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DIFFERENCES BETWEEN RATS AND CHICKENS IN RESPONSE TO SYNTHETIC GLUCOCORTICOSTEROID

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SUMMARY

The effects of fasting and triamcinolone diacetate, a synthetic glucocorticosteroid, on blood glucose levels, hepatic glycogen and TNA contents, activities of phosphorylase *a*, glucose-6-phosphatase, fructose-1,6-diphosphatase, alanine and aspartate aminotransferases and ribonuclease (at pH 7·6) and protein synthesizing capacity in rats and chickens have been compared. Forty-eight h fasting altered blood glucose levels by 25 and 5% respectively and caused complete depletion of hepatic glycogen. Significant reduction in total liver RNA, phosphorylase *a*, fructose-1,6-diphosphatase activities and considerable increases in glucose-6-phosphatase, aminotransferases and ribonuclease activities have also been observed under this condition. Fasting also lowered significantly the amino acid incorporating activities of post-mitochondrial fractions. Refeeding restored blood glucose levels, hepatic glycogen and RNA contents, and phosphorylase *a*, fructose-1,6-diphosphatase and amino acid incorporating activities to near, or slightly above, normal levels in fasted rats and chickens. Hormone administration was effective only in fasting rats in restoring the various biochemical parameters (with the exception of ribonuclease) to near normal or slightly above fasting levels, and glucose-6-phosphatase and aminotransferase activities above fasting levels. The cause of the failure of the synthetic glucocorticosteroid to elicit any such changes in fasting chickens has been discussed.

INTRODUCTION

AN INCREASE in the *in vitro* production of free fatty acid by mammalian adipose tissue but not by that of the domestic fowl, on the addition of epinephrine, norepinephrine or other adipokinetic substances has been reported [1]. Similarly, an elevation of plasma FFA and liver triglycerides by catecholamines has also been observed in mammals, but not in ducks [2, 3]. Other marked differences between rats and chickens, particularly in their capacity to utilize gluconeogenic amino acids for glucose synthesis and in the production of ketone bodies have been recently reported from this laboratory [4, 5].

While investigating the physiological effects of synthetic glucocorticosteroids, triamcinolone diacetate in particular, in different species, it became immediately evident that chickens and rats did not respond similarly to triamcinolone diacetate. Gluconeogenesis, as is known, is dependent on the activities of certain enzymes unique to the process [6, 7]. Glucocorticosteroids stimulate glucose production from non-carbohydrate precursors in rats by virtue of their ability to induce *de novo* synthesis of these key enzymes [6–9]. The inability of triamcinolone diacetate, a synthetic glucocorticosteroid, to stimulate glucose production in chickens from non-carbohydrate precursors, initiated the study of the effects

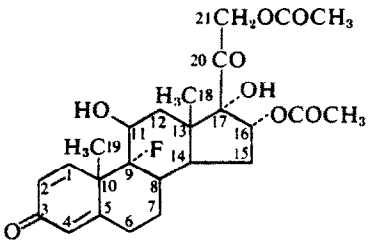
Abbreviations used: ATP: adenosine triphosphate, GTP: guanosine triphosphate, PEP: phosphoenolypyruvate, PK: pyruvate kinase, RNA: ribonucleic acid, mRNA: messenger ribonucleic acid, G-6-Pase: glucose-6-phosphatase, FD Pase: fructose-1, 6-diphosphatase, and FFA: free fatty acid.

of this hormone on blood glucose levels, hepatic glycogen and RNA contents, activities of phosphorylase *a*, glucose-6-phosphatase, fructose-1, 6-di-phosphatase, aspartate and alanine aminotransferases, and ribonuclease (at pH 7·6) in rats and chickens. The purpose of this study was to show whether or not the unresponsiveness of chickens to triamcinolone diacetate can be explained on the basis of its failure to stimulate enzyme induction in this species. The results are reported in this paper.

METHODS AND MATERIALS

Chemicals. ATP, GTP and phosphoenolpyruvate were purchased from Sigma Chemical Co., St. Louis, Mo.; Pyruvate kinase from Boehringer Manheim Corporation, New York, N.Y.; highly polymerized yeast RNA, pancreatic crystalline ribonuclease from Worthington Biochemical Corporation, Freehold, N.J.; DL-C^{14} leucine from New England Nuclear, Boston, Mass. All other chemicals were analytical or reagent grade. Animals; Male rats of Fisher-344 strain, 140–160 g body wt., and chickens of Leghorn-0505 strain, 350–400 g body wt., were used in the present study. The animals were maintained on laboratory diets (viz Purina rat chow for rats and Purina chick starter for chickens) and water *ad libitum*.

The rats were killed after 48 h of fasting and chickens after 48, 96 or 112 h of fasting. The fasting animals received either hormone, 4·0 mg (0·25 ml)/100 g body weight, or the same volume of the medium (polysorbate 80 USP, polyethylene glycol 4000 USP, sodium chloride, benzyl alcohol, water and HCl to make pH 6·0) in which the hormone was suspended. They were both administered intraperitoneally 16 h prior to killing. All animals were killed by decapitation between 9 and 10 a.m. Other experimental details are described in previous papers[10]. The hormone used was triamcinolone diacetate (16α,21-diacetoxy-9α-fluro-11,17β-dihydroxy-1,4-pregnadiene-3,20-dione). It is a highly potent synthetic glucocorticoid steroid and was purchased from Lederle Products Department, Cyanamid of Canada Limited, Montreal, Canada. The chemical structure of this hormone is shown below:



Estimation of blood glucose, hepatic glycogen and RNA, and the activities of phosphorylase a, glucose-6-phosphatase, fructose-1,6-diphosphatase and aspartate and alanine aminotransferases

Blood glucose, hepatic glycogen and RNA content, protein and inorganic phosphorous (Pi) were estimated by the methods previously described by Somogyi[11], Kemp and van Heinjningen[12], Cleland and Slator[13], Fleck and Begg[14] and Fiske and Subbarow[15] respectively. Phosphorylase *a* and glucose-6-phosphatase were assayed in cell-free preparations from rat and chicken livers by the methods previously described by Cori, Illingworth, and

Keller[16] and Swanson[17] respectively. The activities of fructose-1,6-diphosphatase and aspartate and alanine aminotransferase were assayed in cell-free preparations and in high-speed supernatant fractions from rat and chicken livers by the methods described by Freedland and Harper[18], Reitman and Frankel [19], and Wrobleski and Ladue[20] and ribonuclease activity (at pH 7·6) in the particulate fraction by the method of Kunitz[21]. Experimental details of the various methods employed in this study were the same as described previously from this laboratory[10, 22, 23].

Incorporation of [C^{14}]-leucine into proteins by post-mitochondrial preparation

The animals were decapitated, livers were quickly removed, washed twice with cold TKM-sucrose buffer (medium A), blotted dry on filter paper, minced and homogenized in 2·5 vol of medium A in a Potter–Elvehjam homogenizer at 0°C. Medium A contained: 0·25 M sucrose, 50 mM tris-HCl, 25 mM KCl and 5 mM $MgCl_2$, with final pH 7·6. The post-mitochondrial supernatant fraction was prepared by centrifuging the homogenate in a Spinco (Model L) preparative ultracentrifuge at 2000 *g* for 10 min.

The amino acid incorporating system employed here was similar to that previously described[23]. The reaction mixture in 1 ml contained: ATP 1 μmol, GTP, 0·2 μmol, phosphoenolpyruvate 10 μmol, pyruvate kinase 40 μg, $MgCl_2$ 5 μmol, KCl 10 μmol, tris-HCl pH 7·6 50 μmol and post-mitochondrial supernatant fraction 0·2 ml (5·0 mg protein). The final pH was 7·6. The reaction was carried out at 37°C for 15 min and terminated by adding an equal volume of 10% cold TCA solution. Each sample was washed, dried and dissolved in 1 ml Hyamine Hydroxide (Packard 10-X), and the radioactivity was counted to an efficiency of 80% (background 22 c.p.m.) in an Ansitron scintillation counter.

RESULTS

Complete depletion of glycogen from the livers of rats and chickens occurred after 48 h of fasting, but blood glucose levels dropped by 25% in rats compared to only 5% in chickens (Table 1). A further drop in blood glucose levels was noted in chickens when fasting was continued up to 96 h. Considerable decreases in liver weights and their RNA contents (expressed in mg/100 g body weight) were also noticed in both species after 48 h of fasting. Refeeding the fasted animals with their respective normal diets for the next 24 h restored their blood glucose levels, liver weights and glycogen and RNA contents to near or slightly above normal values. Administration of triamcinolone diacetate was effective in fasting rats in restoring blood glucose levels, liver weights and hepatic glycogen and RNA contents to their corresponding normal levels, but failed to elicit these changes in fasting chickens (Table 1).

Significant decreases in phosphorylase *a* and fructose-1,6-diphosphatase activities and considerable increases in glucose-6-phosphatase, aspartate and alanine aminotransferases and ribonuclease (measured at pH 7·6) activities from their respective normal levels of activities, were noted in both of the species after 48 h of fasting. However, the changes in the activities of aminotransferases were less marked in chickens than in rats. Further decreases in phosphorylase *a* and fructose-1,6-diphosphatase activities and increases in glucose-6-phosphatase, aminotransferase and ribonuclease activities from their 48 h fasting levels could be induced in chickens by prolonging the fasting to 96 h. These activities could

Animal	Blood glucose (mg/100 ml)	Liver wt. (g/100 g body wt.)	Liver glycogen (mg/100 mg liver)	Liver RNA (mg/g liver)	Liver RNA (mg/100 g body wt.)
Rat (fed)	102 ± 8*	5·10 ± 0·36*	4·6 ± 0·44*	7·6 ± 0·26*	38·76 ± 2·8*
Rat (fasted 48 h)	75 ± 6	3·20 ± 0·23	< 0·1	7·2 ± 0·32	23·64 ± 2·2
Rat (fasted 48 h, then refed)	106 ± 9	5·26 ± 0·38	5·1 ± 0·48	7·8 ± 0·29	40·46 ± 3·3
Rat (fasted 48 h + hormone administered 32 h after fasting)	102 ± 10	4·96 ± 0·41	4·5 ± 0·42	6·9 ± 0·37	34·42 ± 2·6
Rat (fasted 48 h + medium administered 32 h after fasting)	79 ± 6	3·26 ± 0·24	< 0·20	7·4 ± 0·29	24·46 ± 2·5
Chicken (fed)	216 ± 14	2·46 ± 0·26	2·60 ± 0·27	8·4 ± 0·38	20·56 − 2·1
Chicken (fasted 48 h)	205 ± 10	1·64 ± 0·18	< 0·10	7·9 ± 0·47	12·80 ± 1·6
Chicken (fasted 96 h)	185 ± 9	1·42 ± 0·19	< 0·10	6·2 ± 0·41	9·26 ± 1·1
Chicken (fasted 96 h, then refed)	221 ± 11	2·52 ± 0·27	2·78 ± 0·29	8·6 ± 0·40	21·67 ± 1·9
Chicken (fasted 96 h + hormone administered 80 h after fasting)	180 ± 8	1·26 ± 0·16	< 0·20	6·2 ± 0·36	7·82 ± 0·86
Chicken (fasted 96 h + medium administered 80 h after fasting)	188 ± 10	1·45 ± 0·21	< 0·20	6·4 ± 0·43	9·42 ± 0·98

*S.D. of mean.

Table 1. The effects in rats and chickens of fasting, refeeding and hormone administration on their blood glucose levels, liver weights, hepatic glycogen and RNA contents. Hormone (or medium A) was administered in rats 32 h after fasting and in chickens 80 h after fasting. Rats were fed 48 h after fasting and chickens 96 h after fasting. Number of animals involved in each set of experiments was 6. Experimental details are described in the text under Methods and Materials.

Animal (condition)	Phosphorylase *a*	G-6-Pase	FDPase	Alanine Aspartic aminotransferase (in Sigma Units)		Ribonuclease (increase in optical density at 260 mμ)
				× 10^4	× 10^4	
Rat (fed)	13·6 ± 1·06*	10·4 ± 0·81	5·7 ± 0·50	3·2 ± 0·16	5·2 ± 0·38	0·51 ± 0·060
Rat (fasted 48 h)	10·3 ± 0·92	12·6 ± 0·96	3·8 ± 0·29	7·2 ± 0·24	9·6 ± 0·59	0·62 ± 0·063
Rat (fasted 48 h, then refed)	14·2 ± 1·10	10·8 ± 0·88	5·9 ± 0·46	3·5 ± 0·18	5·4 ± 0·37	0·48 ± 0·039
Rat (fasted 48 h + hormone administered 32 h after fasting)	14·6 ± 1·13	14·4 ± 1·02	6·9 ± 0·53	10·6	18·8 ± 1·04	0·42 ± 0·038
Rat (fasted 48 h + medium administered 32 h after fasting)				× 10^2	× 10^4	
Chicken (fed)	14·8 ± 1·09	9·8 ± 0·78	5·2 ± 0·43	2·9 ± 0·13	4·2 ± 0·29	0·55 ± 0·044
Chicken (fasted 48 h)	11·1 ± 0·93	11·6 ± 0·86	3·7 ± 0·28	3·7 ± 0·19	4·6 ± 0·32	0·69 ± 0·057
Chicken (fasted 48 h, then refed)	15·1 ± 1·14	10·1 ± 0·83	5·4 ± 0·41	3·1 ± 0·14	4·3 ± 0·35	0·58 ± 0·049
Chicken (fasted 48 h + hormone administered 32 h after fasting)	11·3 ± 1·01	11·9 ± 0·89	3·9 ± 0·30	3·6 ± 0·13	5·4 ± 0·41	0·67 ± 0·056
Chicken (fasted 48 h + medium administered 32 h after fasting)	11·5 ± 1·04	11·8 ± 0·92	3·8 ± 0·36	3·7 ± 0·16	5·7 ± 0·16	–

*S.D. of the mean.

Table 2. The effects in rats and chickens of fasting, refeeding and hormone administration on their phosphorylase *a*, glucose-6-phosphatase, fructose-1,6-diphosphatase, alanine and aspartate aminotransferase and ribonuclease activities. Phosphorylase *a* activity was assayed in a reaction mixture of 1 ml, containing: 0·1 ml of the cell-free preparation from a liver homogenate made in 0·1 M NaF (1 in 12), 0·3 ml of 0·15 M NaF, 0·2 ml of 0·1 M citrate buffer pH 5·8, 0·05 ml of 0·25 M glucose-1-phosphate (dipotassium salt), and 100 mg of glyogen. The reaction was carried out at 30°C for 30 min and terminated thereafter by adding an equal volume of cold 10% TCA. Inorganic phosphorous (Pi) released was determined in TCA-extract. The results are expressed as μmol of Pi released/min/g of liver. The animals were fasted for 48 h. They were refed 48 h after fasting. Hormone (or medium A) was administered 32 h after fasting. Number of animals used in each set of experiments was 6.

Glucose-6-phosphatase activity was assayed in a reaction mixture of 1 ml, containing: glucose-6-phosphate 10 μmol, citrate buffer (pH 6·4) 10 μmol, and 0·1 ml of the cell-free preparation from a 10% liver homogenate made in 0·1 M citrate pH 6·4. The reaction was carried out at 30°C for 30 min and terminated thereafter by adding an equal volume of cold 10% TCA. Pi released was determined in TCA-extract. The results expressed as μmol of Pi released/min/g of liver. The other conditions were the same as described above

Fructose-1,6-diphosphatase activity was assayed in a reaction mixture of 1 ml containing: FDP 10 μmol, Mg^{++} and Mn^{++} 0·25 μmol each, cysteine 20 μmol, serine 10 μmol and 0·1 ml of the cell-free preparation from a 10% liver homogenate made in 0·25 M sucrose in 0·01 M tris-HCl buffer, pH 7·6. The pH of the reaction mixture was adjusted to pH 9·5. The reaction was carried out at 37°C for 30 min and terminated by adding an equal volume of cold 10% TCA. Pi released was determined in TCA-extract. The results expressed as μmol of Pi released/min/g of liver. Each value is the mean of results obtained from 6 different animals.

The activities of hepatic aspartate and alanine aminotransferases were assayed in the high-speed supernatant fraction obtained from the cell-free preparation (used for fructose-1,6-diphosphatase activity determination) by centrifuging it at 105,000 *g* for 2 h in Spinco (Model L) preparative centrifuge. Details of the methods are described in the text under Methods and Materials. Values are expressed in Sigma Units (Sigma Bulletin No. 505). The other conditions were the same as described above.

Ribonuclease activity was assayed in a reaction mixture of 1 ml containing 20 μmol of tris-HCl buffer pH 7·6, 2 mg yeast RNA, 0·5 μmol of Mg^{++}, 0·2 ml particulate fraction, obtained by centrifuging the cell-free preparation (used for fructose-1,6-diphosphatase activity determination) at 30,000 *g* for 2 h and 0·1 ml of 1% Triton X-100. The reaction was carried out at 30°C for 20 min and terminated by adding 0·5 ml of cold 0·75% uranyl acetate in 25% $HClO_4$. 0·1 ml of the TCA extract was diluted to 3 ml and its optimal density at 260 m was determined. This, minus the control value, is a measure of the increase in acid soluble material released by the action of ribonuclease on RNA considered as an index of ribonuclease activity. The results are expressed as an increase in E_{260} min/g of liver. The other conditions were the same as described above.

be restored to their normal or slightly above their normal levels by refeeding the animals with their respective normal diets for 24 h (Table 2). Administration of triamcinolone diacetate was effective in elevating the activities of glucose-6-phosphatase and aminotransferases above their fasting levels in rats but not in chickens and also in bringing back the activities of phosphorylase *a* and fructose-1,6-diphosphatase to near or slightly above normal levels. In rats, ribonuclease activity (measured at pH 7·6) was suppressed by hormone, whereas in chickens this activity remained unaffected (Table 2).

The results in Table 3 show that amino acid incorporating activity of the post-mitochondrial supernatant fractions from chicken livers was only about 35% of that for similar preparations from rat livers. The post-mitochondrial supernatant fractions from fasting rat and checken livers were 34 and 35% less active than similar preparations from fed rat and chicken livers (Table 3). Refeeding the

Source of post-mitochondrial supernatant fraction from the liver of:	Counts/min/mg protein
Rat (fed)	1015 ± 56*
Rat (fasted (48 h)	676 ± 35
Rat (fasted 48 h then refed)	1038 ± 62
Rat (fasted 48 h + hormone administered 32 h after fasting	934 ± 49
Rat (fasted 48 h + medium administered 32 h after fasting	662 ± 32
Chicken (fed)	362 ± 30
Chicken (fasted 48 h)	236 ± 22
Chicken (fasted 48 h then refed)	386 ± 36
Chicken (fasted 48 h + hormone administered 32 h after fasting)	237 ± 27
Chicken (fasted 48 h + medium administered 32 h after fasting)	248 ± 26
Chicken (fed) (homogenized in rat liver cell sap)	689 ± 42
Chicken (fasted 48 h) (homogenized rat liver cell sap)	406 ± 38

*S.D. of the mean.

Table 3. The effects in rats and chickens of fasting, refeeding and hormone administration on incorporation of C^{14} leucine into proteins by their liver post-mitochondrial fractions. The reaction mixture contained in 1 ml, ATP 1 μmol phosphoenolypyruvate, 10 μmol pyruvate kinase 40 μg, GTP 0·2 μmol, $MgCl_2$ 5 μmol, KCl 10 μmol of tris-HCl pH 7·6 50 μmol, and microsome-cell sap preparation 0·2 ml (5·0 mg protein). The reaction was carried out at 37°C for 15 min and terminated by adding an equal volume of cold 10% TCA. The samples (TCA-insoluble material) were washed, dried, dissolved in Hyamine Hydroxide (Packard 10-X) and counted with 80% efficiency in an Ansitron Liquid Scintillation Counter. Washing and counting procedures were as described in the text under Materials and Methods. The other conditions were the same as described in Table 2

fasted animals restored their protein synthesizing capacities to near normal values. However, hormone was found to be effective only in rats (Table 3).

Administration of the medium in which the hormone was suspended could provoke only insignificant changes in blood glucose levels, hepatic glycogen and RNA content, activities of phosphorylase *a*, glucose-6-phosphatase, fructose-1, 6-diphosphatase, aminotransferases, and amino acid incorporation in fasted rats or chickens (Tables 1–3).

DISCUSSION

Although glucose concentration in avian blood has long been known to be very high, the effects in rats and chickens of fasting or glucocorticosteroid, (triamcinolone diacetate in particular) on blood glucose, hepatic glycogen and RNA contents, and the activities of certain enzymes unique to gluconeogenesis, do not appear to have been reported. The results of this paper demonstrate different effects of fasting on blood glucose levels in rats and chickens, but the effects on hepatic glycogen and RNA contents and phosphorylase *a*, glucose-6-phosphatase, fructose-1,6-diphosphatase, alanine and aspartate aminotransferases, and ribonuclease (measured at pH 7·6) activities are similar. The effects of refeeding the fasting animals on these biochemical parameters have also been found to be

similar. Despite this similarity, the responsiveness of the fasting animals to triamcinolone diacetate is different, namely blood glucose levels, hepatic glycogen and RNA contents, protein synthesizing capacity, and the activities of the gluconeogenic enzymes, investigated here (with the exception of ribonuclease) are greatly increased in fasting rats by hormone, but no such effects could be provoked in fasting chickens. The changes observed in rats after fasting and hormone administration are compatible with those reported by others [10, 25–28]. The activity of phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenic pathway, in chicken liver (not shown) like in guinea pig and pigeon livers but unlike in rat liver, is affected neither by fasting nor by glucocorticosteroid [29, 30].

Marked differences in responsiveness between mammals and domestic fowls to epinephrine and nonepinephrine and between mammals and ducks to catecholamines have been mentioned earlier [1–3]. Similarly, rats respond to all glucocorticosteroids, including the triamcinolone diacetate. Contrary to this, chickens respond only to some glucocorticosteroids, namely hyperglycaemia and an increase in hepatic glycogen content could be induced in adult chickens by intramuscular injections of hydrocortisone and corticosterone while cortisone and deoxycorticosterone fail to provoke these changes [31]. It thus appears that chickens unlike rats respond differently to different steroid hormones. The failure of triamcinolone diacetate to act in chickens can be explained by any of the three following possibilities: (1) triamcinolone diacetate is not absorbed in blood in the species, (2) if it is absorbed, it is not hydrolyzed to the free alcohol which might be the active form, and (3) if it is absorbed and transformed into its active form, it is unable to stimulate the transcription and translation processes prerequisite for the induction of the enzymes unique to gluconeogenesis. As stated earlier, this synthetic steroid hormone is very active in rats but to the best of my knowledge, no one showed that it requires hydrolysis to alcohol (active form) for its activity. It would be interesting to find out which one of the first two possibilities is responsible for the failure of triamcinolone diacetate to provoke enzyme induction in chickens and what enzyme is involved in the hydrolysis of this steroid hormone in rats if hydrolysis to active form is a prerequisite condition for its activity in rats.

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