Divergent Effects of Actinomycin D on Cortisol and on Glucose Stimulation of Glycogenesis in Mouse Liver

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1. The administration of cortisol and of other glucocorticoid steroids to starved mice produced an increase in liver glycogen content, an elevation of glycogen-synthetase activity and a predominantly particulate localization of both phosphorylase and glycogen-synthetase enzymes. 2. Three daily doses of actinomycin D caused a marked glycogen depletion, a significant decrease in glycogen-synthetase activity, the solubilization of phosphorylase and glycogen synthetase and the following effects on the activities of various other enzymes: a decrease in UDP-glucose pyrophosphorylase and phosphoglucomutase, an increase in glucose 6-phosphate dehydrogenase and no change in glucose 6-phosphatase, 6-phosphogluconate dehydrogenase, pyruvate kinase and UDP-glucose dehydrogenase. 3. Glucose ingestion, but not cortisol administration, reversed the effects of actinomycin D on liver glycogen content and on the activities of phosphorylase and glycogen synthetase.

Actinomycin D specifically blocks DNA-dependent RNA synthesis (Goldberg, Rabinowitz & Reich, 1962), and inhibits glucocorticoid stimulation of a number of liver gluconeogenic enzymes such as glucose 6-phosphatase (EC 3.1.3.9), fructose 1,6-diphosphatase (EC 3.1.3.11) (Weber & Singhal, 1964), phosphopyruvate carboxylase (EC 4.1.1.32) (Ray, Foster & Lardy, 1964) and tyrosine aminotransferase (EC 2.6.1.5) (Greengard & Acs, 1962). Weber, Singhal & Stamm (1963) showed that multiple doses of this antibiotic, given concurrently with cortisone for 5 days, caused depletion of liver glycogen in rats fed *ad libitum*. These findings were again interpreted to mean that glucocorticoids are primarily inducers of gluconeogenic enzymes. Ray *et al.* (1964), on the basis of short-term (1 day) studies, came to an opposite conclusion and attributed the glycogenolysis to decreased food intake after the administration of actinomycin D.

We have investigated both the activities and physical state in the cell of glycogen synthetase (UDP-glucose–glycogen glycosyltransferase, EC 2.4.1.11) and phosphorylase (EC 2.4.1.1) under conditions of glycogenesis and glycogenolysis in mice (Sie & Fishman, 1964; Sie, Hablanian & Fishman, 1964). In particular, glycogen-synthetase activity is decreased in starvation-induced glycogenolysis and is restored to normal by the administration of cortisol. It seemed desirable to extend these studies to other glucocorticoids and to examine the consequences of administering actinomycin D on various enzymes involved in the formation of glycogen. The effects of this antibiotic over a period of 3 days were studied with respect to glycogenic rather than to gluconeogenic phenomena.

Our results indicate that actinomycin D does cause glycogenolysis and preferentially prevents the stimulation of glycogenesis by cortisol but has no effect on the stimulation of glycogenesis by glucose.

MATERIALS AND METHODS

Chemicals and enzymes. AMP, ADP, ATP, UDP-glucose (90% pure), pyruvate kinase (EC 2.7.1.40) (type 1; Sigma Chemical Co.), NAD^+, $NADP^+$, glucose 1-phosphate (dipotassium salt), glucose 6-phosphate (disodium salt), 6-phosphogluconate (tricyclohexylammonium salt), phosphoenolpyruvate (tricyclohexylammonium salt), rabbit-muscle phosphoglucomutase (EC 2.7.5.1) and yeast glucose 6-phosphate dehydrogenase (EC 1.1.1.49), of highest purity, were obtained from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.; rabbit-liver glycogen was from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.; actinomycin D was a gift from Merck and Co., Rahway, N.J., U.S.A. All other chemicals were the best reagent grades available from commercial sources.

Animals. In all experiments, 3-month-old male Ajax mice, each weighing 20–25g., were maintained on Purina

Chow and water *ad libitum*. The conditions for glucocorticoid-induced glycogenesis were as follows. Animals in groups of five were deprived of food at 9 a.m. After 12 hr. the controls were injected subcutaneously with 0·1 ml. of aq. 0·85% NaCl and the experimental animals with 0·1 ml. of hormone suspension (1 mg.). The animals were killed 12 hr. after the injection and the livers removed quickly and chilled on ice.

To study the changes in enzyme activities as a function of dosage of actinomycin D, animals were given, by intraperitoneal injection, daily 10 μg. doses of antibiotic in 0·1 ml. of aq. 0·85% NaCl for 1, 2 or 3 days. Groups of six animals each were killed 24 hr. after the final injection. The control animals received an equal amount of aq. 0·85% NaCl. Body weight and food intake were recorded daily for the animals treated with actinomycin D for 3 days and their controls. The animals were killed by decapitation and the liver weights were determined. The experiments were of 3 days' duration as a longer period showed the same effects. Toxicity did not limit the experimental period to 3 days.

The ability of cortisol to affect glycogenesis in animals pretreated with actinomycin D for 1, 2 or 3 days was studied in the following manner. Immediately after the last actinomycin D injection, food was withheld from the animals for a total of 24 hr., and 0·1 ml. of a suspension of cortisol (1 mg.) in aq. 0·85% NaCl was administered subcutaneously at the twelfth hour of starvation. Two groups of control animals not receiving actinomycin D were set up similarly except that at the twelfth hour of starvation on the last day of each experiment one group was given 0·1 ml. of aq. 0·85% NaCl and the other group received 0·1 ml. of a suspension of cortisol (1·0 mg.) in aq. 0·85% NaCl. All the animals were killed 12 hr. after the injection.

Glycogenesis was studied in animals receiving a daily dosage of actinomycin D for 3 days. Starvation was imposed during the third day at 9 a.m.; after 12 hr. 0·3 ml. of glucose (180 mg.) solution was given by stomach tube to a group of six control animals (no actinomycin D) and six experimental animals. The animals were killed 12 hr. after the glucose solution had been administered.

Table 1. *Effect of glucocorticoids on liver glycogen content and on specific activities of glycogen synthetase and phosphorylase*

Each animal was starved for 24 hr.; after the first 12 hr. 1 mg. of glucocorticoid in 1 ml. of 0·85% NaCl was administered. Individual livers of five to 24 animals were assayed in each experimental group. For assay procedures see the text. Results are presented as means ± S.E.M. Glycogen-synthetase activities are expressed as μmoles of UDP formed/mg. of protein/hr. Phosphorylase activities are expressed as μmoles of P_i formed/mg. of protein/hr.

Treatment	No. of mice	Glycogen content (g./100 g. of liver)	Sp. activity of glycogen synthetase		Sp. activity of phosphorylase	
			Supernatant fraction	Particulate fraction	Supernatant fraction	Particulate fraction
None	11	2·77 ± 0·43	0·09 ± 0·07	4·37 ± 0·64	1·02 ± 0·29	6·33 ± 2·58
Starved, but no steroid given	24	0·28 ± 0·14	0·96 ± 0·46	2·30 ± 0·32	4·51 ± 3·32	7·27 ± 3·16
Cortisol	9	1·88 ± 0·60	0·23 ± 0·19	4·17 ± 0·46	1·42 ± 0·83	9·37 ± 2·55
11β,17-Dihydroxy-6α-methyl-pregn-4-ene-3,20-dione	5	1·42 ± 0·19	0·15 ± 0·11	4·22 ± 0·44	1·19 ± 0·59	10·50 ± 5·30
6α-Fluoro-11β,17-dihydroxy-pregn-4-ene-3,20-dione	5	2·33 ± 0·26	0·19 ± 0·03	4·11 ± 0·36	1·16 ± 0·66	7·54 ± 1·58
9-Fluoro-11β,17-dihydroxy-pregn-4-ene-3,20-dione	5	1·22 ± 0·17	0·17 ± 0·11	4·28 ± 0·85	1·55 ± 0·37	9·10 ± 2·46
21-Acetoxy-9-chloro-11β,16α,-17-trihydroxy-pregn-4-ene-3,20-dione	5	2·47 ± 0·21	0·18 ± 0·13	3·78 ± 0·58	0·63 ± 0·82*	7·13 ± 1·43
9-Fluoro-11β,16α,17-trihydroxy-pregn-4-ene-3,20-dione	5	2·82 ± 1·14	0·12 ± 0·10	4·12 ± 0·43	0·79 ± 0·65	6·84 ± 0·77
6α,9-Difluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione	5	3·01 ± 0·67	0·13 ± 0·11	5·02 ± 0·79	1·42 ± 0·40	6·34 ± 1·51
9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione	5	2·50 ± 0·67	0·05 ± 0·04	4·93 ± 1·71	1·00 ± 0·95	6·93 ± 0·95
6α-Fluoro-11β,17,21-trihydroxy-pregna-1,4-diene-3,20-dione	5	2·10 ± 0·66	0·07 ± 0·06	5·74 ± 1·40	1·21 ± 0·46	8·94 ± 1·52
Cortisone	5	2·15 ± 0·54	0·12 ± 0·10	4·80 ± 0·73	1·65 ± 0·49	6·57 ± 2·15
Cortisone acetate	5	1·49 ± 0·46	0·14 ± 0·12	4·68 ± 0·80	1·74 ± 0·30	7·47 ± 1·91
Prednisone	5	2·15 ± 0·50	0·21 ± 0·08	4·93 ± 1·25	1·40 ± 0·47	6·14 ± 1·00
Prednisolone	5	2·68 ± 0·33	0·15 ± 0·11	5·24 ± 0·45	1·58 ± 0·46	6·50 ± 0·50

* Wide standard errors such as this are due to a heterogeneous population of responses, i.e. 0, 0, 0·12, 1·40 and 1·64 in this case.

Enzyme assay procedures. For phosphorylase and glycogen synthetase, a solution containing sucrose (0·25 M), NaF (0·1 M) and EDTA (1 mM) was used to prepare the 10% (w/v) tissue homogenates. The supernatant fractions were obtained after centrifugation of the homogenates at 100000 *g* for 30 min. at 0° in a refrigerated Spinco model L centrifuge.

Glycogen synthetase and phosphorylase were measured in both homogenate and supernatant fractions, glucose 6-phosphatase in the homogenate and the other enzymes in the supernatant fractions.

The individual enzyme activity and the reference to the method employed for its measurement are indicated as follows: phosphoglucomutase (Najjar, 1948); glucose 6-phosphatase (Cori & Cori, 1952); phosphorylase (Cori, Illingworth & Keller, 1955; Leloir & Goldemberg, 1960); glycogen synthetase (Leloir & Goldemberg, 1960); UDP-glucose dehydrogenase (EC 1.1.1.22) (Strominger, Maxwell & Kalckar, 1955); UDP-glucose pyrophosphorylase (EC 2.7.7.9) (Munch-Peterson, 1955); pyruvate kinase (Nigam, MacDonald & Cantero, 1962); phosphogluconate dehydrogenase (EC 1.1.1.44); glucose 6-phosphate dehydrogenase (Fitch, Hill & Chaikoff, 1959).

The specific activity of each enzyme is expressed as μmoles of substrate utilized or of product liberated/mg. of protein/hr. Protein was estimated according to the method of Lowry, Rosebrough, Farr & Randall (1951), with crystalline bovine albumin serving as reference standard.

Other assays. Blood glucose was determined by the Nelson–Somogyi methods (Nelson, 1944; Somogyi, 1945). Glycogen was extracted with hot KOH and precipitated by 95% (w/v) ethanol. It was estimated as glucose units by the cysteine–H_2SO_4 reaction (Dische, 1949).

RESULTS

Starvation-induced glycogenolysis in mice decreased the total glycogen-synthetase activity in the liver but increased that of both glycogen synthetase and phosphorylase in the supernatant fraction of the centrifuged liver homogenate (Sie & Fishman, 1964). However, these changes are reversed to give normal values under the influence of glucocorticoids, as shown by the increase in glycogen-synthetase activity and by the relocation of both enzymes to the particulate fraction (Table 1). These phenomena are paralleled by a rise in liver

Table 2. *Effect of actinomycin D on body weight, food consumption and liver weight*

Results are presented as means ± S.E.M. of six mice/group.

Treatment	None (control)	Actinomycin D for 3 days
Net change in body wt. (g.)	−0·75 ± 0·71	−4·6 ± 0·25*
Food intake (g./day)	3·80 ± 0·71	0·51 ± 0·23*
Liver wt. (g.)	1·22 ± 0·03	0·89 ± 0·08*

* $P < 0·01$ as compared with control.

Table 3. *Effect of actinomycin D on liver glycogen content, on blood glucose concentration and on specific activities of glycogen synthetase and phosphorylase*

Individual livers of six mice were assayed in each experimental group. For assay procedures see the text. Results are presented as means ± S.E.M. Glycogen-synthetase activities are expressed as μmoles of UDP formed/mg. of protein/hr. for supernatant and particulate fractions and as μmoles of UDP formed/g. of wet liver/hr. for homogenates. Phosphorylase activities are expressed as μmoles of P_i formed/mg. of protein/hr. for supernatant and particulate fractions and as μmoles of P_i formed/g. of wet liver/hr. for homogenates.

Duration of actinomycin treatment (days)	Glycogen content (g./100g. of liver)	Concn. of blood glucose (mg./100 ml).	Sp. activity of glycogen synthetase: Supernatant fraction	Sp. activity of glycogen synthetase: Particulate fraction	Sp. activity of glycogen synthetase: Homogenate	Sp. activity of phosphorylase: Supernatant fraction	Sp. activity of phosphorylase: Particulate fraction	Sp. activity of phosphorylase: Homogenate
0	2·82 ± 0·23	120·1 ± 13·70	0·15 ± 0·08	4·65 ± 0·49	406·8 ± 28·9	1·05 ± 0·23	8·65 ± 2·88	831 ± 261
1	1·91 ± 0·37*	155·5 ± 40·50†	0·28 ± 0·16	4·60 ± 0·86	415·8 ± 47·2	2·05 ± 0·93*	14·68 ± 2·81*	1450 ± 136*
2	0·27 ± 0·35*	166·5 ± 32·80*	0·79 ± 0·64*	2·62 ± 1·23*	294·3 ± 112·5†	7·05 ± 3·58*	9·48 ± 7·81	1309 ± 394
3	0·15 ± 0·17*	118·0 ± 55·00	1·42 ± 0·58*	1·16 ± 0·69*	209·7 ± 45·5	13·47 ± 2·02*	0·72 ± 1·33*	1157 ± 113

* $P < 0·01$ as compared with control.
† $P < 0·05$ as compared with control.

glycogen content, as expected. All the glucocorticoids studied behaved like cortisol without exception.

Table 2 indicates that animals receiving actinomycin D for 3 days showed a significant decrease in body weight, food consumption and liver weight as compared with the control animals.

Table 3 records the sequence of changes produced by actinomycin D treatment as a function of its daily dosage on blood glucose concentration, on liver glycogen content and on the distribution of glycogen synthetase and phosphorylase between the supernatant and particulate fractions of centrifuged homogenates. The glycogen content declined progressively, almost disappearing by the third day. No effect on glycogen synthetase was observed on the first day, but on the second day there was a distinct decrease in the activity of this enzyme in the particulate fraction and a rise in the supernatant fraction. These changes had progressed further by the third day. For phosphorylase, there was initially a marked elevation of enzyme activity in the particulate fraction and a corresponding increase in the supernatant fraction. Finally, on the third day, the enzyme was found almost completely in the supernatant fraction. Also, glycogen synthetase and not phosphorylase exhibited a significant loss in total activity as shown by the homogenate values. A normal or elevated blood glucose concentration was observed in all the actinomycin D-treated animals.

In addition to the decrease in glycogen-synthetase activity, the effects of actinomycin D on other liver enzymes are listed in Table 4. Three days of antibiotic treatment caused a diminution in the activities of UDP-glucose pyrophosphorylase by 50% and in phosphoglucomutase by 25%. In sharp contrast with these enzyme deficits there was a statistically significant though moderate (35%) elevation in glucose 6-phosphate-dehydrogenase activity. Finally, the activities of the following enzymes remained unchanged: glucose 6-phosphatase, 6-phosphogluconate dehydrogenase, pyruvate kinase and UDP-glucose dehydrogenase.

Cortisol-induced glycogenesis can be produced in animals previously treated with actinomycin D for no longer than 24hr. (Table 5), as evidenced by the increase in glycogen content, the increase of glycogen-synthetase activity and the relocation of glycogen synthetase and phosphorylase to the particulate fraction. For phosphorylase, an initial increase in its total activity due to the particulate fraction remains unaltered.

After 48hr. of actinomycin D treatment, cortisol induced glycogenesis was limited, as indicated by diminished liver glycogen content, a decreased synthesis of glycogen synthetase and a more pronounced evidence of solubilization of phosphorylase and of glycogen synthetase. After 3 days of actinomycin D administration, the glucocorticoid effect was almost entirely blocked.

Although cortisol-stimulated glycogenesis was prevented by actinomycin D (3 days), this was not the case for glycogenesis after glucose ingestion (Table 5). There was, in the latter case, a return to the normal values for glycogen-synthetase activity and liver glycogen content, and the relocation of glycogen synthetase and phosphorylase to their particulate sites. Maltose could partially replace glucose, since maltose induced a moderate increase in glycogen-synthetase activity and liver glycogen content (H.-G. Sie, A. Hablanian & W. H. Fishman, unpublished work).

Table 4. *Effects of actinomycin D on mouse-liver enzymes other than phosphorylase and glycogen synthetase*

Individual livers of six animals were assayed in each experimental group. For assay procedures see the text. Results are presented as means ± S.E.M. Enzyme activities are expressed as μmoles of product formed or substrate utilized/mg. of protein/hr.

Treatment	Sp. activity of enzyme: None (control)	Sp. activity of enzyme: Actinomycin D for 3 days
Glucose 6-phosphatase	2·00 ± 0·26	1·82 ± 0·34
6-Phosphogluconate dehydrogenase	0·46 ± 0·14	0·51 ± 0·05
Pyruvate kinase	1·08 ± 0·17	1·10 ± 0·16
UDP-glucose dehydrogenase	0·34 ± 0·03	0·31 ± 0·06
Phosphoglucomutase	0·104 ± 0·011	0·077 ± 0·004*
UDP-glucose pyrophosphorylase	18·90 ± 1·47	9·10 ± 2·48*
Glucose 6-phosphate dehydrogenase	0·35 ± 0·17	0·47 ± 0·08†

* $P < 0·01$ as compared with control.
† $P < 0·05$ as compared with control.

Table 5. *Effects of cortisol or of glucose on liver glycogen content, on blood glucose concentration and on specific activities of glycogen synthetase and phosphorylase of mice pretreated with actinomycin D*

Each animal was starved for the 24hr. of the day of autopsy; after the first 12hr. the inducer was administered, and all animals were killed after 24hr. Individual livers of six animals were assayed in each experimental group. For assay procedures see the text. Results are presented as means ± S.E.M. Glycogen-synthetase activities are expressed as μmoles of UDP formed/mg. of protein/hr. for supernatant and particulate fractions and as μmoles of UDP formed/g. of wet liver/hr. for homogenates. Phosphorylase activities are expressed as μmoles of P_i formed/mg. of protein/hr. for supernatant and particulate fractions and as μmoles of P_i formed/g. of wet liver/hr. for homogenates.

Duration of actinomycin D pretreatment (days)	Inducer	Glycogen content (g./100g. of liver)	Concn. of blood glucose (mg./100ml.)	Sp. activity of glycogen synthetase			Sp. activity of phosphorylase		
				Supernatant fraction	Particulate fraction	Homogenate	Supernatant fraction	Particulate fraction	Homogenate
0	0	0·28 ± 0·14	79·6 ± 19·5	0·96 ± 0·46	2·30 ± 0·32	280·0 ± 48·7	4·51 ± 3·32	7·27 ± 3·16	1111 ± 195
0	Cortisol	1·90 ± 0·60	106·3 ± 35·5	0·12 ± 0·12	4·17 ± 0·46	373·0 ± 33·6	1·41 ± 0·83	9·37 ± 2·55	913 ± 224
1	Cortisol	2·01 ± 0·31	125·3 ± 18·5	0·17 ± 0·13	3·94 ± 0·72	363·0 ± 50·7	2·38 ± 0·48†	15·6 ± 2·60*	1595 ± 157*
2	Cortisol	1·21 ± 0·61†	186·0 ± 30·2*	0·61 ± 0·52*	2·88 ± 0·73*	310·0 ± 29·7†	4·78 ± 4·36	7·94 ± 3·92	1114 ± 300
3	Cortisol	0·25 ± 0·16*	138·0 ± 47·3	1·24 ± 0·47*	1·55 ± 0·68*	241·2 ± 30·2*	9·13 ± 4·45*	4·49 ± 4·58†	1154 ± 117
0	Glucose	1·38 ± 0·27		0·37 ± 0·24	4·43 ± 0·75	488·6 ± 50·4	4·01 ± 2·58	8·98 ± 2·73	1280 ± 180
3	Glucose	1·84 ± 0·54		0·41 ± 0·15	3·49 ± 0·57	332·6 ± 31·4*	2·06 ± 0·98	12·05 ± 2·65†	1166 ± 154

* $P < 0·01$ as compared with control without actinomycin D pretreatment.
† $P < 0·05$ as compared with control without actinomycin D pretreatment.

DISCUSSION

The administration of cortisol to starved animals produces an increase in the activity of glycogen synthetase (Sie & Fishman, 1964; Sie *et al.* 1964), an enzyme whose major catalytic reaction is the synthesis of glycogen (Leloir & Goldemberg, 1960). The study of 12 glucocorticoids other than cortisol demonstrates further that the increase in glycogen-synthetase activity in the liver after the administration of hormone consistently parallels an increase of its reaction product, glycogen. Further, Kreutner & Goldberg (1966) reported that an early glycogenic response to hydrocortisone may be mediated in part by an activation of glycogen synthetase.

Villar-Palasi & Larner (1966) demonstrated that glycogen, at a concentration similar to that normally found in muscle tissue, inhibited some enzyme systems involved in the interconversion *in vitro* of glucose 6-phosphate-dependent and -independent glycogen synthetase and two forms of phosphorylase. Therefore they stated that, in muscle, glycogen could act in a double-feedback mechanism controlling its own synthesis and degradation. Even though their results cannot be directly extrapolated to liver, similar enzyme systems do exist in the liver. From previous observations (Mommaerts, Illingworth, Pearson, Guillory & Seraydarian, 1959; Friedmann, Goodman & Weinhouse, 1963; Steiner & King, 1964) one could infer that glycogen may exert a regulatory effect on its accumulation when tissue content is low and prevents its formation when tissue content is high.

Glycogenolysis resulting from the administration of actinomycin D induces alterations in the intracellular distribution of glycogen synthetase and phosphorylase, which are normally associated with the glycogen pellet (Leloir & Goldemberg, 1960; Sie *et al.* 1964). Phosphorylase is solubilized without significant change in its activity except a transitory rise, whereas glycogen synthetase is solubilized and most of it disappears from the particulate fraction.

The solubilization of phosphorylase has now been observed in three conditions of glycogen depletion, namely starvation (Sie *et al.* 1964; Tata, 1964), ethionine administration (Sie & Hablanian, 1965) and actinomycin D administration (the present work). The most reasonable interpretation is that the enzyme remains attached to glycogen molecules of ever-decreasing size as the glycogenolysis proceeds to the point at which they can no longer be sedimented at 100000*g* (Barber & Allen, 1966).

Glycogen synthetase would therefore be expected to behave similarly, since it is a component of the glycogen pellet. On the other hand, Tata (1964) explained the normal distribution of phosphorylase in terms of its pre-existing preferential binding of enzyme to glycogen of high molecular weight from

which it is completely released when glycogen is degraded.

For glycogen synthetase, the binding of enzyme protein to glycogen depends on the maintenance of an active enzyme configuration by certain intracellular metabolites, e.g. glucose 6-phosphate, acting as an allosteric effector (Steiner, Younger & King, 1965). Since mouse-liver glycogen synthetase has been found by biochemical and histochemical techniques to be glucose 6-phosphate-dependent (Sie, Sawyer & Fishman, 1966), the decrease in glycogen-synthetase activity after the antibiotic treatment is attributed to the decrease of enzyme protein and not to any variation in glucose 6-phosphate concentration, since glucose 6-phosphate was added to the digest during the determination of the enzymic activity.

Actinomycin D interferes with glycogen synthetase in other systems. Thus Steiner & King (1964) administered insulin to alloxan-diabetic rats and demonstrated an increase in glycogen-synthetase activity and a rapid deposition of glycogen during early hours of treatment. This increase in glycogen-synthetase activity was prevented by actinomycin D. Accordingly, they suggested the involvement of RNA in the synthesis of enzyme protein, since actinomycin D has been shown to be a highly specific inhibitor of DNA-dependent synthesis of cellular RNA by RNA polymerase (Reich, Franklin, Shatkin & Tatum, 1961). Recent findings indicate that actinomycin D may block RNA synthesis only transiently in normal and regenerating rat liver (Schwartz, Sodergren, Garofalo & Steinberg, 1965), although Revel & Hiatt (1964) reported a 50% inhibition of nuclear RNA synthesis in liver 17hr. after a (3mg./kg. body wt.) dose of actinomycin D.

In spite of the decreased food intake of the animals injected with actinomycin D, the induced glycogenolysis cannot be attributed to involuntary starvation since the concentration of blood glucose was observed to remain at a normal level. In addition, the administration of glucose but not cortisol stimulated glycogenesis under the present experimental conditions. Ray *et al.* (1964) observed that actinomycin D did not suppress glycogen formation in the rat given both glucose and hydrocortisone. However, these authors did not investigate the separate effects of glucose and of hydrocortisone. Therefore the glycogenesis *in vivo* stimulated by glucose ingestion but not cortisol under our experimental condition is especially noteworthy. This effect of glucose is undoubtedly related to the demonstration (Honig & Rabinovitz, 1966) that incubation of sarcoma 37 ascites cells *in vitro* with actinomycin D resulted in inhibition of synthesis of nuclear and cytoplasmic protein. The presence of glucose in the incubation mixture could prevent or alleviate these inhibitions, even though a 90% decrease in RNA synthesis was still in evidence (Honig & Rabinovitz, 1966).

The present results show divergent effects of actinomycin D on various enzyme systems, many of which are important in catalysing the sequential reactions leading to glycogenesis. The administration of actinomycin D has been found to decrease not only the activity of glycogen synthetase, but also those of phosphoglucomutase and UDP-glucose pyrophosphorylase by 25% and 50% respectively. Glucose 6-phosphate-dehydrogenase activity was slightly stimulated to a significant but moderate increase of 35% in its activity, in contrast with a 300–500% increase in the activity of this enzyme in ethionine-fed mice (Sie & Hablanian, 1965). Some enzymes, e.g. glucose 6-phosphatase, 6-phosphogluconate dehydrogenase, pyruvate kinase and UDP-glucose dehydrogenase, are not affected by the antibiotic.

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