed in formalin were stained with hematoxylin and eosin while sections from the tissue fixed in absolute alcohol were stained by a PAS reaction method with or without diastase digestion. In addition, oil red-O staining for fat was made on the frozen section from the tissue fixed in formalin.

RESULTS

Hepatic bile flow: Although the total bile acid and bilirubin in the hepatic bile following the installation of total biliary fistulas in dogs decreased with time the bile flow during the first 2 to 3 hours after preparation was relatively consistent. The administration of corticosteroids to dogs did not provoke any observable responses except insignificant minor changes of body weight. However, significant changes of hepatic bile flow and bile acid content in response to secretory stimulants were noticed in glucocorticoid-treated animals (Table 1). In the control animal the bile flow was increased following secretin or pancreozymin stimulation and both the bile flow and bile acid content were significantly increased by the stimulation with the choleretics, taurocholate and cholate.

Following administration of glucocorticoids for 10 days the bile flow in response to secretin or pancreozymin was increased more than double in comparison with that of control values, but the bile acid content in bile was essentially unchanged(Table 1 & 3). The response of bile flow induced by the choleretics, particularly by sodium cholate, was enhanced in glucocorticoid-treated animals. Unlike the control, in these animals bile acid content in bile was not raised by the taurocholate administration.

In the DOCA treated group the bile secretion after various secretory stimulation was approximately similar to that seen in cortisone-treated animals. The response of bile secretion in testosterone-treated animals showed little difference from control and the flow was rather erratic.

Hepatic weight: The weight of the liver as a
fraction of body weight in the animals receiving glucocorticoids daily for 10 days was significantly increased to 46.4 g/kg body weight from the control value of 37.8 g/kg body weight (Table 2 & 3). The hepatic weight was also increased in the adrenalectomized animals receiving supplementary cortisone for 2 weeks. The testosterone-treated animals or DOCA treated animals showed a slight reduction of liver weight.

The weight of the pancreas was relatively constant in all animals and the weight of the kidneys was decreased slightly in case of prednisolone, dexamethasone, DOCA treated animals and adrenalectomized animals with supplementary cortisone.

**Histologic changes:** The most conspicuous histologic alterations were clear vesicular and vacuolar ballooning of hepatic cells at mid and periporal zones of the liver. The cells with lesser degree of ballooning showed vesicular appearance of cytoplasm with fine reticular pattern while the cells showing a marked degree of ballooning consisted of cystic or vacuolated appearance of cytoplasm (Fig. 1 to 9). These cells reacted negatively to oil red-O but reacted positively to PAS reaction. Vesicular cells contained granular PAS positive material while the vacuolated cells contained flaky form of PAS positive substance. Most of the PAS positive substance disappeared after digestion with diastase. The ballooned cells at the central part of the liver frequently remained unstained by the PAS while most of the ballooned cells at the subcapsular area stained strongly (Fig. 6 & 9). Some of the cells at the central part of the liver stained stronger when sections were cut thicker.

Vacular ballooning of hepatic cells were seen in the animals treated with cortisone, prednisolone and dexamethasone, namely glucocorticoid only, whereas no such changes were observed in the animals treated with testosterone, DOCA, or adrenalectomized.
animals and normal control animals. Among the glucocorticoids, dexamethasone produced the most marked ballooning change. Serial biopsies from the liver following prednisolone administration showed beginning of cell ballooning from 2 to 3 days after the initial administration of prednisolone and the changes disappeared around 5 to 6 days after the discontinuation of the drug injections.

The degree and time sequence of hepatocellular ballooning correlated closely with the increase of glycogen content of the liver measured by chemical methods (Table 4). Serum transaminases and alkaline phosphatase were elevated significantly during serial biopsies, which may due to the hepatic tissue damage rather than prednisolone administration.

### Table 3. Changes of Liver Weight and Bile Secretion in Control and Glucocorticoid Treated Dogs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Group</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Glucocorticoid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>10</td>
<td></td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver wt, g/kg body wt</td>
<td>37.8</td>
<td></td>
<td>46.4*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bile Flow, cc/10kg/10min</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Secretin 10u</td>
<td>0.88</td>
<td></td>
<td>1.73*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sod. Cholate 100gm</td>
<td>1.93</td>
<td></td>
<td>2.53*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Bile Acid, mg/10kg/10min</td>
<td>56.0</td>
<td></td>
<td>39.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretin 10u</td>
<td>100.0</td>
<td></td>
<td>45.6*</td>
<td></td>
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*indicates the sum of animals treated with cortisone, prednisolone and dexamethasone.

*P < 0.05

### Table 4. Changes of Serum Enzyme Value and Liver Glycogen Content During Serial Biopsies in Prednisolone-treated Dogs

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Prednisolone acetate, 2 mg/kg I.M. Daily</th>
<th>Withdrawal</th>
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<tr>
<td></td>
<td>Days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1-2</td>
</tr>
<tr>
<td>Serum Glucose mg/100cc</td>
<td>41.2</td>
<td>63.3</td>
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<tr>
<td>Serum GOT, u.</td>
<td>18.0</td>
<td>66.3</td>
</tr>
<tr>
<td>Serum GPT, u.</td>
<td>11.0</td>
<td>62.3</td>
</tr>
<tr>
<td>Serum A.P. u.</td>
<td>2.24</td>
<td>4.77</td>
</tr>
<tr>
<td>Liver Glycogen, mg/100g tissue</td>
<td>500</td>
<td>—</td>
</tr>
<tr>
<td>Liver Tissue dry wt, mg/g, wet wt</td>
<td>258</td>
<td>260</td>
</tr>
<tr>
<td>Ash wt, mg/g dry wt</td>
<td>58.1</td>
<td>42.3</td>
</tr>
</tbody>
</table>

GOT: Glutamic-oxalacetic transaminase
GPT: Glutamic-pyruvic transaminase
A.P.: Alkaline phosphatase

**DISCUSSION**

In response to secretory stimulants the bile volume recovered from the cortisone, prednisolone or dexamethasone treated dogs was significantly greater than that recovered from the untreated control group. However, bile salt secretion was not increased. These indicate that long term administration of glucocorticoids results in a hydrocholeretic influence on bile secretion in response to secretory stimulants. In the relation of glandular secretion to adrenocortical hormone Villereal et al (1955) have shown that daily administration of ACTH or cortisone increases basal and stimulated acid gastric secretion after a week or so. However, Ritchie et al (1965) have observed that the parietal cell count increases in canine stomach following long term administration of cortisone. Consequently our results may seem to explain that a larger amount of gastric acid passed from the stomach into the duodenum of the dogs treated with glucocorticoid resulting in augmented secretin release which led to the increase in bile
secretion. However, this explanation is unlikely particularly in the case of secretin or pancreozymin stimulated secretion since the secretin or pancreozymin was found to inhibit gastric secretion according to Greetee et al (1957) and others (Jordan and Peterson, 1962; Jordan and De la Rosa, 1964; Wormsley and Grossman, 1964).

Our finding that long term administration of glucocorticoids resulted in an increase in liver weight was of note. Delaney and Grim (1966) have observed a somewhat analogous hyperplasia of the pancreas in the dogs following cortisone administration. This fact, together with the finding that daily administration of cortisone caused an increase of parietal mass according to Ritchie et al (1965), may serve to explain that the augmented cholerisis in glucocorticoid treated animals is dependent on the enlargement of the secretory gland. In addition, the amino acids mobilized from peripheral tissues by the catabolic effect or the antianabolic effect of corticosteroids funnel into the liver and the organ might actually be increased in mass (Christensen, 1961) and the stimulated bile secretion would be augmented. The cholepoeitic effect of dietary amino acids has been reported by Coburn and Annegers (1950) and Magee et al (1952, 1954).

In the other hand Swingle et al (1959) have claimed that glucocorticoid maintains the properties necessary to induce shifts of body fluid and certain electrolytes between intra and extracellular compartments, particularly in kidneys. The liver may be similarly susceptible to corticosteroid induced shift in body fluid, and this corticosteroid effect, which is favoured by Gans and McEntee (1961) who observed augmented bile secretion during the administration of prednisolone, may be reflected in an increase of bile secretion.

The weight of the pancreas as a fraction of body weight was 2.4 g/kg body weight in control and 2.5 g/kg body weight in corticosteroid treated animals in our experiment. Delaney and Grim (1966) found that only in the case of animals receiving cortisone daily for as long a period as a month the pancreatic weight showed 2.5 g/kg body weight and in the control or animals receiving nonsteroids it showed 1.9 g/kg body weight which is a significant difference from the value of cortisone treated animals. Why the control value of pancreatic weight in our animals was already elevated as the corticosteroid group was not known. It may be due to the dietary and environmental adjustment of adrenocortical function in our dogs, however, it is beyond our speculation.

A peculiar change characterized by vesicular and vacuolar ballooning of hepatic cells at mid- and periportal zones of the liver were seen all animals treated with glucocorticoids, most marked with dexamethasone. The materials accumulated in ballooned cells were diastase sensitive PAS positive substances, namely, glycogen. Gans and McEntee (1961) also found similar changes of liver cells in prednisolone treated dogs. However, they could not prove the nature of the material contained in the ballooned cells and presumed that it is due to the accumulation of intracellular water leading to the hydropic swelling. Considering the finding that some of ballooned cells at the inner part of the liver remained unstained by the PAS reaction while cells at the subcapsular area or periphery of the tissue block stained positive, the failure of positive results from PAS reaction in some of our materials and findings of Gans and McEntee are probably due to the poor fixation of glycogen when it accumulated in massive amount. The evidence that appearance and the degree of ballooning of hepatic cells in serial biopsy specimens of the liver following prednisolone administration paralleled closely with the increase of glycogen content of the liver determined by chemical methods, and the fact that these changes were observed only in animals treated with glucocorticoids especially in an order of their glycogenetic potency, and not in the animals treated with non-glucocorticoids or normal controls, strongly support the glycogen nature of the materials which accumulated in ballooned cells.

In DOCA treated animals the stimulated bile secretion was enhanced as in cortisone group. However, the animals had neither hepatic weight gain nor glycogen stores in the cells. These results indicate that the hepatic effect of DOCA, a mineralocorticoid, is not at all similar to glucocorticoids.
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ACKNOWLEDGEMENTS

The authors are grateful to Professor Woo Choo Lee for the critical advice in this study and also to Professor Roberta G. Rice for the kind help in preparing the manuscript.
Fig. 1. Liver from normal control dog. H & E staining, x100.

Fig. 2. Liver from the dog treated with prednisolone, 2.0 mg/kg daily for 10 days. Zonal distribution of clear vacuolar ballooning of hepatic cells at mid- and periportal zone is conspicuously seen. H & E staining, x 100.

Fig. 3. Liver from the dog treated with prednisolone. Higher magnification of ballooned cells showing clear vesicular, granular and vacuolated cytoplasm. H & E staining, x 430.
Fig. 4. Liver from the dog treated with dexamethasone, 0.4 mg/kg daily for 10 days. Ballooning changes are more pronounced than those seen in the dog treated with prednisolone. H. & E. staining, x 100.

Fig. 5. Liver from the dog treated with cortisone 8.0 mg/kg daily for 10 days. Note much milder ballooning change of hepatic cells in comparison with prednisolone or dexamethasone treated dog. H. & E. staining, x 100.

Fig. 6. Liver from the dog treated with dexamethasone. Cells at the periphery of ballooned liver cell mass are stained positively while cells at inner part remained unstained. PAS reaction, x 100.
Fig. 7. Liver from the dog treated with dexamethasone. All of ballooned liver cells stained heavily in flake form. PAS reaction, x 100.

Fig. 8. Liver from the dog treated with dexamethasone. Higher magnification of ballooned cells which contain large flake form of PAS positive substance in the cytoplasm. PAS reaction, x 430.

Fig. 9. Liver from the dog treated with dexamethasone. Note intense PAS positive reaction at subcapsular area whereas ballooned cells at inner part remained unstained. PAS reaction, x 430.