Prednisolone and Glucose-6-phosphatase Activity in Liver Cells

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Prednisolone, a cortisol analogue, was given intraperitoneally to rats with 5, 10 or 15 doses of 5 mg. per kg. of body weight per day. Sacrificing the animals 24 hours after the last injection, unfixed frozen sections from small pieces of liver tissue were incubated and stained by a modification from the method of Wachstein and Meisel (1965) for glucose-6-phosphatase (G-6-Pase) activity. Some of the tissue blocks were processed for staining with hematoxylin and eosin for histopathological observation. Glucose-6-phosphatase activity, being represented histochemically by brownish-black deposits, was progressively increased after administrations 5 or 10 times. With 15 doses of prednisolone the general histochemical picture of G-6-Pase activity appeared to be similar to that of the control group, except for a different distribution of hepatocytes possessing strong activity. In prednisolone treated rats, the swollen hepatocytes showed a marked, cytoplasmic vacuolization and nuclear pyknosis, particularly in the periportal and midzonal areas of hepatic lobules. Some discussion of the G-6-Pase corticosteroids are presented in terms of their metabolic effects.

It is well known that corticosteroids and their analogues have been widely used clinically in order to modify the course of various diseases, because of their effects in suppressing the immune response and their anti-inflammatory effects (Colbert et al., 1953; Gray et al., 1971; Balow and Rosenthal, 1973) and especially their favorable influence on the common chronic diseases. However, the variability in response to cortisone treatment in liver disease (Streeten, 1959) and in necrosis of skeletal muscle (Ellis, 1956; Tice & Engel, 1968) presents a thorny therapeutic problem. Clinical observations and animal experiments suggest that the response may vary according to acuteness of the disease (Aterman, 1954; Bottiglioni et al., 1956; Goldegraber and Kirsner, 1959).

It has been shown that high doses of cortisone resulted in permanent diabetes in the rat, characterized by hyperglycemia and glycosuria (Ingle, 1941). Insulin-resistant "cortisone diabetes" showing very high blood sugar levels and glycosuria has also been attributed to increased glucogenesis. There is also evidence

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that certain phases of carbohydrate utilization (Colowick et al., 1974) and glycogen breakdown (Kerppola, 1952) are inhibited. On the other hand, it was demonstrated that cortisone administration caused a striking increase in the rate of glucose production (Welt et al., 1952).

Glucose-6-phosphatase (G-6-Pase), a specific enzyme occurring in greatest amount in the liver, participates in the conversion of glycogen to glucose. The site of G-6-Pase activity can be demonstrated histochemically and is indicated by a brown-black lead sulfide deposit, and liver cells give cytoplasmic staining with accentuation at the periphery of the lobule. The enzyme is absent from the liver in cases of glycogen storage disease (Cori, 1952-53) but present otherwise in all mammalian organs (liver, kidney, small intestine, rectum) capable of releasing glucose into the blood stream. According to Shull et al. (1956), the level of G-6-Pase in the livers of hereditary obese hyperglycemic mice is higher than in control animals. After cortisone administration, a marked increase in the enzyme level in homogenates was noted by Weber et al. (1956).

It is of interest to study the histochemical changes of G-6-Pase activity in the liver of animals treated with large doses of prednisolone acetate, a synthetic analogue of cortisol (hydrocortisone) in order to elucidate the mechanism of action of prednisolone on the enzyme in terms of glucose production and glucose-6-phosphate ester.

MATERIALS AND METHODS

Young adult males, with an initial weight of 200 to 220 gm., Sprague-Dawley strain, bred from the same colony in this laboratory, were used throughout this investigation and had free access to the laboratory diet and water to the experiment. They were divided into two groups; a normal control group and an experimental group. Control rats received no pre-treatment or physiologic saline intraperitoneally in volumes and frequencies identical with those of the prednisolone acetate-treated experimental group. To each rat of the experimental group was given intraperitoneally 5, 10 or 15 doses of prednisolone acetate (Preson; Chong Kun Dang, Corporation, Seoul, Korea) in saline suspension, in a dose of 5 mg. per kg. of body weight per day.

Animals were sacrificed 24 hours after the last injection of prednisolone, and unfixed frozen sections by a freezing microtome were used for histochemical study of glucose-6-phosphatase activity. Each section was washed for 2-3 minutes in a 0.4 M sucrose solution and incubated for 15-20 minutes at room temperature in a modification (Tice and Barnett, 1962; Pearse, 1968) of Wachstein and Meisel medium (1956), which contained 0.125% potassium glucose-6-phosphatase solution, 3 ml.; 0.2 M Tris-maleate buffer, pH 6.7, 20 ml.; 0.4 M sucrose solution, 5 ml.; 2% lead nitrate solution, 3 ml. and distilled water to make 50 ml. After washing in distilled water, the sections were developed in dilute ammonium sulfide solution for 5 minutes and then were treated by rinsing, dehydration and mounting in glycerin jelly. Some of the sections were incubated in a medium lacking the substrate, glucose-6-phosphate, and we found the staining negative in these sections, indicating that the granular deposits in the preparations, if any, might represent enzyme activity.

Some pieces of liver from the animals used were fixed in 10% formalin, embedded in, sectioned 6 µ thick, and stained with hematoxylin-eosin for observation of histopathological findings.
RESULTS

A. Glucose-6-phosphatase activity

1. Control group

Brownish-black, granular deposits of lead sulfide indicating the localization of enzymatic activity were found in parenchymal cells with cytoplasmic staining and without nuclear staining. Neither bile canaliculi, blood vessels, nor other cell types occurring in the sections were stained. The distribution of the final product was most marked in liver cells with strongly-staining reaction in the third, peripheral zone of the hepatic lobule (Fig. 1, 2 and 3). In the centrilobular and intermediate regions, glucose-6-phosphatase activity was much weaker than in the periportal zone, and the reaction granules were relatively dispersed in cytoplasm (Fig. 2).

2. Experimental group

After 5 injections of prednisolone the swollen hepatocytes, in which the reaction granules were closely packed to ectoplasm, were widely distributed in the whole lobule, especially in centrilobular and intermediate zones. But there were also some hepatocytes having weak enzyme-activity (Fig. 4 and 5).

In rats treated with 10 dosings, the hepatocytes with compact, strongly-stained granules were increased much more than in the 5-day group. However, loss of enzyme activity occurred within occasional liver cells (Fig. 6, 7 and 8).

After 15 prednisolone-administrations, the general histochemical picture of glucose-6-phosphatase activity became similar to that of the control group, but the distribution of the liver cells with strong activity of the enzyme was markedly extensive, from the periportal zone-centrilobular zone (Fig. 9).

B. Histological findings:

1. Control group

In all the the control animals, the livers revealed normal liver histology: pale, large, round nuclei and basophilic cytoplasm in hepatic cell plates. (Fig. 10 and 11).

2. Experimental group

In animals with 5 and 10 injections of prednisolone, the swollen hepatocytes in most of the lobule showed the presence of apparently empty spaces in their cell bodies representing extensive cytoplasm is vacuolation, and their nuclei were pyknotic. These changes were more pronounced and extensive in the 15-day group than in the 10-day group (Fig. 12, 13, 14 and 15).

After 15 administrations, the above changes of hepatocytes were demonstrated in the periportal and middle zones of the hepatic lobule. Contrarily, near and in the centrilobular zone the hepatocytes showed increased basophilia, but they were decreased in vesicular appearance and in nuclear pyknosis (Fig. 16 and 17).

DISCUSSION

The histopathological picture in this investigation agrees well with other reports demonstrating the presence of apparent empty spaces, and extensive cytoplasmic vacuolization in the swollen hepatocytes of cortisone or prednisolone treated animals (Weber et al., 1956; Kang, 1971 and Ro, 1974). The changes were progressively increased in extent with increasing doses up to 10 injections, but no further increase, even was lesser degree, was observed thereafter. These findings are also similar to the observations of Kang (1971). However, the exact nature of these clear spaces in cytoplasm is still obscure and awaits further studies, although their contents can be speculated to be
glucagon and/or lipids (Hill, 1961; Hill and Droke, 1963; Wiener et al., 1968; Mahley et al., 1969; Kang, 1971 and Ro, 1974) or as diluted protein solutions (Gans and Mencentee, 1961 and Thomson et al., 1971).

Glucose–6–phosphatase is present in all mammalian organs releasing glucose into the blood stream, such as the liver, kidney and small intestine. In normal liver tissue brownish-black granular deposits, which represent the histochemical localization of enzyme activity by the method of Wachstein and Meisel (1956), are found in hepatocytes without nuclear staining. It cannot be demonstrated in blood vessels and bile canaliculi. This histochemical feature was observable in sections of the authors' control group.

However, Tice and Barnett (1962) established that deposits of final reaction-product were, at an ultrastructural level, regularly found within the nuclear envelope of hepatic cells, suggesting the non-artifactual nature of these deposits. In this connection, it should be cited that the nuclear envelope clearly relates this structure to the enzymatically active endoplasmic reticulum and that evidence has been provided of morphological continuity of the nuclear membrane with the ER in many cell types (Waston, 1955). Furthermore, G–6–Pase was found predominantly, if not exclusively, in the microsomal fraction of homogenates, and the enzyme activity was found in both smooth-and rough-surfaced elements of the endoplasmic reticulum of hepatic cells (Tice and Barnett, 1962; Reynolds, 1963; Dallner et al., 1966 and Chauveau et al., 1967).

In addition, it is not uncommon to find profiles of the ER variety surrounding glycogen areas (Porter & Bruni, 1959). The ER in the liver, being an intracellular transport system of tubules, could be concerned with the transport of glucose from glycogen areas.

Weber et al. (1956), in their biochemical determinations of liver glucose–6–phosphatase activity after cortisone administration, reported a significant increase in the G–6–Pase activity in the liver homogenate. In addition to this study, Weber and others (1961) reported biochemical data that cortisone administration had increased liver G–6–Pase in both adrenalectomized and hypophysectomized rats.

These biochimical studies are supported by our histochemical findings, but their discussion on the increase of the enzyme activity in all three particulate fractions should be noted. They stated that the increases in the enzyme activities in the nuclear and mitochondrial fractions could be partly due to an increased contamination from the microsomal fraction, because G–6–Pase activity was found to be mainly concentrated in the microsomal fraction (59%) and about equally distributed in the nuclear and mitochondrial fractions (25% each) in the control animals. They also suggested that the mitochondrial activity-increase could be more marked than it seemed from the data, because the number of mitochondria was decreased to half as a result of cortisone administration (Allard et al., 1954).

It has been shown that high doses of cortisone resulted in permanent diabetes in rats, characterized by hyperglycemia and glycosuria (Ingle, 1941). Insulin-resistant "cortisone diabetes" showing very high blood sugar levels and glycosuria has also been reported in humans (Celler et al., 1951). The mechanism of the action of cortisone in increasing the blood sugar level has been attributed to increased gluconeogenesis. There is also evidence that certain phases of carbohydrate utilization (Colowick et al., 1947) and glycogen breakdown (Kerppola, 1952) are inhibited. On the
other hand, it was demonstrated that cortisone administration caused a striking increase in the rate of glucose production (Welt et al., 1962). Glucose-6-phosphatase is a specific enzyme capable of preferentially dephosphorylating glucose-6-phosphate, catalyzing the transfer of phosphoryl groups from glucose-6-phosphate to glucose or fructose and participating in the conversion of glycogen to glucose and in gluconeogenesis (Barka and Anderson 1963 and Pearse 1968). Since glucose production from the liver is related to the rate of hydrolysis of the glucose-6-phosphate ester, the highly increased G-6-Pase activity after cortisone administration may be responsible, partly at least, for the high blood glucose levels in cortisone treated animals (Weber et al., 1956). This finding may explain the report of Welt et al. (1962) on the great increase in the rate of glucose production after cortisone treatment. The finding of high liver G-6-Pase levels in hyperglycemic animals also agrees well with the report of Ashmore et al. (1954) on increased liver G-6-Pase activity in alloxan diabetic rats.

Although a broad outline of the action of the corticosteroids is emerging and there are a number of instances in which the synthesis of specific proteins is known to be induced by the hormones, the links between the initial actions of the hormones and the final metabolic effects have not been elucidated for the most part, and the specific mechanism by which the glucocorticosteroids act on the synthesis of specific enzymes is still unknown. The corticosteroids react with receptor proteins in the cytoplasm of sensitive cells to form a steroid-receptor complex. Such receptors have been identified in many mammalian tissues (Ballard et al., 1974). The steroid-receptor complex moves into the nucleus, where it binds to chromatin. Information carried by the receptor protein directs the genetic apparatus to transcribe RNA of all types. Presumably the most important of these RNAs is the giant heterogenous RNA, which contains nucleotide sequences that act as templates (mRNA) after a complex processing of the polymer has taken place (Scherrer, 1973). Steroid hormones appear to stimulate transcription and ultimately the synthesis of specific proteins, such as G-6-Pase (which is one of the key enzymes in the regulation of gluconeogenesis). The enzyme is increased, possibly by the derepression of a functional genetic unit in the nucleus which controls its synthesis. This seems to be a comparatively specialized action of the steroids since many other hepatic enzymes are not increased (Harper, 1973; Haynes & Larner, 1975). Weber et al. (1963) described an interesting fact that the cortisone-induced de novo synthesis of liver glucose-6-phosphatase was prevented by injections of actinomycin D in the rat.

This histochemical demonstration of the effect of cortisone on liver G-6-Pase activity brings up the possibility that the adrenal cortex may play a role in the physiological maintenance and regulation of this enzyme and in turn of homeostatic mechanisms through the regulation of glucose production.

REFERENCES

- Aterman K: Studies in fibrosis of liver induced by carbon tetrachloride. AMA Arch Pathol 57:12, 1954
- Ballow JE, Rosenthal AS: Glucocorticoid suppression of macrophage migration inhibitory
Cori CT, Harvey Lectures, p 145, 1952-1953
Cori CT, Cori CF: Glucose-6-phosphatase of the liver in glycogen storage disease. J Biol Chem 199:661, 1952
Ellis JT: Necrosis and regeneration of skeletal muscles in cortisone-treated rabbits. Amer J Path 32:993, 1956
Geller W, LaDue JS, Glass GBJ: Insulin-resistant diabetes precipitated by cortisone and reversed by nitrogen mustard. AMA Arch of Internal Med 87:124, 1951
Goldgraber MB, Kirsner JB: Corticotropin( ACTH) and adrenal steroids in liver disease. Arch Intern Med 104:469, 1959
Kerppola W: Inhibition of phosphorylase with cortisone and its activation with adrenaline in the rabbit. Endocrinology, 51:192, 1952
Ro JY: An effect of Vitamin A and cortisone acetate administration on thioacetamide induced hepatic necrosis in rats. Yonsei J Med Sci 7:47, 1974
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Streetsen DHP: The hepatic metabolism of adrenocortical steroids and some clinical implications thereof. Gastroenterology 36: 643, 1959


Tice LW, Barrnett RJ: The fine structural localization of glucose-6-phosphatase in rat liver. J Histochem Cytochem 10:754, 1962

Tice LW, Engel AG: The effects of glucocorticoids on red and white muscles in the rat.

Amer J Path, 50:311, 1967


Fig. 1. G-6-Pase activity in the control rat, Wachstein & Meisel method. ×40.

Fig. 2. The enzyme activity, the control group. Note that the enzyme activity is much weaker in centrilobular and middle zones than in periportal. ×100.

Fig. 3. The enzyme activity in the periportal area of the control group. ×450.

Fig. 4. The enzyme activity, centrilobular and intermediate zones, after 5 injections of prednisolone. The swollen hepatocytes, in which deposits were closely packed to ectoplasm, were widely distribute. ×100.

Fig. 5. The enzyme activity, periportal region, after 5 injections. ×450.

Fig. 6. Liver with 10 times of administration. ×100.
Fig. 7. G-6-Pase activity, 10 injections of prednisolone, periportal area. ×450.

Fig. 8. Same as Fig. 7, in centrilobular region. ×450.

Fig. 9. The enzyme activity after 15 injections. ×100.

Fig. 10. Liver of the control group, H-E and vital staining with India ink. ×100

Fig. 11 Control liver, periportal area, H-E stain. ×450.

Fig. 12. After 5 injections of prednisolone, H-E stain. ×100.
Fig. 13. Periportal region, 5 injections of prednisolone. H-E stain. ×450.

Fig. 14. Centrilobular region, 5 injections. H-E stain. ×450.

Fig. 15. After 10 injections of prednisolone. H-E stain. ×100

Fig. 16. After 15 injections of prednisolone. H-E stain. ×100.

Fig. 17. Centrilobular region after 15 injections. H-E stain. ×450.