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REGULATION OF ENZYMES INVOLVED IN GLUCONEOGENESIS

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INTRODUCTION

INVESTIGATORS of the nature of the role of enzymes in homeostasis can hardly find a more interesting or more suitable model system for analysis than the processes of gluconeogenesis. Since mammalian organisms depend almost entirely on glucose as an energy source for the central nervous system, complicated and apparently foolproof emergency and long-term adaptation systems operate to maintain adequate glucose levels in the blood. In this homeostatic system, on which the life of animals depends in the absence of exogenous glucose, blood sugar is maintained through the process of glucose formation from non-glucose precursors which is called gluconeogenesis. In this process of homeostatic adaptation it is necessary to increase the rate of certain reaction sequences in order to elevate the level of glucose precursors, which is achieved through activation and synthesis of strategic enzyme systems. Thus, the maintenance of blood glucose homeostasis takes place at the molecular level through the phenomenon of activation and synthesis of strategic enzyme systems.

For visualizing and, at the same time, limiting the scope of this discussion, a simplified scheme of gluconeogenesis is shown in Fig. 1. The blood sugar level embodying the triggering and the end product of the whole process occupies the center of this picture.

1. *Glycogenolysis.* The decrease of blood glucose level starts the chain of events by bringing into play the immediate, acute, emergency reactions, which do not involve enzyme synthesis, but still result in increased enzyme activity, (a) by activation of enzyme systems (liver phosphorylase) and (b) by saturating pre-existent, active enzyme systems (phosphoglucomutase) through a sudden rise in substrate concentration. These metabolic events take place because the decrease in blood sugar results in epinephrine release from the adrenal medulla. Epinephrine activates phosphorylase, causing immediate breakdown of hepatic stores of glycogen, which saturates phosphoglucomutase by the increased G-1-P levels which, in turn, result in high

levels of G-6-P. This saturates liver glucose-6-phosphatase (G-6-Pase)* and this enzyme then releases glucose into the blood stream.

2. *Glycogenolysis and gluconeogenesis.* Epinephrine triggers two more mechanisms, namely, the release of glycerol from adipose tissue and activation of the anterior pituitary to release ACTH. Both these functions are part and parcel of the subsequent mechanisms of gluconeogenesis started by these stimuli. The decrease in blood sugar level also results in

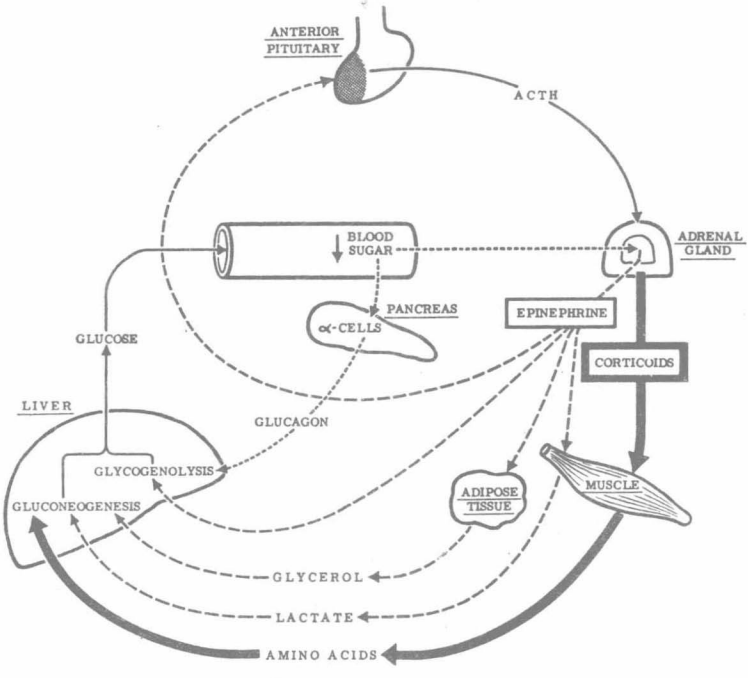


FIG. 1
Processes of gluconeogenesis.

glucagon secretion from the alpha cells of the pancreas which again promotes glycogen breakdown through phosphorylase activation. These emergency reactions supply an immediate rise in blood sugar and assist in maintaining normal glycemia for a few hours while the processes of gluconeogenesis come into play with reactions more time-consuming such as release and transport of precursors from the periphery to the liver and increased biosynthesis of hepatic enzyme systems involved in gluconeogenesis.

*The following abbreviations are used in the text, tables and figures: G-6-P = glucose-6-phosphate; G-6-Pase = glucose-6-phosphatase; G-1-P = glucose-1-phosphate; FDPase = fructose-1,6-diphosphatase; TAC = triamcinolone; ADX = adrenalectomized.

3. *Gluconeogenesis.* The epinephrine-activated anterior pituitary releases and continues to produce ACTH which, in turn, results in increased production and secretion of glucocorticoid hormones. The glucocorticoids cause release of lactate, various amino acids and three-carbon precursors which arrive to the liver through the blood stream. First, the increased level of metabolites saturates the pre-existing enzymes and thus a rise in pyruvate to glucose production can be achieved. Later, the increased biosynthesis of gluconeogenic enzymes becomes noticeable in terms of significant increases in enzyme assay systems in which rate is determined by the enzyme protein in question.

There are a number of outstanding papers on various aspects of gluconeogenesis although no book or monograph has been dedicated to this subject. Because of the exceptional advantages of the model system of gluconeogenesis in the study of the role of enzymes in homeostasis the first day of this symposium is dedicated to various aspects of the regulation of gluconeogenesis.

EXPERIMENTAL BACKGROUND

My contribution will be limited to that portion of the process given in Fig. 1 which deals with the liver enzymes involved in gluconeogenesis from the point of view of regulatory mechanisms uncovered in our laboratories.

Ten years ago in selecting the enzymes for our studies I started with hepatic G-6-Pase because this enzyme is the final common path of both glycogenolysis and gluconeogenesis, and thus, it is a pathway in itself. Extensive investigations on the behavior of G-6-Pase in a number of laboratories showed that hormonal regulation could be well demonstrated on this enzyme.[1–3] FDPase, along with a chain of enzymes participating in other carbohydrate pathways, was also investigated.[4] Because of its rate-limiting role, it soon became clear that it was necessary to carry out systematic and correlative studies on all enzymes of gluconeogenesis to achieve a more complete knowledge of the phenomena involved. A most important advance in our understanding of the mechanism of hepatic gluconeogenesis was outlined in a series of papers by Krebs which emphasized the key differences between the enzymatic steps of glycolysis and of gluconeogenesis.[5–7]

Krebs pointed out that there are three steps of gluconeogenesis which are crucial for the reversal of glycolysis. Because of thermodynamic and enzymatic considerations the reversibility of glycolysis depends on the operation of specific gluconeogenic enzymes. These are G-6-Pase, which reverses G-6-P formation by a hydrolytic reaction; and FDPase, which reverses the fructose-1,6-diphosphate formation, again by hydrolysis. Finally, the reversal of pyruvate production from phosphoenolpyruvate is

accomplished by insertion of a small cycle consisting of the conversion of pyruvate to oxalacetate and the yielding of phosphoenolpyruvate from this compound, as described at the present meeting by Utter[8] and by Krebs.[9]

In an attempt to pinpoint key enzyme reactions in terms of rate-limiting steps, the problems involved are visualized in Figs. 2 and 3. The activities of enzymes of hepatic gluconeogenesis are compared in Fig. 2 by expressing

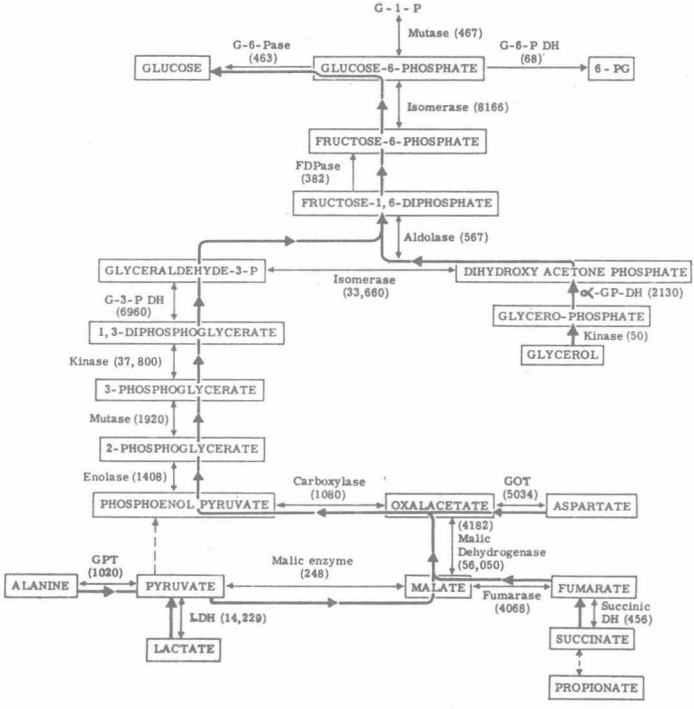


FIG. 2

Comparison of activities of enzymes involved in hepatic gluconeogenesis.

all activities on the same basis, as micromoles of substrate metabolized per wet weight per hour at 37°C. This compilation forms a chart which, with modifications, was designed after that of Krebs.[5,6] Although it is superceded at the present meeting by presentations of Utter[8] and Krebs[9] referring to pyruvate to phosphoenolpyruvate conversion, this figure is used since it demonstrates the problem clearly. In the path of gluconeogenesis G-6-Pase and FDPase are about the "slowest" enzymes with aldolase and the malic enzyme also exhibiting low activities. It is noteworthy that the glycerokinase reaction, which could serve as the starting point of a

rapid short-circuit to glucose formation from glycerol, is the slowest reaction[10,11] in this figure. Thus, enzymes of low activity, the pacemakers[12] of metabolic sequences, occur in gluconeogenesis at the spots of thermodynamically crucial reactions. This further emphasizes the rate-limiting role of these enzymes, as marked on Fig. 3, which presents the current status of our knowledge of gluconeogenesis brought up to date at this meeting.

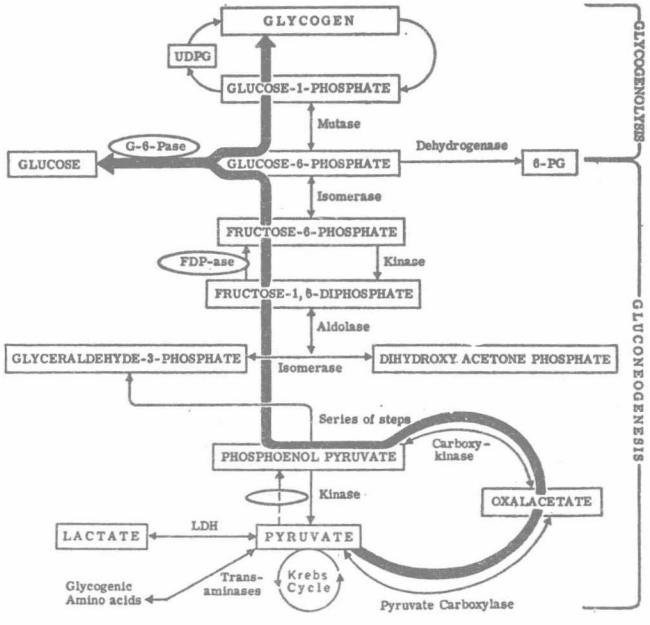


FIG. 3

The main path of gluconeogenesis in the liver. The crucial reactions posing thermodynamic obstacles are circled.

In the following presentation we will explore the regulatory influences involved in controlling hepatic gluconeogenesis at the enzyme level.

MATERIALS AND METHODS

The various biochemical methods, counting of cells and expression of enzymatic results, have been described and are referred to by citing the pertinent previous works published elsewhere.[2,13–15] Specific experimental conditions are referred to in conjunction with the presentation of results.

Cortisone, hydrocortisone, medrol (Merck, Sharp and Dohme) and triamcinolone (9-alpha-fluoro-11-beta,16-alpha,17-alpha, 21-tetrahydroxy-1,4-pregnandiene-3,20-dione) (Lederle) were purchased as commercial preparations. The structures of the glucocorticoids used in this work are shown in Fig. 4. Most of the early studies utilized cortisone, but all the current experimentation is being conducted with triamcinolone.

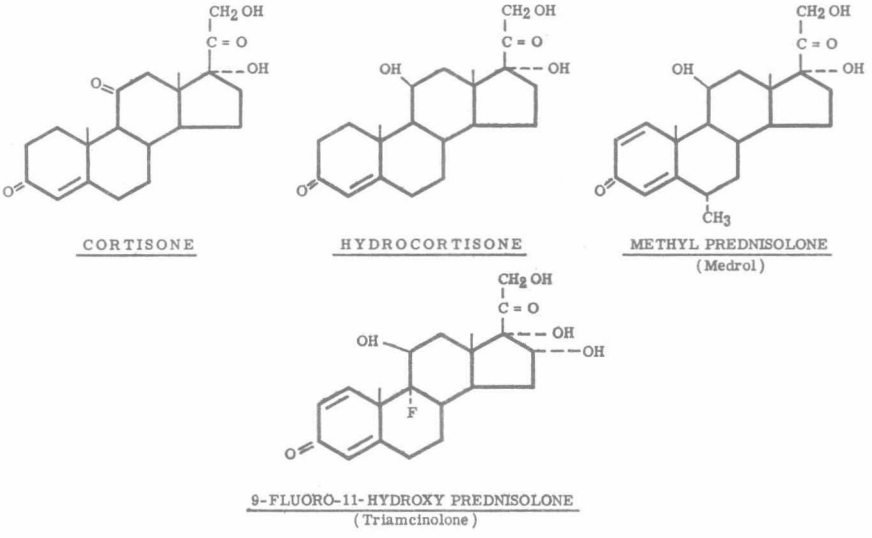


FIG. 4

Comparison of the structures of steroid hormones which exert pronounced effects on enzymes involved in gluconeogenesis.

RESULTS AND DISCUSSION

Study of Cortisone Effects in Rats of Different Weight Groups

Since we observed that animals in different weight groups exhibit different responses to cortisone administration in terms of G-6-Pase activity increases, a detailed investigation was carried out on this subject. This study led to the preference of young rats of about 100 g weight for enzyme induction experiments. Evidence for such a conclusion is now presented.

Effect of cortisone on hepatic nitrogen in rats of different weight groups. Figure 5 shows that when cortisone was given to rats of different weight groups the nitrogen content of the average cell was statistically significantly elevated in all weight groups, but the largest increases were obtained in the 50 g rats. When nitrogen amount was expressed per 100 g body weight basis, as shown in Fig. 6, a similar situation was found with the highest rises

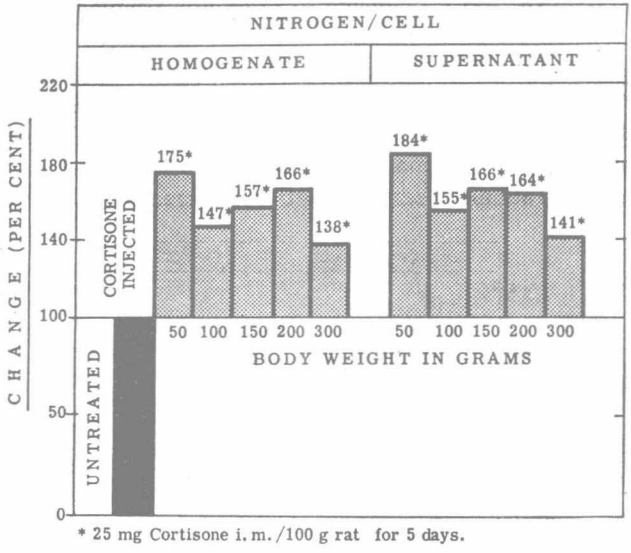


FIG. 5

Effect of cortisone on liver nitrogen content in rats of different weight groups. The nitrogen values are calculated on a per average cell basis. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four to five rats in each group.

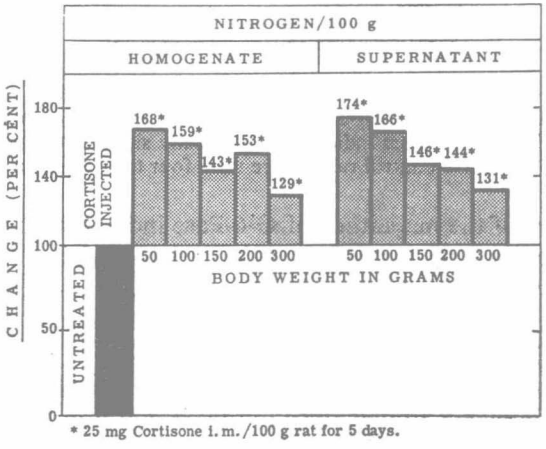


FIG. 6

Effect of cortisone on liver nitrogen content in rats of different weight groups. Nitrogen values are calculated on a per 100 g body weight basis. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four to five rats in each group.

in 50 and 100 g rats and a tendency to gradually smaller increases in the larger rats.

Response of hepatic G-6-Pase and FDPase to cortisone induction in rats of different weight groups. Figure 7 shows that cortisone administration induced statistically significant increases in hepatic G-6-Pase level in all groups. However, the most marked increases were obtained in the smallest rats and the rise gradually decreased with the increasing weight of the rats.

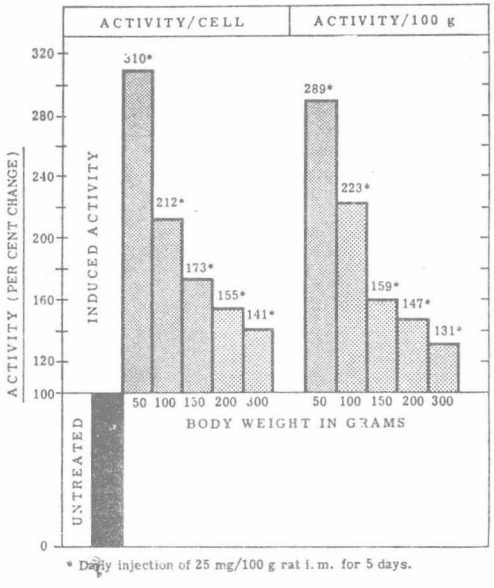


FIG. 7

Response of liver glucose-6-phosphatase to induction by cortisone. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four to five rats in each group.

The dependence of the magnitude of G-6-Pase induction on the weight (or age) of the animals holds true whether the enzyme activity is calculated per average cell or per 100 g body weight basis. In contrast, FDPase activity was statistically significantly increased to about the same extent in all age groups (Fig. 8).

In conclusion, on the basis of these experiments 100 g rats were used in most of the subsequent studies since in this weight group the induction of marked increases for G-6-Pase activity was achievable.

Dose Response Studies with Cortisone

In our original investigations doses of 25 mg cortisone acetate were given intramuscularly for 5 days and animals were sacrificed for biochemical

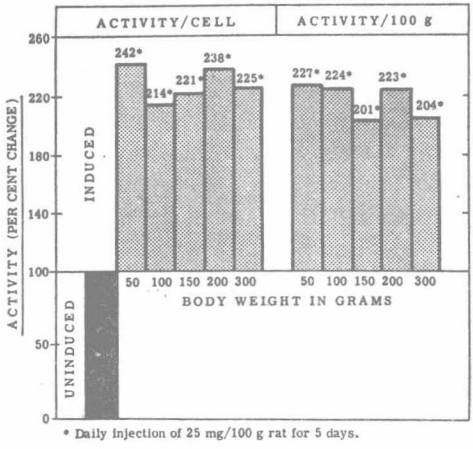


FIG. 8

Response of liver fructose-1,6-diphosphatase to induction by cortisone. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four to five rats in each group.

studies on the 6th day.[16,17] Recently we worked up this subject systematically in order to decrease the dose employed and establish the shortest time required for demonstration of enzyme-inducing effects.

Effect of different doses of cortisone on liver nitrogen levels. Figure 9 shows that when cortisone doses of 2.5–25 mg were injected in groups of rats the

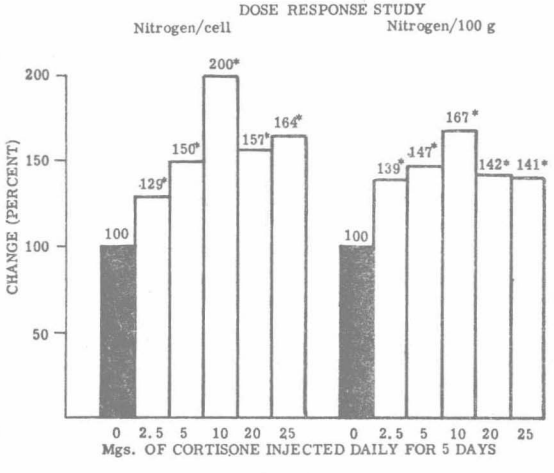


FIG. 9

Effect of different doses of cortisone on liver homogenate nitrogen content. The results are expressed per cell and per 100 g body weight. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four or more rats in each group.

most pronounced response on the homogenate nitrogen level of the average cell was achieved by a dose of 10 mg. However, significant increments were noted for all dose levels, including the lowest ones employed in this study, 2.5 mg. Similar results were found when values were expressed on a per 100 g body weight basis (Fig. 9). The nitrogen content of the supernatant fluid (cytoplasm) of the average cell showed behavior essentially similar to that of the homogenate nitrogen (Fig. 10). Again the highest increases were noted for the group receiving 10 mg cortisone, with significant increases in all groups. Similar information is gained when the data are calculated on a 100 g body weight basis.

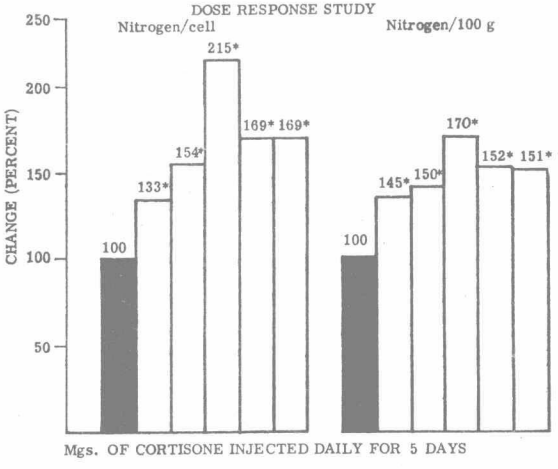


FIG. 10

Effect of different doses of cortisone (0.25–25 mg, as in Fig. 11) on liver supernatant nitrogen content. The results are expressed per cell and per 100 g body weight. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four or more rats in each group.

Effect of different doses of cortisone on hepatic G-6-Pase and FDPase activity. All doses of cortisone used in this study (2.5–25 mg/100 g rat) resulted in statistically significant increases in both G-6-Pase (Fig. 11) and FDPase activity (Fig. 12). The 10 mg dose achieved the highest inductions in these gluconeogenic enzyme activities (280 per cent or more). However, the 2.5 mg dose already gave enzyme increases to 145 per cent or higher. Beyond the dose of 10 mg the extent of enzyme induction remained the same or decreased. The results were essentially the same whether they were expressed per average cell or on a per 100 g body weight basis.

In conclusion, the experimental data indicate that with cortisone statistically significant, well measurable enzyme increases were found with doses of 2.5 mg and optimum induction was achieved with a dose of 10 mg.

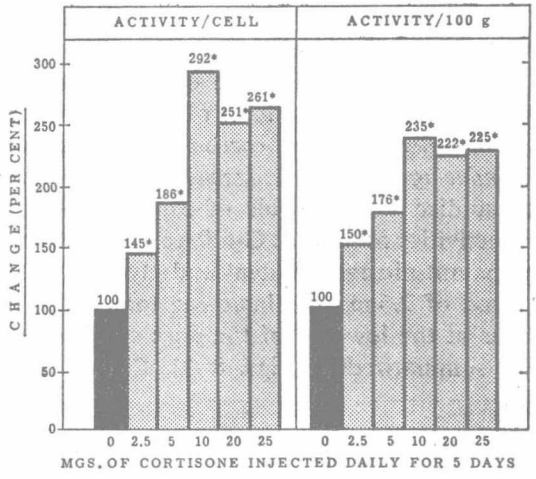


FIG. 11

Effect of different doses of cortisone on liver glucose-6-phosphatase activity. The results are expressed per cell and per 100 g body weight. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four or more rats in each group.

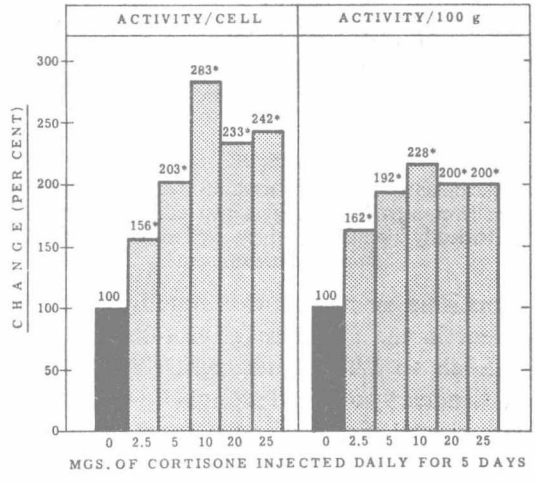


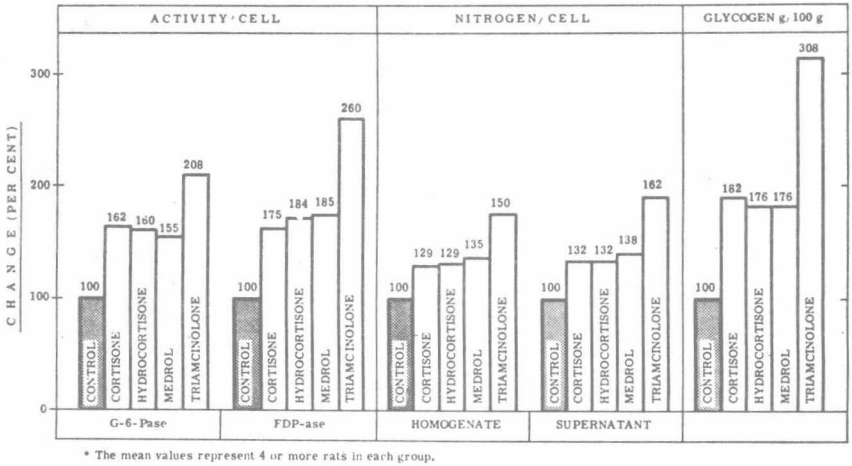
FIG. 12

Effect of different doses of cortisone on liver fructose-1,6-diphosphatase activity. The results are expressed per cell and per 100 g body weight. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were 4 or more rats in each group.

Comparison of the Effectiveness of Different Glucocorticoids in Induction of Hepatic Gluconeogenic Enzymes

Recently we evaluated the activities of different corticoids in order to establish which is the most potent inducer of the gluconeogenic enzymes.

Comparison of the effectiveness of cortisone, hydrocortisone, medrol and triamcinolone in inducing hepatic gluconeogenic enzymes. Figure 13 shows that all steroids studied were capable of causing statistically significant elevations in the activities of hepatic G-6-Pase and FDPase, and in homogenate and supernatant nitrogen content and glycogen level. With a daily intraperitoneal dose of 2.5 mg the increases took place in 3 days. This hormone level was at the low end of the dose spectrum evaluated in the cortisone studies summarized in Figs. 8–12. Figure 13 points out that



* The mean values represent 4 or more rats in each group.

FIG. 13

Comparison of the effectiveness of 4 steroid hormones on hepatic gluconeogenic enzymes and on liver nitrogen and glycogen content. All biochemical parameters were statistically significantly increased as compared to the values of control untreated rats.

cortisone, hydrocortisone or medrol were equally effective in causing rises of 150–180 per cent in the activities of G-6-Pase and FDPase, and in raising nitrogen levels to 129–138 per cent. The three steroids elevated glycogen content to a nearly identical extent (176–182 per cent of the values of control, uninjected rats). However, equal doses of triamcinolone achieved much higher responses, increasing G-6-Pase to 208, FDPase to 260, homogenate nitrogen to 150, supernatant nitrogen to 162 and glycogen levels to 308 per cent of the normal values.

In conclusion, among the steroids tested triamcinolone was the most effective in increasing gluconeogenic enzyme activities and liver nitrogen and glycogen levels.

Dose Response Studies with Triamcinolone

In view of these findings investigation was carried out to study the dose responses for triamcinolone. Figure 14 summarizes the analysis of the effectiveness of various doses of triamcinolone (0.25 mg to 10 mg/100 g rat i.p. for 3 days). All triamcinolone doses employed were capable of inducing statistically significant increases in hepatic G-6-Pase and FDPase activities and in homogenate and supernatant nitrogen content. The

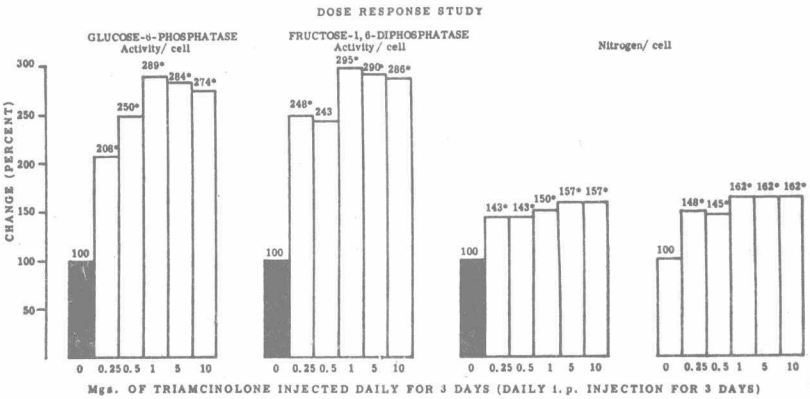


FIG. 14

Effect of different doses of triamcinolone on hepatic gluconeogenic enzymes and nitrogen content. The asterisks beside the numbers indicate a statistically significant alteration from the values of untreated control rats. There were four or five rats in each group.

enzyme activities rose to 200 per cent or higher and the nitrogen level increased to 143–148 per cent with the smallest dose used in this study (0.25 mg). An optimum effect for the enzyme activity induction was reached with a dose of 1 mg which resulted in increases of 289 and 295 per cent, respectively, for G-6-Pase and FDPase activity. Then the extent of increases leveled off or decreased slightly. The 1 mg dose raised the homogenate and supernatant nitrogen levels to 150 and 162 per cent, respectively, and higher doses failed to achieve any further increase under the present experimental schedule.

In conclusion, triamcinolone is considerably more effective than cortisone, hydrocortisone or medrol and achieved higher enzyme responses than these steroids with markedly smaller doses. The minimum effective dose of triamcinolone was 0.25 mg and optimum induction was achieved by 1 mg/ 100g rat.