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ROLE OF ENZYMES IN HOMEOSTASIS—VI.

EFFECT OF TRIAMCINOLONE AND OTHER STEROIDS ON ENZYMES INVOLVED IN GLUCONEOGENESIS*

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Abstract—In view of the reports that adrenocorticosteroids induced *de novo* synthesis of enzymes involved in gluconeogenesis, systematic studies were carried out to compare the potency of the various steroids and to establish the smallest dose necessary and shortest time required to detect enzyme synthesis for the key hepatic gluconeogenic enzymes, glucose 6-phosphatase and fructose 1,6-diphosphatase.

The potency of triamcinolone, cortisone, hydrocortisone, and methylprednisolone (Medrol) as inducers of the gluconeogenic enzymes was compared in a 3-day injection schedule in rats and it was found that triamcinolone was the most effective inducer. Dose-response studies showed that daily injections of 10 mg of cortisone i.m. for 5 days or 1 mg of triamcinolone i.p. for 3 days achieved the highest increases in hepatic enzyme activities and nitrogen and glycogen content.

In an effort to achieve a more rapid induction in enzyme synthesis cortisone and triamcinolone (25 mg/100 g) were injected i.p. Both steroids brought about a rapid rise in fructose 1,6-diphosphatase activity (128–145%) in 4–6 hr. Only triamcinolone, however, was capable of causing a significant increase in glucose 6-phosphatase activity during the same period.

Further dose-response studies with triamcinolone revealed that a single i.p. dose of 0·25 mg/100 g was sufficient to induce statistically significant increases in hepatic glucose 6-phosphatase (140%) and in fructose 1,6-diphosphatase (159%) activity in 24 hr. A dose of 0·5 mg was capable of causing a statistically significant rise (117–121%) in hepatic gluconeogenic enzyme activity in 6 hr.

Since rapid increases in liver glucose 6-phosphatase and fructose 1,6-diphosphatase activity can be induced by triamcinolone administration, it appears that the mechanism of effect of the gluconeogenic corticosteroid hormones at the molecular level entails an early stepping up of the rate of synthesis of these key gluconeogenic enzymes.

SINCE the discovery that cortisone injection increases hepatic glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase; 3.1.3.9†) activity in the rat,[1, 2] studies in various laboratories have confirmed and extended these findings.[3, 4] Recently it was shown that cortisone administration specifically and preferentially induced liver gluconeogenic enzymes without affecting other enzymes involved in carbohydrate

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† See *Report of the Commission on Enzymes*. Pergamon Press, Oxford (1961).

metabolism.[5, 6] That the steroid-induced increases recorded for hepatic G-6-Pase,* FDPase (D-fructose-1,6-diphosphate 1-phosphohydrolase; 3.1.3.11), lactic dehydrogenase, phosphohexose isomerase, malic dehydrogenase, and aldolase were due to new enzyme synthesis was revealed in current investigations by abolition of the increases by treatment with actinomycin,[7-9] puromycin,[8, 10] or ethionine.[6, 11-13] In conducting further research into the mechanism of hormone action on enzyme synthesis it has become necessary to analyze carefully the dose and regimen used to demonstrate enzyme induction. The present work describes the effect of various types of steroid hormones and presents the sequence of events during cortisone- and triamcinolone-induced enzyme increases. Triamcinolone is the most potent inducer of the gluconeogenic enzymes in this series, and hepatic enzyme activity increases as early as 4–6 hr after its administration.

MATERIALS AND METHODS

Animals and experimental conditions

Young male Wistar rats with an initial weight of 90–110 g were used in these studies. Rats kept in separate cages were maintained on Purina laboratory chow and water *ad libitum*. The following experimental arrangements were used.

Dose-response studies with cortisone. Rats were given i.m. cortisone injections daily for 5 days and killed on the sixth day. Cortisone was given in doses of 2·5, 5, 10, 20, and 25 mg per 100 g rat.

Comparison of the effect of various steroids. Groups of rats were given i.p. injections of cortisone, hydrocortisone, Medrol, or triamcinolone for 3 days and killed on the fourth day. The steroids were given in doses of 2·5 mg per 100 g rat.

Dose-response studies with triamcinolone. Rats were injected i.p. with triamcinolone and killed on the fourth day. Triamcinolone was given to different groups of rats in doses of 0·25, 0·50, 1·0, 5·0, and 10·0 mg per 100 g rat.

Sequence of events after a single injection of cortisone or triamcinolone. Groups of rats were killed at 2, 4, 6, 12, and 24 hr after a single i.p. injection of cortisone or triamcinolone. The steroids were given in a dose of 25 mg per 100 g rat.

Early (6 and 24 hr) dose-response studies with triamcinolone. Groups of rats were injected i.p. with triamcinolone and were sacrificed after 6 and 24 hr. Triamcinolone was administered to different groups of rats in doses of 0·25, 0·50, 1·0, 5·0, 10·0, and 25·0 mg per 100 g.

Tissue preparation and biochemical methods

The preparation of liver homogenates and supernatant fluids was described previously.[5, 8] The various biochemical methods,[14-17] cellularity determination,[18] expression and statistical treatment of results have also been referred to elsewhere.[8]

All experimental values of the steroid-injected animals were compared with normal, untreated control rats sacrificed in each series. The control rats were not injected with saline because we have found that physiological saline given in volumes corresponding to those used in these experiments has no effect on the biochemical parameters studied.[7]

* The following abbreviations are used: G-6-Pase = glucose 6-phosphatase, FDPase = fructose 1,6-diphosphatase.

Chemicals

The sodium salts of glucose-6-phosphate and fructose-1,6-diphosphate were purchased from Sigma Chemical Co. Cortisone acetate, hydrocortisone 21-phosphate, Medrol (methylprednisolone sodium succinate) (Merck Sharp and Dohme) and triamcinolone (9-α-fluoro-11-β,16-α,17-α,21-tetrahydroxy-1,4-pregnandiene-3,20-dione,16-α-21-diacetate) (Lederle) were purchased as commercial preparations.

RESULTS AND DISCUSSION

Effect of cortisone on liver/body ratio, cellularity, nitrogen level, and gluconeogenic enzyme activity during a 5-day injection schedule

Table 1 shows that the lowest dose used in this experiment, 2·5 mg, was sufficient to result in a statistically significant increase in liver/body ratio and homogenate and supernatant nitrogen levels. The cellularity was significantly decreased. With higher doses the trend of alteration in these parameters became more pronounced. The optimum rise for liver/body ratio and nitrogen content was found with the 10-mg dose level.

Table 1 shows that 2·5 mg cortisone resulted in a statistically significant increase of 145–156% in G-6-Pase and FDPase activity in the average cell. The largest rises for G-6-Pase (292%) and FDPase (283%) in the average cell were achieved by 10 mg cortisone. Similar values were obtained when results were expressed on a 100-g body-weight basis. The 5-day injection schedule used in experiments just described (Table 1) was originally adopted after Lowe and associates[19–21] and was used in previous studies to provide a comparison.[1, 2, 5–8] The present results show that the daily cortisone dose of 25 mg i.m. can be decreased to 10-fold less and still achieve enzyme induction; however, 10 mg was the optimum dose for this steroid. Since the nitrogen and enzyme increases were lower in the groups injected with 20–25 mg of cortisone than in those receiving 10 mg, it is thought that toxic effects of cortisone might be operating at these high dose levels. A similar interpretation was attached to similar findings on the effect of hydrocortisone on tyrosine ketoglutarate transaminase activity by Kenney and Flora.[22]

Effect of various corticoids on liver/body ratio, nitrogen level, and gluconeogenic enzyme activity

In an effort to select the most potent steroid as an inducer for the gluconeogenic enzymes, the smallest effective dose (2·5 mg) reported in Table 1 was chosen for these experiments. In an attempt to shorten the induction process and to achieve a rapid rise in enzyme activities the drugs were given i.p. for a 3-day period. Table 2 compares the effect of cortisone, hydrocortisone, Medrol, and triamcinolone. Table 2 shows that triamcinolone caused the most marked increase in liver/body ratio (162%) and in homogenate nitrogen (150%) and supernatant nitrogen (162%) in the average cell. The increment in nitrogen content induced by the other steroids ranged from 129 to 138% of the normal values.

Table 2 shows that cortisone, hydrocortisone, and Medrol caused approximately 2-fold increases in hepatic glycogen content. However, triamcinolone resulted in a 3- to 4-fold rise in the average cell. Triamcinolone increased G-6-Pase activity in the average cell to 208% and FDPase activity to 260%. The increments caused by the other steroids ranged from 155 to 185% in the average cell. Similar values were

TABLE 1. EFFECT OF VARIOUS DOSES OF CORTISONE AS INDUCERS OF LIVER GLUCONEOGENIC ENZYMES

The mean values and standard errors represent 4 or more animals in each group. The rats were injected i.m. with various doses of cortisone for 5 days and killed on the 6th day. Enzyme activities expressed per cell are calculated as micromoles $\times 10^7$ of substrate metabolized per hour at 37°. Data in parentheses express results in percentages, taking the values of untreated normal rats as 100%.

	Control (untreated)	Cortisone injected daily for 5 days (mg)				
		2·5	5·0	10·0	20·0	25·0
Liver/body × 100	4·5 ± 0·08 (100)	5·9 ± 0·2 (133)*	6·0 ± 0·1 (131)*	6·9 ± 0·2 (154)*	6·5 ± 0·1 (145)*	6·5 ± 0·1 (145)*
Cellularity, millions†	211 ± 4·4 (100)	168 ± 4·6 (79)*	150 ± 3·8 (71)*	111 ± 2·5 (53)*	130 ± 4·2 (62)*	126 ± 2·6 (60)*
Homogenate nitrogen per cell‡	1·4 ± 0·05 (100)	1·8 ± 0·03 (129)*	2·1 ± 0·04 (150)*	2·8 ± 0·07 (200)*	2·2 ± 0·09 (157)*	2·3 ± 0·04 (164)*
Supernatant nitrogen per cell‡	0·65 ± 0·02 (100)	0·86 ± 0·03 (133)*	1·0 ± 0·007 (154)*	1·4 ± 0·03 (215)*	1·1 ± 0·05 (169)*	1·1 ± 0·05 (169)*
Glucose-6-phosphatase per cell	41·7 ± 0·2 (100)	60·4 ± 2·4 (145)*	77·7 ± 2·7 (186)*	121·7 ± 1·6 (292)*	104·6 ± 2·5 (251)*	109·0 ± 1·8 (261)*
Fructose-1,6-diphosphatase per cell	19·5 ± 0·8 (100)	30·3 ± 0·6 (156)*	39·6 ± 0·2 (203)*	55·2 ± 1·3 (283)*	45·5 ± 2·4 (233)*	47·2 ± 1·4 (242)*

* Statistically significant as compared with values of untreated normal rats ($P = < 0·05$).
† Expressed in millions of counted nuclei per gram wet tissue.
‡ Calculated as milligrams of nitrogen $\times 10^7$.

TABLE 2. EFFECT OF VARIOUS CORTICOIDS AS INDUCERS OF LIVER GLUCONEOGENIC ENZYMES

The mean values and standard errors represent 4 or more animals in each group. The animals were injected i.p. with 2·5 mg of the steroid indicated/day for 3 days and killed on the 4th day. Enzyme activities expressed per cell are calculated as micromoles $\times 10^7$ of substrate metabolized per hour at 37°. Data in parentheses express results in percentages, taking the values of untreated normal rats as 100%.

	Control (untreated)	Steroids injected			
		Cortisone	Hydrocortisone	Medrol	Triamcinolone
Liver/body × 100	3·9 ± 0·05 (100)	4·8 ± 0·12 (120)*	4·7 ± 0·07 (118)*	4·7 ± 0·07 (118)*	6·3 ± 0·10 (162)*
Cellularity, millions†	212 ± 2·3 (100)	168 ± 2·0 (78*)	168 ± 1·6 (78*)	166 ± 5·1 (77)*	145 ± 1·5 (68)*
Homogenate nitrogen per cell‡	1·4 ± 0·00 (100)	1·8 ± 0·00 (129)*	1·8 ± 0·00 (129)*	1·9 ± 0·05 (135)*	2·1 ± 0·00 (150)*
Supernatant nitrogen per cell‡	0·68 ± 0·02 (100)	0·90 ± 0·01 (132)*	0·90 ± 0·02 (132)*	0·95 ± 0·02 (138)*	1·10 ± 0·00 (162)*
Glucose-6-phosphatase per cell	53·2 ± 1·10 (100)	86·0 ± 1·7 (162)*	85·8 ± 3·3 (160)*	82·4 ± 2·8 (155)*	110·0 ± 1·7 (208)*
Fructose-1,6-diphosphatase per cell	19·4 ± 0·85 (100)	33·9 ± 0·50 (175)*	35·7 ± 0·43 (184)*	36·0 ± 0·85 (185)*	50·4 ± 0·57 (260)*
Glycogen, mg per cell $\times 10^7$	2·0 ± 0·10 (100)	4·4 ± 0·05 (220)*	4·3 ± 0·26 (215)*	4·3 ± 0·07 (215)*	8·7 ± 0·28 (435)*

* Statistically significant difference as compared with values of untreated normal rats ($P = <0·05$).
† Expressed in millions of counted nuclei per gram of wet tissue.
‡ Calculated as milligrams of nitrogen $\times 10^7$.

obtained when results were expressed on a 100-g body-weight basis. The results indicate that triamcinolone is the most potent inducer of liver G-6-Pase and FDPase synthesis. Triamcinolone was also the most powerful in causing hepatomegaly, glycogen deposition, and a rise in nitrogen content. The superior effectiveness of triamcinolone in causing glycogen deposition confirms previous reports on the comparison of potencies of glucocorticoids.[23, 24]

Effect of various doses of triamcinolone on hepatic nitrogen and gluconeogenic enzyme activity

Having found triamcinolone the most potent inducer of liver gluconeogenic enzymes, our attention turned to establishing the lowest amount of triamcinolone necessary to achieve marked increases in enzyme activity. In this experiment different doses of triamcinolone (0·25 to 10 mg) were injected i.p. for 3 days, and the rats were killed on the fourth day. Thus the experimental arrangement is comparable to that in Table 2. The dose-response studies with triamcinolone are summarized in Table 3 which shows that 0·25 mg of triamcinolone significantly elevated liver/body ratio (128%), homogenate nitrogen (143%), and supernatant nitrogen (148%) in the average cell. A 4-fold increment in this dose was sufficient to cause maximal or near maximal increase in these parameters, and even doses 40-fold higher were little, if any, more effective.

In Table 3 it is interesting that 0·25 mg triamcinolone was capable of increasing G-6-Pase activity to 208% and FDPase activity to 248% in the average liver cell. The 1-mg dose of triamcinolone was the most effective in increasing G-6-Pase (289%) and FDPase (295%) in the average cell. Raising the triamcinolone doses to 5 or 10 mg failed to achieve any further elevation in the enzyme activities. Similar values were obtained when results were expressed on a 100-g body-weight basis. These results demonstrate that intraperitoneal doses of triamcinolone given for 3 days can achieve marked increases with as little as 0·25 mg triamcinolone; 1 mg is the minimal dose producing the plateau effect with respect to the induction of gluconeogenic enzymes examined.

Effect of cortisone and triamcinolone at 2, 4, 6, 12, *and* 24 *hr after injection*

In the investigation of primary effects of hormones it is of special importance to elucidate how early the biochemical response is detectable after the hormone is administered. It has been suggested that the earliest biochemical effects probably belong to the primary mechanism of hormone action and therefore are crucial to the understanding of the action of the hormone at the molecular level.[25] In the analysis of enzyme increases after adrenocorticoid hormone administration detailed studies have been carried out by Rosen and Harding and associates with special emphasis on the behavior of transaminases,[26–29] and advances in this field have been reviewed by Rosen and Nichol.[30, 31]

We previously conducted a time-sequence study, using daily i.m. injections of 25 mg cortisone for 6 days,[6] which showed that under these conditions hepatic FDPase and G-6-Pase rose slowly, since FDPase activity was significantly increased statistically at 24 hr, and G-6-Pase at 48 hr, after beginning cortisone treatment. However, recent preliminary investigations have indicated that by using the more potent triamcinolone through the i.p. route a rise occurs in a few hours.[9, 32] Because

TABLE 3. EFFECT OF VARIOUS DOSES OF TRIAMCINOLONE AS INDUCERS OF LIVER GLUCONEOGENIC ENZYMES

The mean values and standard errors represent 3 or more animals in each group. The rats were injected i.p. with various doses of triamcinolone for 3 days and killed on the 4th day. Enzyme activities expressed per cell are calculated as micromoles $\times 10^7$ of substrate metabolized per hour at 37°. Data in parentheses express results in percentages, taking the values of untreated normal rats as 100%.

	Control (untreated)	Triamcinolone injected daily for 3 days (mg)				
		0·25	0·50	1·0	5·0	10·0
Liver/body × 100	4·0 ± 0·04 (100)	5·1 ± 0·06 (128)*	5·6 ± 0·06 (140)*	6·0 ± 0·04 (150)*	6·0 ± 0·10 (150)*	6·6 ± 0·13 (165)*
Cellularity, millions†	216 ± 2·4 (100)	154 ± 1·4 (71)*	146 ± 2·8 (68)*	130 ± 1·4 (60)*	130 ± 1·4 (60)*	127 ± 3·6 (59)*
Homogenate nitrogen per cell‡	1·4 ± 0·004 (100)	2·0 ± 0·00 (143)*	2·0 ± 0·004 (143)*	2·1 ± 0·04 (150)*	2·2 ± 0·06 (157)*	2·2 ± 0·004 (157)*
Supernatant nitrogen per cell‡	0·68 ± 0·01 (100)	1·00 ± 0·00 (148)*	0·98 ± 0·01 (145)*	1·1 ± 0·00 (162)*	1·1 ± 0·00 (162)*	1·1 ± 0·00 (162)*
Glucose-6-phosphatase per cell	49·0 ± 0·80 (100)	101·8 ± 3·4 (208)*	123·1 ± 3·8 (250)*	141·7 ± 2·2 (289)*	138·6 ± 3·0 (284)*	133·5 ± 5·2 (274)*
Fructose-1,6-diphosphatase per cell	21·0 ± 0·6 (100)	51·4 ± 1·2 (245)*	51·0 ± 3·3 (243)*	61·8 ± 0·6 (295)*	60·9 ± 1·2 (290)*	60·0 ± 2·5 (286)*

* Statistically significant difference as compared with values of untreated normal rats ($P = < 0·05$).
† Expressed in millions of counted nuclei per gram of wet tissue.
‡ Calculated as milligrams of nitrogen $\times 10^7$.

of the importance of demonstration of early effects of corticoid hormones on gluconeogenic enzymes, a detailed investigation was undertaken which is now reported.

Tables 4 and 5 compare the sequence of events after a single i.p. injection of cortisone or triamcinolone during a period of 24 hr. Table 4 shows that with cortisone the liver/body weight ratio became significantly increased at 12 hr. However, homogenate and supernatant nitrogen content was already significantly elevated at 4 hr. G-6-Pase activity did not increase, but FDPase activity was significantly increased at 2 hr to 115% and continued to rise to 128, 145, 159, and 178% at 4, 6, 12, and 24 hr.

Table 5 demonstrates that when a single injection of triamcinolone was given the alterations in the biochemical parameters occurred earlier or were more pronounced than with cortisone. For instance, the liver/body weight ratio was already significantly increased at 4 hr to 120% and to 170% at 24 hr. Homogenate and supernatant nitrogen content was also significantly increased to 115% at 4 hr and rose to 150% at 24 hr. Whereas cortisone had no effect on G-6-Pase, triamcinolone significantly increased it to 123% at 4 hr, and the activity was further elevated to 126, 132, and 180% at 6, 12, and 24 hr after injection. FDPase activity was significantly increased to 146% at 4 hr and continued to rise to 148, 164, and 203% at 6, 12, and 24 hr after treatment.

Effect of different concentrations of triamcinolone at 6 and 24 hr after injection

Since a single triamcinolone injection of 25 mg resulted in marked increases in hepatic G-6-Pase, FDPase, and nitrogen content in 4 hr, it became of interest to establish the smallest dose of this hormone necessary to achieve the early biochemical effects. For this dose-response study triamcinolone was injected i.p. in concentrations of 0·25, 0·50, 1·0, 5·0, 10·0, and 25·0 mg per 100 g rat, and groups of animals were killed after 6 and 24 hr. Since the early effects of triamcinolone may be of particular relevance in the elucidation of the role of this hormone in inducing synthesis of gluconeogenic enzymes, the results are given in detail in Tables 6–8.

Table 6 tabulates the effect of different doses of triamcinolone in rats killed 6 hr after hormone injection. A hormone dose of 0·5 mg was sufficient to cause a statistically significant rise in liver/body ratio and a drop in liver cellularity. A further increase in the triamcinolone dose resulted in an additional increase in liver/body ratio and a decrease in cellularity. The nitrogen content was significantly increased by 0·25 mg triamcinolone, and higher hormone doses further increased the nitrogen content which finally leveled off after the 5-mg steroid dose.

Table 7 shows the dose-response studies of triamcinolone in animals killed 24 hr after injection. The lowest dose, 0·25 mg, caused statistically significant increases to 124–131% in homogenate and supernatant nitrogen content. The liver/body ratio was elevated, and hepatic cellularity was significantly decreased. By administering increasing doses of triamcinolone, higher increases were found in nitrogen content with a tendency to level off at the 5-mg dose. However, the most pronounced alterations for nitrogen as well as liver/body ratio and cellularity were found at the 25-mg dose.

Table 8 gives the results of triamcinolone dose-response studies on hepatic gluconeogenic enzymes in animals killed at 6 and 24 hr after injection. In rats killed at 6 hr both G-6-Pase and FDPase activities were significantly increased statistically by the dose of 0·50 mg (117–121%). When triamcinolone doses were increased to 1,

TABLE 4. SEQUENCE OF EVENTS AFTER A SINGLE INJECTION OF CORTISONE; BEHAVIOR OF LIVER WEIGHT, LIVER/BODY RATIO, NITROGEN CONTENT, AND GLUCONEOGENIC ENZYMES

The mean values and standard errors represent 3 or more animals in each group. The rats were killed at indicated time intervals after a single intraperitoneal injection of cortisone (25 mg/rat). Enzyme activities are calculated as micromoles of substrate metabolized per gram of liver × liver to body weight ratio × 100. Data in parentheses express results in percentages, taking the values of untreated rats as 100%.

	Control (untreated)	Hours after cortisone injection				
		2	4	6	12	24
Body weight, g	96 ± 6 (100)	94 ± 6 (98)	90 ± 2 (93)	92 ± 4 (94)	82 ± 2 (85)	88 ± 1 (92)
Liver weight, g	4·0 ± 0·3 (100)	4·1 ± 0·3 (102)	4·1 ± 0·1 (102)	4·4 ± 0·3 (110)	4·0 ± 0·2 (100)	5·2 ± 0·2 (130)
Liver/body × 100	4·1 ± 0·06 (100)	4·4 ± 0·06 (107)	4·6 ± 0·06 (112)	4·8 ± 0·06 (117)	4·9 ± 0·06 (120)*	6·0 ± 0·08 (145)*
Homogenate nitrogen per 100 g†	128·0 ± 0·8 (100)	132·0 ± 1·0 (103)	138·0 ± 2·6 (108)*	145·8 ± 0·8 (114)*	151·0 ± 1·5 (119)*	162·0 ± 3·1 (126)*
Supernatant nitrogen per 100 g†	63·8 ± 0·8 (100)	65·6 ± 0·5 (103)	68·8 ± 1·0 (108)*	72·1 ± 0·8 (113)*	74·5 ± 0·8 (120)*	81·0 ± 1·5 (127)*
Glucose-6-phosphatase per 100 g	4,210 ± 257 (100)	4,440 ± 78 (106)	4,372 ± 90 (104)	4,256 ± 80 (101)	4,374 ± 34 (104)	4,600 ± 186 (110)
Fructose-1,6-diphosphatase per 100 g	1,681 ± 33 (100)	1,937 ± 48 (115)*	2,138 ± 45 (128)*	2,445 ± 45 (145)*	2,681 ± 12 (159)*	3,000 ± 175 (178)*

* Statistically significant difference as compared with values of untreated rats ($P = < 0.05$).
† Calculated as milligrams of nitrogen per gram of liver × liver to body weight ratio × 100.

TABLE 5. SEQUENCE OF EVENTS AFTER A SINGLE INJECTION OF TRIAMCINOLONE; BEHAVIOR OF LIVER WEIGHT, LIVER/BODY RATIO, NITROGEN CONTENT, AND GLUCONEOGENIC ENZYMES

The mean values and standard errors represent 3 or more animals in each group. The rats were killed at indicated time intervals after a single i.p. injection of triamcinolone (25 mg/rat). Enzyme activities are calculated as micromoles of substrate metabolized per gram of liver × liver to body weight ratio × 100. Data in parentheses express results in percentages, taking the values of untreated rats as 100%.

	Control (untreated)	Hours after triamcinolone injection				
		2	4	6	12	24
Body weight, g	98 ± 1 (100)	97 ± 2 (100)	96 ± 5 (99)	93 ± 3 (94)	90 ± 2 (92)*	87 ± 2 (88)
Liver weight, g	4·0 ± 0·2 (100)	4·2 ± 0·2 (105)	4·6 ± 0·3 (115)	4·5 ± 0·2 (113)	4·7 ± 0·2 (118)	6·0 ± 0·2 (150)*
Liver/body × 100	4·0 ± 0·1 (100)	4·3 ± 0·1 (108)	4·8 ± 0·1 (120)*	4·8 ± 0·06 (120)*	5·3 ± 0·1 (133)*	6·8 ± 0·3 (170)*
Homogenate nitrogen per 100 g†	126·2 ± 2·5 (100)	131·5 ± 2·6 (104)	144·7 ± 2·0 (115)*	148·7 ± 3·4 (119)*	160·0 ± 5·0 (127)*	197·3 ± 0·36 (156)*
Supernatant nitrogen per 100 g†	62·0 ± 0·9 (100)	65·6 ± 1·4 (105)	71·3 ± 1·0 (115)*	74·2 ± 1·2 (120)*	80·3 ± 1·9 (129)*	93·0 ± 0·14 (150)*
Glucose-6-phosphatase per 100 g	3,850 ± 111 (100)	4,100 ± 125 (107)	4,746 ± 11 (123)*	4,836 ± 263 (126)*	5,096 ± 67 (132)*	6,924 ± 490 (180)*
Fructose-1,6-diphosphatase per 100 g	1,753 ± 45 (100)	1,931 ± 66 (111)	2,574 ± 44 (146)*	2,606 ± 87 (148)*	2,876 ± 148 (164)*	3,567 ± 118 (203)*

* Statistically significant difference as compared with values of untreated rats ($P = < 0.05$).
† Calculated as milligrams of nitrogen per gram of liver × liver to body weight ratio × 100.

TABLE 6. SHORT-TERM DOSE-RESPONSE STUDIES WITH TRIAMCINOLONE; BEHAVIOR OF LIVER/BODY RATIO, LIVER WEIGHT, CELLULARITY, AND NITROGEN CONTENT

The mean values and standard errors represent 3 or more animals in each group. The rats were injected i.p. with various doses of triamcinolone and groups of rats were killed 6 hr later. Data in parentheses express results in percentages, taking the values of the control, untreated rats as 100%.

	Control (untreated)	Triamcinolone injected (mg)					
		6-hr					
		0·25	0·50	1·0	5·0	10·0	25·0
Body weight, g	96 ± 2 (100)	92 ± 4 (96)	95 ± 2 (99)	90 ± 5 (94)	90 ± 5 (94)	91 ± 6 (95)	94 ± 2 (98)
Liver weight, g	3·8 ± 0·08 (100)	3·8 ± 0·22 (110)	4·2 ± 0·13 (100)	4·2 ± 0·22 (110)	4·3 ± 0·33 (113)	4·3 ± 0·30 (113)	4·7 ± 0·06 (124)*
Liver/body × 100	3·9 ± 0·00 (100)	4·0 ± 0·04 (103)	4·4 ± 0·12 (113)*	4·7 ± 0·06 (120)*	4·7 ± 0·10 (120)*	4·7 ± 0·04 (120)*	5·1 ± 0·04 (131)*
Cellularity, millions†	204 ± 2 (100)	207 ± 5 (101)	190 ± 1 (93)*	180 ± 4 (89)*	176 ± 5 (86)*	176 ± 5 (86)*	168 ± 2 (82)*
Homogenate nitrogen per cell‡	1·4 ± 0·04 (100)	1·5 ± 0·00 (107)	1·7 ± 0·06 (121)*	1·7 ± 0·06 (129)*	1·9 ± 0·06 (136)*	1·9 ± 0·08 (136)*	1·9 ± 0·06 (136)*
Supernatant nitrogen per cell‡	0·70 ± 0·02 (100)	0·77 ± 0·01 (110)*	0·87 ± 0·00 (125)*	0·93 ± 0·08 (133)*	0·96 ± 0·03 (137)*	0·96 ± 0·03 (137)*	0·99 ± 0·02 (141)*
Homogenate nitrogen per 100 g§	120·0 ± 1·0 (100)	131·5 ± 1·8 (110)*	145·0 ± 5·3 (121)*	154·2 ± 2·1 (128)*	160·0 ± 2·7 (133)*	156·5 ± 2·6 (131)*	163·7 ± 2·0 (137)*
Supernatant nitrogen per 100 g§	56·2 ± 0·8 (100)	64·8 ± 0·2 (115)*	73·5 ± 2·7 (130)*	77·5 ± 1·4 (138)*	80·2 ± 2·1 (142)*	78·8 ± 1·2 (141)*	82·5 ± 1·0 (146)*

* Statistically significant difference as compared with the values of control untreated rats ($P = < 0.05$).
† Expressed in millions of counted nuclei per gram of wet tissue.
‡ Calculated as milligrams of nitrogen × 10^7]
§ Calculated as milligrams of nitrogen per gram of liver × liver to body weight ratio × 100.

TABLE 7. SHORT-TERM DOSE-RESPONSE STUDIES WITH TRIAMCINOLONE; BEHAVIOR OF LIVER/BODY RATIO, LIVER WEIGHT, CELLULARITY, AND NITROGEN CONTENT

The mean values and standard errors represent 3 or more animals in each group. The rats were injected i.p. with various doses of triamcinolone and groups of rats were killed 24 hr later. Data in parentheses express results in percentages, taking the values of the control, untreated rats as 100%.

	Control (untreated)	Triamcinolone injected (mg) 24-hr					
		0·25	0.50	1·0	5·0	10·0	25·0
Body weight, g	96 ± 2 (100)	100 ± 3 (104)	93 ± 4 (96)	92 ± 4 (95)	90 ± 6 (94)	91 ± 5 (93)	93 ± 3 (96)
Liver weight, g	3·8 ± 0·08 (100)	4·6 ± 0·13 (121)*	4·7 ± 0·21 (124)*	5·2 ± 0·22 (139)*	5·4 ± 0·50 (141)*	6·0 ± 0·27 (158)*	6·2 ± 0·20 (163)*
Liver/body × 100	3·9 ± 0·00 (100)	4·7 ± 0·06 (120)*	5·1 ± 0·04 (130)*	5·8 ± 0·10 (148)*	6·0 ± 0·18 (153)*	6·5 ± 0·06 (165)*	6·7 ± 0·04 (170)*
Cellularity, millions†	204 ± 2 (100)	175 ± 5 (85)*	167 ± 4 (82)*	162 ± 4 (79)*	156 ± 6 (76)*	145 ± 4 (71)*	134 ± 1 (65)*
Homogenate nitrogen per cell‡	1·4 ± 0·04 (100)	1·8 ± 0·04 (128)*	1·9 ± 0·06 (136)*	1·9 ± 0·06 (136)*	1·9 ± 0·08 (136)*	1·9 ± 0·08 (136)*	2·1 ± 0·04 (150)*
Supernatant nitrogen per cell‡	0·70 ± 0·02 (100)	0·92 ± 0·01 (131)*	0·94 ± 0·01 (134)*	0·93 ± 0·02 (133)*	0·93 ± 0·04 (133)*	0·96 ± 0·02 (137)*	1·08 ± 0·00 (154)*
Homogenate nitrogen per 100 g§	120·0 ± 1·0 (100)	148·0 ± 2·1 (124)*	157·6 ± 1·8 (132)*	169·2 ± 3·5 (142)*	169·0 ± 4·7 (142)*	180·0 ± 3·0 (150)*	186·2 ± 0·8 (155)*
Supernatant nitrogen per 100 g§	56·2 ± 0·8 (100)	74·3 ± 1·2 (131)*	79·2 ± 0·8 (141)*	83·1 ± 1·5 (147)*	85·2 ± 2·3 (151)*	90·0 ± 1·5 (160)*	94·2 ± 0·5 (168)*

* Statistically significant difference as compared with the values of control untreated rats ($P = <0·05$).
† Expressed in millions of counted nuclei per gram of wet tissue.
‡ Calculated as milligrams of nitrogen × 10^7.
§ Calculated as milligrams of nitrogen per gram of liver × liver to body weight ratio × 100.

TABLE 8. SHORT-TERM DOSE-RESPONSE STUDIES WITH TRIAMCINOLONE; BEHAVIOR OF LIVER GLUCOSE-6-PHOSPHATASE AND FRUCTOSE-1,6-DIPHOSPHATASE

The mean values and standard errors represent 3 or more animals in each group. The rats were injected i.p. with various doses of triamcinolone and groups of rats were killed 6 and 24 hr later. Enzyme activities expressed per cell are calculated as micromoles × 10^7 of substrate metabolized per hour at 37°. When expressed per 100-g body-weight basis, they are calculated as micromoles of substrate metabolized per gram of liver × liver to body weight ratio × 100. Data in parentheses express results in percentages, taking the values of control, untreated rats as 100%.

	Control (untreated)	Triamcinolone injected (mg)					
		6-hr					
		0·25	0·50	1·0	5·0	10·0	25·0
Glucose-6-phosphatase per cell	42·2 ± 0·4 (100)	42·6 ± 1·3 (101)	49·3 ± 1·2 (117)*	53·7 ± 1·7 (127)*	56·7 ± 2·0 (135)*	57·4 ± 1·5 (137)*	58·3 ± 1·00 (139)*
Fructose-1,6-diphosphatase per cell	21·2 ± 0·3 (100)	21·9 ± 1·0 (103)	24·6 ± 1·2 (116)*	30·8 ± 0·3 (148)*	31·0 ± 1·0 (149)*	31·6 ± 0·9 (150)*	34·2 ± 0·3 (162)*
Glucose-6-phosphatase per 100 g	3,354 ± 32 (100)	3,542 ± 49 (106)	4,090 ± 180 (121)*	4,517 ± 126 (134)*	4,680 ± 118 (139)*	4,754 ± 17 (142)*	4,857 ± 96 (146)*
Fructose-1,6-diphosphatase per 100 g	1,704 ± 56 (100)	1,831 ± 65 (108)	2,050 ± 129 (121)	2,590 ± 43 (151)*	2,596 ± 73 (152)*	2,637 ± 29 (155)*	2,901 ± 46 (170)*
		24-hr					
Glucose-6-phosphatase per cell	42·2 ± 0·4 (100)	59·3 ± 1·8 (141)*	61·3 ± 1·4 (146)*	61·3 ± 0·8 (146)*	62·0 ± 2·4 (147)*	67·8 ± 2·1 (161)*	70·3 ± 0·2 (167)*
Fructose-1,6-diphosphatase per cell	21·2 ± 0·3 (100)	33·1 ± 1·3 (159)*	33·4 ± 0·5 (160)*	34·4 ± 1·3 (162)*	33·8 ± 1·5 (160)*	36·5 ± 1·0 (172)*	42·3 ± 0·8 (200)*
Glucose-6-phosphatase per 100 g	3,354 ± 32 (100)	4,800 ± 47 (144)*	5,126 ± 81 (154)*	5,683 ± 300 (168)*	5,780 ± 170 (171)*	6,410 ± 152 (191)*	6,241 ± 134 (186)*
Fructose-1,6-diphosphatase per 100 g	1,704 ± 56 (100)	2,680 ± 66 (157)*	2,824 ± 23 (166)*	3,242 ± 17 (191)*	3,144 ± 118 (185)*	3,465 ± 5 (203)*	3,752 ± 74 (220)*

* Statistically significant difference as compared with values of control, untreated rats ($P = <0·05$).

5, 10, and 25 mg, G-6-Pase in the average cell increased to 127, 135, 137, and 139%, respectively, and FDPase to 148, 149, 150, and 162%. A similar type of dose response was found when enzyme activities were expressed on a 100-g body-weight basis. Thus 0·5 mg of this steroid was sufficient to induce detectable enzyme synthesis in 6 hr. However, 5 mg caused more pronounced increases, but further doses of 10 or 25 mg resulted in little additional increase.

In animals killed 24 hr after triamcinolone injection the dose of 0·25 mg was sufficient to give enzyme increases of 141–159% for G-6-Pase or FDPase in the average cell. A 20-fold increase of the dose failed to give any further increase in this time period; however, 10 and 25 mg caused increases to 167 and 200%. Similar findings were observed when results were expressed on a 100-g body-weight basis.

The data in Tables 6–8 reveal that triamcinolone is the most potent inducer of G-6-Pase and FDPase activity and that marked increases in hepatic nitrogen and gluconeogenic enzyme activities can be detected as early as 4 hr after triamcinolone injection. Since these results demonstrated an early rise in these two key gluconeogenic enzyme activities, it is of considerable interest that an early increase in phosphoenolpyruvate carboxykinase activity was reported by Lardy and associates.[33] These three enzymes are placed at strategic points of the gluconeogenic process where they are involved in circumventing thermodynamic barriers in the reversal of glycolysis, as pointed out by Krebs.[34] In consequence, in the mechanism of effect of the gluconeogenic steroid hormones at the molecular level, the early rise in the rate-limiting triad of gluconeogenic enzymes may have a crucial role.[9] In this connection it is of interest that the same three enzymes, G-6-Pase, FDPase, and phosphoenolpyruvate carboxykinase, are markedly decreased or absent in rapidly growing liver tumors,[16, 35, 36] where failure of gluconeogenesis was shown by isotope methods in incubating tumor slices with pyruvate.[37]

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