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EFFECTS OF TOXIC CHEMICALS ON SOME ADAPTIVE LIVER ENZYMES, LIVER GLYCOGEN, AND BLOOD GLUCOSE IN FASTED RATS*

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Abstract—After poisoning by an irritant (acrolein) and several organophosphate insecticides, the liver glycogen content of fasted rats was increased. This effect and the effects of these chemicals on blood glucose and some adaptive rat liver enzymes were compared in adrenalectomized, hypophysectomized, and adrenal demedullated rats, and in rats treated with inhibitors of protein synthesis. The results suggested that acrolein increased glycogen deposition by stimulating the secretion of increased amounts of adrenal glucocorticoids which in turn stimulate the synthesis of adaptive liver enzymes that are required for gluconeogenesis and glycogenesis. Increased liver glycogen deposition after administration of the organosphate Guthion appeared to be dependent upon adrenal hormones but was apparently independent of stimulation of adaptive enzyme synthesis. Increased availability of carbohydrate precursors resulting from increased muscle glycogenolysis appears to contribute to the hyperglycemia and liver glycogen depositions following Guthion poisoning.

PREVIOUS studies have shown that rat liver alkaline phosphatase and tyrosine-α-ketoglutarate transaminase activities are increased markedly after inhalation or injection of several irritant chemicals[1, 2] and after sublethal poisoning by several organophosphate insecticides.[3] Investigations of the mechanism of these effects of toxic chemicals on liver enzymes suggested that the pituitary–adrenal cortex system responded to toxic stress to increase the secretion of adrenal glucocorticoids, which in turn stimulated the rates of synthesis of the enzyme proteins. The physiologic or pathologic consequences of increased hepatic alkaline phosphatase and tyrosine transaminase activities induced by toxic chemicals are not known. Rosen *et al.*,[4] Greengard *et al.*,[5] and Weber *et al.*[6] have reported studies suggesting that the glycogenic and gluconeogenic action of cortisone and hydrocortisone are accompanied by and apparently dependent upon the inductive synthesis of adaptive liver enzymes. Hyperglycemia has been reported as one feature of poisoning by organophosphate insecticides.[7] It seemed possible, therefore, that the induction of alkaline phosphatase and tyrosine transaminase activities that occurred in the livers of rats subjected to toxic stress might have an effect on the regulation of carbohydrate metabolism. The

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present investigation was undertaken to test this possibility. In most of the experiments the organophosphate insecticide Guthion* or the irritant acrolein was used. Two other organophosphates, Delnav† and Dipterex,‡ were tested in preliminary experiments. The results of the study showed that several organophosphate insecticides and acrolein stimulate deposition of liver glycogen in fasted rats. For acrolein this effect appeared to be related to stimulation of synthesis of adaptive liver enzymes and in this respect resembled the glycogenic action of hydrocortisone. The increase in liver glycogen and tyrosine transaminase activity that was produced by Guthion appears to occur through a different mechanism.

METHODS

Adult male Holtzman rats (Holtzman Co., Madison, Wis.) weighing between 200 and 300 g were used in this investigation. Bilaterial adrenalectomies were performed under pentobarbital anesthesia. Hypophysectomized and adrenal demedullated Holtzman rats were purchased from the Hormone Assay Laboratories (Chicago, Ill.) and were supplied with drinking water containing 0·9% sodium chloride or 5% glucose respectively. The animals were used in experiments 7–10 days after surgery. A commercial rat diet was supplied *ad libitum* except that before experiments the rats were fasted between 6 p.m. and 2 p.m. of the following day when they were sacrificed by decapitation. During this interval, glucose water was replaced with tap water for the hypophysectomized rats. The animals were injected intraperitoneally with the test chemicals at appropriate times so that the same 20-hr fast period was used for all experiments. Acrolein, Dipterex, and hydrocortisone were given as aqueous solutions. Delnav was dissolved in corn oil and Guthion in propylene glycol. All experiments included control animals which received equal-volume doses (0·5 ml/kg) of the solvents. Actinomycin D, puromycin dihydrochloride, and ethionine were given intraperitoneally in aqueous solution.

The procedures for measuring alkaline phosphatase, tyrosine-α-ketoglutarate transaminase, glucose 6-phosphatase, acetylcholinesterase, adrenal ascorbic acid, and blood glucose have been described previously.[2, 3] For glycogen measurements duplicate samples of 100–300 mg of fresh liver or muscle were digested by boiling with 30% potassium hydroxide, washed, and precipitated twice with 95% ethanol. The precipitate was resuspended in an appropriate volume of water so that the color that developed when a 0·5-ml aliquot was added to 5 ml of anthrone reagent[8] could be read at 620 mμ on a Bausch and Lomb Spectronic 20 colorimeter that was calibrated for each experiment against a standard glucose solution. The lactic acid content of whole blood was measured according to the procedure of Barker and Summerson.[9] All assays were performed in duplicate.

RESULTS

Table 1 summarizes the relationships among blood glucose, liver glycogen, tyrosine transaminase, and alkaline phosphatase activities that were observed in preliminary

* Guthion is the trademark of the Chemagro Corp. for 0,0-dimethyl-S-(4-oxo-1,2,3-benzotriazine-3(4H)-ylmethyl)phosphorodithioate.

† Delnav is a trademark of the Hercules Powder Co., for 2,3-*p*-dioxanedithiol-S,S′-bis(0,0-diethyl phosphorodithioate).

‡ Dipterex is the trademark of the Chemagro Corp. for 0,0-dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate.

experiments with several toxic chemicals. The results are expressed as the average percentage of controls for four or more fasted rats in each group. Under the conditions shown, blood glucose was not appreciably affected by any of the chemicals. Liver glycogen levels were considerably greater than control levels. It should be pointed out that liver glycogen levels for fasted control rats were only 20–100 mg/100 g. An

TABLE 1. BLOOD GLUCOSE, LIVER GLYCOGEN, AND ADAPTIVE ENZYMES IN 20-hr FASTED MALE RATS AFTER TOXIC CHEMICALS

Compound	Dose (mg/kg i.p.)	Time after injection (hr)	Per cent of controls*			
			Blood glucose	Liver glycogen	Liver alkaline phosphatase	Liver tyrosine transaminase
Acrolein	1·5	5	95 ± 9·3	548 ± 81	270 ± 48	446 ± 39
Delnav	40	15	122 ± 7·6	1345 ± 350	313 ± 70	379 ± 55
Dipterex	200	5	101 ± 6·5	670 ± 31	183 ± 19	131 ± 7
Guthion	3	5	103 ± 4·0	1566 ± 251	110 ± 8	72 ± 5

* Figures are mean percents of solvent-treated controls ± S.E. for four to six animals in each group.

increase in liver glycogen after administration of toxic chemicals to fasted rats might be a result of either increased rates of glycogen formation and deposition or decreased rates of glycogenolysis during the period between the time of injection and the time of sacrifice. To test this, food was withdrawn from 12 rats at 6 p.m.; at 9 a.m. the following day the rats were injected with 0·5 ml propylene glycol (solvent for Guthion)/kg. Six rats were sacrificed within 30 min after injection and the remaining six were sacrificed 5 hr later. The means ± standard errors for the glycogen contents of the livers were 38 ± 18 mg/100 g for the group sacrificed immediately after injection of solvent and 65 ± 17mg/100 g for those sacrificed 5 hr later. This experiment indicated that no glycogen depletion occurred during the last 5 hr of the 20-hr fast period. Since all animals were fasted during the same 20-hr interval before sacrifice, and since in most experiments injections were made during the last 5 hr of this period, an increase in liver glycogen after injection of the chemicals must represent increased deposition.

Table 1 also shows that increased activities of hepatic alkaline phosphatase and tyrosine transaminase accompanied the increase in liver glycogen that occurred after acrolein, Delnav, and Dipterex. Previous studies[2, 3] on adrenalectomized rats had indicated that the increased hepatic enzyme activities that occurred after acrolein and Delnav were mediated through increased secretion of endogenous adrenal glucocorticoids. It appeared, therefore, that the increased liver glycogen deposition after these toxic chemicals could be the result of increased adaptive enzyme synthesis, since reports of studies of rats given exogenous glucocorticoids indicated that the glycogenic action of these hormones is dependent upon inductive synthesis of adaptive liver enzymes.[4–6] In the case of Guthion, however, the increase in liver glycogen levels 5 hr after injection was not accompanied by an increase in tyrosine transaminase or alkaline phosphatase activity. This suggested that the glycogenic effect of this compound was not dependent on adaptive enzyme synthesis, and it seemed that further

studies with Guthion might provide information concerning additional factors involved in the regulation of liver glycogen levels under conditions of toxic stress. Most of the remainder of this work was devoted to experiments with Guthion. In some experiments the effects of Guthion were compared with the effects of acrolein in order to illustrate that the stress of exposure to chemically and pharmacologically unrelated compounds can result in qualitatively similar effects but through different mechanisms.

Dosage and time relationships among the biochemical effects of Guthion

Table 2 shows the results of a series of experiments in which groups of rats were sacrificed 5 hr after various doses of Guthion. Assays of liver glucose 6-phosphatase

TABLE 2. BIOCHEMICAL EFFECTS IN 20-hr-FASTED MALE RATS 5 hr AFTER GUTHION

Assay	Dosage (mg/kg i.p.)			
	0·0	2·0	3·0	4·0
Brain cholinesterase (μl CO_2/50 mg/10 min)	107·0* ± 0·9	81·7 ± 3·8	75·6 ± 2·1	68·9 ± 3·0
Adrenal ascorbic acid (μg ascorbic acid/mg)	4·22 ± 0·22	3·19 ± 0·34	3·82 ± 0·25	2·76 ± 0·09
Blood glucose (mg/100 ml)	94·3 ± 7·1	84·4 ± 2·1	96·8 ± 3·8	107·4 ± 4·5
Liver glycogen (mg/100 g)	65 ± 18	361 ± 258	1018 ± 163	1211 ± 105
Liver glucose 6-phosphatase (μg phosphorus/mg/hr)	35·2 ± 2·0	32·8 ± 2·2	32·4 ± 0·9	33·7 ± 1·1
Liver alkaline phosphatase (μg phosphorus/mg/hr)	0·51 ± 0·05	0·44 ± 0·08	0·56 ± 0·04	0·65 ± 0·09
Liver tyrosine transaminase (μg *p*-hydroxyphenylpyruvic acid/mg/hr)	15·2 ± 1·1	13·5 ± 0·5	10·9 ± 0·7	15·0 ± 2·8

* Figures are mean values ± S.E.M. for seven, three, seven, and five rats in the 0·0, 2·0, 3·0, and 4·0 mg/kg dosage-groups respectively.

were included because this enzyme has been reported to be associated with the glycogenic acition of adrenocortical steroids.[6] Adrenal ascorbic acid levels can be used as an index of increased secretory activity of the adrenal cortex.[10] Brain cholinesterase measurements were included in the experiments with Guthion to serve as an indicator of the degree of primary toxic action produced by this compound.[11]

It can be seen from the data in Table 2 that at 5 hr after Guthion the significant dose-related effects were inhibition of brain cholinesterase activity, depletion of adrenal ascorbic acid, and increased liver glycogen. The liver enzyme activities and blood glucose were not appreciably affected at this time interval. At the 3·0 and 4·0 mg/kg dosage levels, typical signs of poisoning by anticholinesterase agents were observed, but no deaths occurred.

A study of the effects of Guthion at various intervals after injection revealed other biochemical changes that were not detected at the 5-hr interval. The results of this series of experiments are summarized in Fig. 1. The percentage changes in liver glycogen values were too large to permit convenient plotting of the data on the graph; therefore the percentages of controls are shown as numerals at the appropriate time intervals. The curves represent the average values for groups of four or more animals

that were sacrificed at 0·5, 1, 3, 5, or 8 hr after injection of Guthion 3 mg/kg. This time–response study shows that the maximal degree of inhibition of brain cholinesterase and depletion of adrenal ascorbic acid occurs at or before 30 min after injection. After these biochemical events, blood glucose and liver tyrosine transaminase activities increased to levels approximately twice the control values at 1 hr after

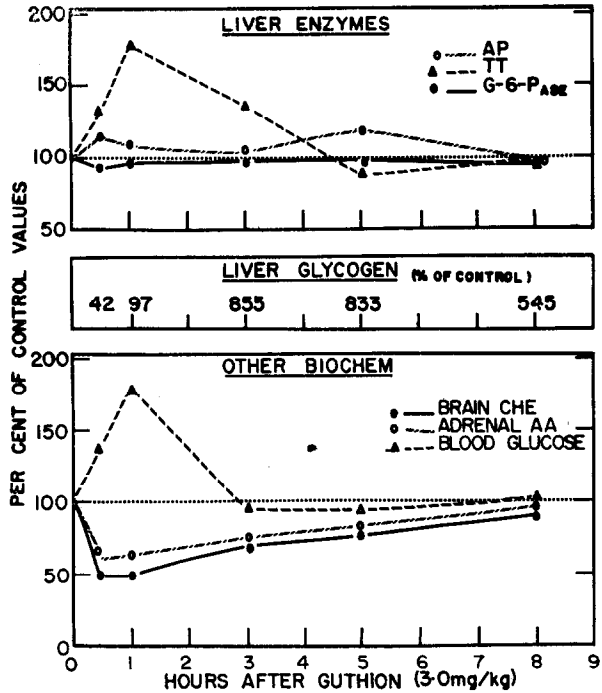

FIG. 1. Biochemical effects in fasted male rats at various intervals after 3 mg Guthion/kg, i.p. The curves represent average per cents of controls for four to seven animals at each test interval. Mean values ± standard errors for 7 control rats are shown in Table 2 (0·0 dosage level). Abbreviations are as follows: AP (alkaline phosphatase); TT (tyrosine-α-ketoglutarate transaminase); G-6-Pase (glucose 6-phosphatase); CHE (cholinesterase); AA (ascorbic acid).

injection and then decreased to normal values by 3 to 5 hr. The mean glycogen contents of the livers were less than control values at 1·5 and 1 hr after injection, but they increased to several times control values at 3 and 5 hr after injection and remained elevated for at least 8 hr. Liver glucose 6-phosphatase and alkaline phosphatase activities were not appreciably affected at any of these time intervals.

The increase in liver tyrosine transaminase activity after Guthion differed from the response of this enzyme after acrolein[2] in that it occurred sooner after administration of the toxic chemical. It appeared that the early increases in tyrosine transaminase activity and blood glucose might be causally related to later glycogen deposition in the liver.

Effects of actinomycin D *on the response to Guthion, acrolein, and hydrocortisone*

Greengard *et al.*[5] reported that actinomycin, an inhibitor of RNA synthesis,[12] prevents both the elevation of liver tyrosine transaminase and liver glycogen deposition

that follow the injection of exogenous glucocorticoids. It seemed probable that actinomycin pretreatment would prevent the glycogenic effect of acrolein and Guthion if this effect were mediated by adrenocorticoid-stimulated enzyme syntheses. The data in Table 3 summarize the results of experiments designed to test this hypothesis.

TABLE 3. EFFECT OF PRETREATMENT WITH ACTINOMYCIN D ON BIOCHEMICAL RESPONSES OF RATS TO HYDROCORTISONE, GUTHION, AND ACROLEIN

Treatment*	Time of sacrifice (hr)	No. of animals	Liver glycogen (mg/100 g)	Blood glucose (mg/100 ml)	Liver tyrosine transaminase (μg/mg/hr)
Water + propylene glycol	3	5	106 ± 43†	72 ± 2·8	14·8 ± 1·4
Actinomycin + propylene glycol	1–3	6	106 ± 41	85 ± 4·9	14·5 ± 0·9
Water + hydrocortisone	3	4	472 ± 102	92 ± 3·0	96·4 ± 13·8
Actinomycin + hydrocortisone	3	4	104 ± 56	97 ± 1·5	20·2 ± 2·0
Water + Guthion	3	5	1031 ± 180	87 ± 6·0	22·5 ± 3·7
Actinomycin + Guthion	3	6	916 ± 134	100 ± 8·0	21·0 ± 3·2
Water + acrolein	5	5	583 ± 86	96 ± 6·7	93·0 ± 5·7
Actinomycin + acrolein	5	4	146 ± 13	121 ± 12·0	19·7 ± 1·6

* Actinomycin D (1 mg/kg) was given 30 min before propylene glycol (0·5 ml/kg), hydrocortisone phosphate (25 mg/kg), Guthion (3 mg/kg), or acrolein (1·5 mg/kg).
† Figures are mean values ± S.E.M.

One mg actinomycin D/kg or 1 ml water was injected i.p. 30 min before treatment with acrolein, Guthion, or hydrocortisone. The animals were sacrificed at the times indicated in the table. The data show that actinomycin prevented the increase in liver glycogen and transaminase activity that was produced by hydrocortisone. This confirmed the observations of Greengard *et al.*[5] Actinomycin also prevented the increases in liver glycogen, alkaline phosphatase, and tyrosine transaminase activities that were produced by acrolein. The increase in liver glycogen that occurred at 3 hr after Guthion was not prevented by actinomycin and appears, therefore, to be produced through a different mechanism.

Table 4 shows that pretreatment with actinomycin D or other inhibitors of protein synthesis failed to prevent the increase in liver tyrosine transaminase activity that occurred 1 hr after Guthion. This suggests that the increased activity of this enzyme after Guthion is not the result of an increased rate of enzyme synthesis. Pretreatment with actinomycin, puromycin, or ethionine did not alter the anticholinesterase or the hyperglycemic action of Guthion.

Endocrine regulation of the biochemical effects of Guthion

The depletion of adrenal ascorbic acid that occurred after Guthion had at first suggested that the effects of Guthion on the liver might be mediated through the adrenals, as had been demonstrated for induction of liver enzymes by acrolein.[2] However, the results of the experiments on animals pretreated with actinomycin indicated that the effects on the liver produced by acrolein and Guthion occur through different mechanisms. In order to obtain additional information concerning the mechanism of the effects of Guthion, experiments were performed on rats which had been bilaterally adrenalectomized, hypophysectomized, or adrenal demedullated.

TABLE 4. EFFECT OF PRETREATMENT WITH INHIBITORS OF PROTEIN SYNTHESIS ON BIOCHEMICAL RESPONSES OF RATS AT ONE HOUR AFTER GUTHION

Treatment*	No. of animals	Brain cholinesterase (μlCO_2/50 mg/10 min)	Blood glucose (mg/100 ml)	Liver glycogen (mg/100 g)	Liver tyrosine transaminase (μg/mg/hr)
Water + propylene glycol	6	101 ± 2·2†	97 ± 3·4	361 ± 95	11·7 ± 2·9
Water + Guthion	5	50 ± 3·3	153 ± 12·0	203 ± 61	27·3 ± 1·5
Actinomycin D + propylene glycol	6	99 ± 1·2	85 ± 4·9	106 ± 41	14·5 ± 0·9
Actinomycin D + Guthion	6	42 ± 2·2	175 ± 5·8	218 ± 57	22·2 ± 0·9
Puromycin + propylene glycol	6	102 ± 3·0	93 ± 1·5	325 ± 37	23·7 ± 2·4
Puromycin + Guthion	5	44 ± 2·1	170 ± 12·1	357 ± 92	35·8 ± 4·8
Ethionine + propylene glycol	3	98 ± 2·3	84 ± 2·1	190 ± 29	12·7 ± 0·9
Ethionine + Guthion	4	34 ± 2·2	148 ± 3·7	603 ± 156	28·2 ± 1·4

* Actinomycin (1 mg/kg), puromycin dihydrochloride (35 mg/kg), and ethionine (100 mg/kg) were injected 30 min before Guthion (3 mg/kg) or propylene glycol (0·5 ml/1 g).

† Figures are means ± S.E.M.

Each surgical group and a group of intact rats of the same age were fasted and subdivided into three treatment groups as follows: (a) controls which received injections of propylene glycol, (b) subjects injected with 3 mg Guthion/kg and sacrificed 1 hr later, and (c) subjects injected with 3 mg Guthion/kg and sacrificed 5 hr later. The results of assays on the tissues of these rats are summarized in Figs. 2 and 3. The bars represent the mean values obtained in assays on five or six animals for each treatment group.

Figure 2 shows that the surgical treatments did not affect the degree of inhibition of cholinesterase by Guthion. Total adrenalectomy or adrenal demedullation failed to

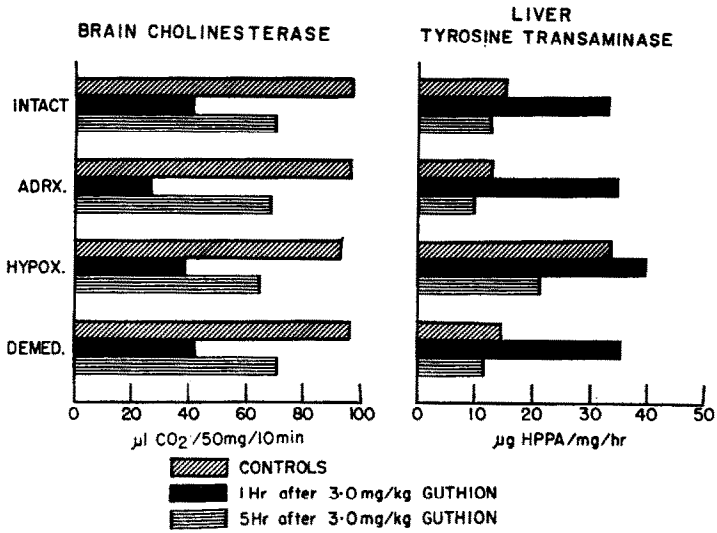

FIG. 2. Role of the pituitary–adrenal axis in controlling the effects of Guthion on brain cholinesterase and liver transaminase. Each bar represents the mean of assays on five or six animals. Abbreviations are as follows: ADRX (bilaterally adrenalectomized); HYPOX (hypophysectomized); DEMED (adrenal demedullated); HPPA (*p*-hydroxyphenyl pyruvic acid).

reduce the increment in liver tyrosine transaminase activity that occurred at 1 hr after Guthion. For unknown reasons, the tyrosine transaminase activity in the hypophysectomized control group was twice as high as that for any of the other controls. Comparison of the 1-hr and 5-hr sacrifice groups suggests, however, that hypophysectomy does not prevent the effect of Guthion on tyrosine transaminase at 1 hr after injection.

Figure 3 shows that total adrenalectomy and adrenal demedullation prevented the elevation of blood glucose at 1 hr after Guthion. Hypophysectomy did not prevent this response. From these data it appears that Guthion is capable of eliciting a hyperglycemic response as long as there is an intact adrenal medulla. Deposition of liver glycogen after Guthion was prevented in all three groups of surgically altered rats. Increased glycogen deposition after Guthion appeared to be dependent upon the normal function of both the adrenal medulla and the adrenal cortex.

Dynamic aspects of the effect of Guthion on blood glucose and liver glycogen

The results of the experiments on adrenalectomized and adrenal demedullated rats suggested that the elevation of blood glucose after Guthion might be accounted for on the basis of epinephrine-stimulated glycogenolysis in the liver. However, in several

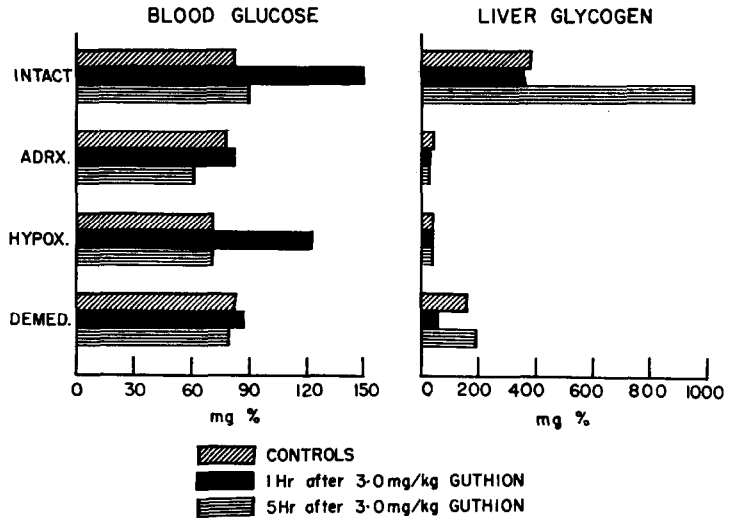

FIG. 3. Role of pituitary–adrenal axis in controlling the effect of Guthion on blood glucose and liver glycogen. Each bar represents the mean of assays on five or six animals. Abbreviations are as follows: ADRX (bilaterally adrenalectomized); HYPOX (hypophysectomized); DEMED (adrenal demedullated).

experiments the total glycogen content of the livers of fasted rats was scarcely adequate to account for the hyperglycemia at 1 hr after injection of Guthion and certainly could not account for the increased liver glycogen at 5 hr after injection. It was felt, therefore, that a more thorough study of the changes that occurred in carbohydrate metabolism during the first hour after Guthion might provide more information concerning the dynamic aspects of these effects. Lactic acid produced from muscle glycogen represents another potential precursor for the increased liver glycogen. Therefore, measurements of blood lactic acid and the glycogen content of skeletal muscle (femoral) were included. Injections of 3 mg Guthion/kg were made at appropriate intervals so that at the end of the 20-hr fast period the times between injection and sacrifice were 7, 15, 30, 60, and 120 min. The results of measurements made on these animals are summarized in Table 5.

At 7 min after injection of Guthion, brain cholinesterase activity was 75 per cent inhibited. The symptoms observed at this time were characteristic of poisoning by organophosphate insecticides and included muscular tremors and convulsions. These symptoms persisted for 15 to 30 min after injection. Blood lactic acid began to increase at 7 min after injection and reached its highest level after 30 min. The rise in lactic acid was accompanied by a decrease in muscle glycogen. Mean liver glycogen levels were lower than controls at 7, 15, and 30 min, but the variability of these values was relatively large. The maximal increase in blood glucose did not occur until 60 min after injection.

TABLE 5. BIOCHEMICAL EFFECTS IN RATS DURING THE FIRST 2 HOURS AFTER INJECTION OF 3 mg GUTHION/kg

Time after injection (min)	No. of animals	Glycogen (mg/100 g)		Blood (mg/100 ml)		Enzymes	
		Liver	Muscle	Lactate	Glucose	Liver TT (μg/mg/hr)	Brain CHE (μl CO_2/50 mg/10 min)
0 (controls)	12	130 ± 30*	243 ± 8	16·0 ± 1·0	85·7 ± 3·1	17·0 ± 2·8	94·0 ± 1·8
7	6	25 ± 5	209 ± 24	18·8 ± 1·1	85·4 ± 3·1	19·7 ± 2·7	21·3 ± 0·7
15	5	40 ± 16	194 ± 12	23·1 ± 2·0	89·1 ± 3·4	20·0 ± 2·2	31·6 ± 0·4
30	5	20 ± 6	221 ± 19	26·2 ± 2·2	115·9 ± 9·2	21·0 ± 3·4	31·0 ± 1·9
60	5	99 ± 41	158 ± 22	22·9 ± 1·4	146·8 ± 7·1	38·2 ± 2·0	35·0 ± 1·2
120	10	844 ± 97	171 ± 14	11·5 ± 0·6	113·6 ± 4·7	19·7 ± 1·8	51·8 ± 1·5

* Figures are means ± S.E.M. Values that differ significantly ($P < 0{\cdot}01$) from the control means are underlined.

At 120 min after injection the blood glucose levels were returning toward normal, and blood lactic acid was below the control values. Muscle glycogen remained low, and liver glycogen was greatly increased. Tyrosine transaminase activities of the liver were significantly increased only at the 60-min interval.

DISCUSSION

This investigation has shown that two different types of toxic stressors, acrolein (an irritant) and Guthion (a cholinergic organophosphate insecticide), will under certain conditions cause an increase in the activity of rat liver tyrosine-α-ketoglutarate transaminase and an increased deposition of liver glycogen. The mechanisms responsible for these two effects differ, however. For acrolein both the increased liver glycogen and the increased tyrosine transaminase activities appear to be mediated through hypersecretion of adrenocorticoids. Acrolein-induced elevations of liver glycogen and tyrosine transaminase activities were prevented by actinomycin D, presumably through inhibition of adaptive enzyme synthesis. These findings are consistent with reports of studies with exogenous glucocorticoids which suggest that these hormones produce their glucogenic and glycogenic actions through the stimulation of adaptive enzyme synthesis.[4–6]

The increase in tyrosine transaminase activity that was produced by Guthion did not appear to involve inductive synthesis of this enzyme. Thus, pretreatment with actinomycin, puromycin, and ethionine, which act to inhibit enzyme synthesis at different levels in the protein synthesis chain,[13, 14] failed to prevent the increase in tyrosine transaminase. It appears from these studies that the elevation of tyrosine transaminase after Guthion is more closely associated with inhibition of esterase activity than with any of the other parameters studied in this investigation. This is suggested by the experiments on bilateraly adrenalectomized rats in which the only responses to Guthion that were not eliminated were the increased hepatic tyrosine transaminase and brain cholinesterase inhibition. Since certain organophosphates are known to inhibit some proteolytic enzymes,[15] it seems possible that the degradation of the tyrosine transaminase enzyme in the liver might be inhibited by Guthion. Tyrosine transaminase has a relatively short half-life[16, 17] and, if its degradation were prevented, a net accumulation of enzyme would be expected if biosynthesis of the enzyme continued at a normal rate. Substrate induction of the synthesis of tyrosine transaminase in livers of rats fed high concentrations of tyrosine has also been demonstrated.[13] If substrate induction is involved in the response to Guthion, this would imply that the insecticide affects other pathways of tyrosine metabolism, permitting an accumulation of the amino acid. Additional studies are required to explain the mechanism of the effect of Guthion on tyrosine transaminase.

The hyperglycemic response to Guthion in fasted rats might be explained in part by inhibition of cholinesterase at neuroeffector sites in the adrenal medulla, leading to hypersecretion of epinephrine which stimulates the breakdown of residual liver glycogen to glucose. The results obtained in this study suggest, however, that other factors may contribute to hyperglycemia. The increased blood lactic acid resulting from increased muscular contraction during the tremors and convulsions produced by Guthion could serve as a precursor for gluconeogenesis. The increased glycogen deposition that occurs at 5 hr after Guthion might be accounted for on the basis of a clearance of lactic acid and glucose from the blood and the synthesis of these precursors

into glycogen in the liver. This latter step appears to require the functional integrity of the pituitary–adrenal cortex axis, since liver glycogen deposition failed to occur in the presence of hyperglycemia in Guthion-treated hypophysectomized rats.

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