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BIOCHEMICAL CHANGES IN RAT LIVER AFTER ACUTE BERYLLIUM POISONING

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Abstract—Rats were injected intravenously with 0·75 mg Be/kg and the effects upon liver metabolism were investigated within the first 24 hr. Poisoned animals showed an impaired loss of liver glycogen during fasting. Studies upon the incorporation of labelled glucose into liver glycogen, the ability of poisoned livers to resynthesize glycogen and determination of the concentration of glucose-6-phosphate failed, however, to establish a relationship between changes in glycogen metabolism *in vivo* and the highly specific inhibition of phosphoglucomutase by beryllium *in vitro*. Microsomal, lysosomal and mitochondrial enzyme systems were little affected. Therefore, no specific action of beryllium was found and though a very powerful necrogenic agent, beryllium differs markedly from other hepatotoxins.

EXPLANATIONS of the mechanism of cellular and tissue injuries have been sought by many studies on the effect of many substances on the liver *in vivo*. This tissue has proved attractive because a considerable part may be damaged or even removed without killing the animal. Many substances damage the liver: for instance carbon tetrachloride, thioacetamide, dimethylnitrosamine, ethionine etc., and the effects they have on liver cell function has been studied a great deal (for review cf. McLean, *et al.*[1] and Magee).[2] However, for some of the compounds there is considerable evidence that the compound is metabolized and the compound administered is not the toxic agent to the liver cell. For carbon compounds in particular, metabolism must be considered a possibility. Indeed the unique susceptibility to damage by such compounds may be due to the ability of the liver to metabolize foreign compounds. It therefore seemed attractive to study the action of beryllium, a compound which cannot be metabolized, on the biochemistry of the liver. Intravenous injection of soluble beryllium compounds causes severe liver necrosis of the midzonal type.[3,4] Death 2 or 3 days later is preceded by biochemical disturbance which may be attributed to the progressive destruction of liver tissue.[5] In the light of new knowledge it seemed profitable to re-examine the biochemical basis of liver necrosis produced by the intravenous injection of beryllium salts.[60]

At a molecular level beryllium ions have been shown to inhibit two enzymes, alkaline phosphatase and phosphoglucomutase.[6,7] An earlier observation that liver glycogen concentrations remain unexpectedly high during fasting in animals poisoned with beryllium has been re-examined in the light of the *in vitro* studies on phosphoglucomutase.

The experiments to be described represent a more critical analysis of the effects of intravenous beryllium salts upon the liver of rats. A preliminary account of some of the findings has been reported.[8]

MATERIAL AND METHODS

Animals

Male albino rats (180–200 g) of the Porton strain, kept on dietM .R.C. 41B[9] were used.

Special chemicals

The following chemicals were obtained from the sources stated: disodium ethylene diamine tetraacetate (EDTA disodium salt); glucose-6-phosphoric acid, disodium salt; glucose-1-phosphoric acid, disodium salt; DL-iso-citric acid, trisodium salt; anthrone; sodium fumarate (British Drug Houses, Poole, Dorset); sodium pyruvate; sodium glutamate (Koch-Light Laboratories Ltd, Colnbrook, Bucks.); Triton X-100 (C. Lennig & Co., London); ATP disodium salt; glucose oxidase, fungal crude; (Sigma Chemical Co., London); peroxidase; glucose-6-phosphate dehydrogenase, phosphoglucomutase, NADP (Boehringer London, Ealing); PPO (2,5 Diphenyl-oxazole); Dimethyl POPOP [1,4 bis-2-(4-methyl-5-phenyloxazolyl)-benzene]; Cab-O-Sil thixotropic gel powder; hydroxide of hyamine 10-X (Packard Instruments, Wembley); bovine serum albumin fraction V (Armour Laboratories, London); Zeocarb 225, De Acidite M-IP (The Permutit Co., London); [U-^{14}C]D-glucose, sp. act. 3·9 mc/m-mole; [U-^{14}C]L-leucine, sp. act. 6·6 mc/m-mole (The Radiochemical Centre, Amersham, Bucks).

Methods, general

0·75 mg Be/kg body wt. was injected into a tail vein between 9.00 and 10.00 a.m. Control animals were injected with 0·9% NaCl. The animals had access to water but not to food after 9 a.m. At different times after beryllium the animals were killed by decapitation. The liver was quickly removed, rinsed with distilled water, superficially dried, weighed and further prepared as described below.

The LD_{50} for the 200 g male rats used, determined according to Weil[10] was 0·44 mg Be/kg (fiducial limits 0·32–0·61).

Gross liver composition was determined according to McLean and McLean[11]. Calcium was extracted from fat free dry liver pieces with HCl and determined by flame-photometry.

Liver glycogen. Pieces of liver (1·0–1·5 g) were digested in hot KOH 60 per cent w/v. The glycogen was isolated by double precipitation with ethanol and estimated using anthrone reagent[12] with the modification that the ice-cold anthrone reagent was added to the sample cooled in ice. The colour was then developed by heating for exactly 6 min in a boiling water bath. Results were calculated as g glycogen/100 g of fresh liver, using glucose as standard.

Incorporation of [^{14}C]*-glucose into liver glycogen.* At different intervals after beryllium, rats were given 2 μc [U-^{14}C]-glucose intravenously and killed 20 or 50 min later. The glycogen from two different parts of the liver was isolated as described above and suspended in scintillation liquid jelly (4% Cab-O-Sil in dioxan three parts, toluene one part, cellosolve three parts, PPO 1%, POPOP 0·5%, naphthalene 8%). Counting

was done in a Tri-Carb Scintillation Counter (Packard). A number of counts/min at least ten times above background was collected and standards, corresponding to a fifty-fold dilution of the actual injected amount of counts/min into the animal were run with each assay. No checks for quenching were made, and any variation due to this is included in the standard errors.

Specific activity of blood glucose. At different times after beryllium, rats were given 6μc[U-^{14}C]-glucose intravenously and killed 50 min later. Blood samples were deproteinized with zinc sulphate–NaOH.[13] Glucose was estimated by the modified method[14] of Huggett and Nixon.[15] Samples of deproteinized extract were deionized as described[16] with the modification that the columns were composed of equal parts of Zeocarb 225 (hydrogen form, 14–52 mesh) and De-Acidite M-IP(chloride form 14–52 mesh). After concentrating, the glucose was further purified by paper chromatography on fenestrated[17] Whatman No. 54 paper with butanol–water (85:15) for 48 hr at 25°. After location of the glucose with the aid of markers it was eluted with water and estimated as above. Radioactivity was determined by liquid scintillation counting as described before.

Determination of glucose-6-phosphate and glucose-1-phosphate. In these experiments rats were stunned by a blow on the head and pieces of liver were removed within 10–15 sec, dropped into liquid nitrogen and, whilst still frozen, homogenized in 6% (w/v) perchloric acid with a Nelco homogenizer. The amount of glucose-6-phosphate was determined in extracts as described by Hohorst *et al*[18] tris HCl buffer being used. Known amounts of glucose-6-phosphate were added to each liver extract after the original reaction had stopped and results were calculated using these internal standards.

In a few experiments attempts were made to determine glucose-1-phosphate as described by Tarnowski *et al*[19]:

Incorporation of [^{14}C]-*leucine into liver protein.* Two, 6 and 24 hr after beryllium rats were injected with 1μc[U-^{14}C]-leucine intravenously and killed 15 min later. At 36 hr after beryllium, the animals could only be injected intraperitoneally and were then killed 30 min later. The livers were homogenized in a Nelco homogenizer in 10% (w/v) trichloroacetic acid and the protein isolated with a procedure similar to that described by Villa-Trevino *et al.*[20] Weighed aliquots of the dry protein powder were dissolved in hyamine and counted in toluene with 0·6% PPO in a Packard Scintillation counter. After counts/min at least ten times over background had been recorded, an internal standard was added and the efficiency was determined for each individual sample.

Plasma isocitric dehydrogenase. Rats injected with beryllium were bled under ether anaesthesia from the carotid artery and plasma isocitric dehydrogenase was estimated as described by McLean and McLean.[11]

Respiration of liver mitochondria. Liver homogenates (10% w/v) were prepared in 0·3 M ice-cold sucrose with the modified[21] homogenizer described by Aldridge *et al.*[22] with a difference in diameter between pestle and tube of 0·02 in. and revolving at 1100 rev/min. A mitochondrial fraction was isolated as described by Aldridge.[23] Oxygen uptake by 0·3 ml of the mitochondrial fraction (average protein content, 4 mg) was measured in a Warburg apparatus at 37°, gas phase air, with the medium and substrate described by Aldridge and Stoner.[24] Readings were taken at 5 or 10 min. intervals and the results calculated in terms of μl O_2 consumed/min/mg protein or μl O_2 consumed/min/liver with the method given by Aldridge *et al.*[25]

TABLE 1. LIVER WEIGHT AND GROSS LIVER COMPOSITION AFTER INJECTION OF BERYLLIUM

	6 hr			24 hr		
	Control	Beryllium	*P*	Control	Beryllium	*P*
Liver weight g	8·5 ± 0·1 (40)	8·9 ± 0·1 (40)	<0·02	6·4 ± 0·1 (40)	7·6 ± 0·1 (40)	<0·01
Water, g/kg fat-free dry liver	2608 ± 42 (6)	2613 ± 67 (6)	⩾0·1	2700 ± 42 (6)	2890 ± 70 (6)	<0·1
Lipid, g/kg fat-free dry liver	75·3 ± 8·4 (6)	71·0 ± 5·9 (6)	⩾0·1	106 ± 18 (6)	141 ± 9 (6)	<0·2
Protein, g/total liver	1·60 ± 0·04 (32)	1·61 ± 0·04 (32)	⩾0·1	1·32 ± 0·04 (32)	1·44 ± 0·05 (32)	<0·1
DNA, mg/total liver	8·0 ± 0·5 (6)	7·4 ± 0·3 (6)	⩾0·1	7·1 ± 0·6 (6)	8·1 ± 0·5 (6)	⩾0·1
Calcium, μmole/g fat-free dry liver	—	—	—	4·2 ± 0·2 (6)	11·5 ± 3·0 (6)	<0·1

Rats (180–200 g) were injected intravenously with 0·75 mg Be/kg or saline.

Glucose-6-phosphatase was estimated by the liberation of P_i in a medium containing 25 mg homogenized liver, 3 mM glucose-6-phosphate, 7 mM histidine buffer pH 6·5 and 1 mM EDTA. Inorganic P was determined according to Berenblum and Chain[26] and protein by the method of Lowry *et al.*[27]

Acid phosphatase. Activity of free acid phosphatase was determined at 37° in a medium containing 100 mg of homogenized liver, sodium citrate–citric acid buffer 0·1 M, pH 5·8 and β-glycerophosphate 0·05 M. Release of the bound enzyme was obtained by pre-incubating one part of 25% liver-homogenate for 30 min in one part of sucrose 0·3 M containing Triton-X100 0·4% (v/v).[28]

Liver DNA was estimated in suitable amounts of liver homogenate according to the modified phenylamine method described by Burton.[29] Commercial DNA (Mann Research Laboratories, New York) was used as reference substance.

Treatment of results. Unless otherwise stated, all results in the tables are given as mean values with S.E.M. The number of animals used in each group is given in brackets. Significance levels were estimated according to Student's *t*-test. Degrees of freedom (d.f.) were estimated in the following way: if $n_1 = n_2$, d.f. $= n - 1$[30]; if $n_1 > n_2$, d.f. $= n_2 - 1$. $P = 0{\cdot}05$ or less was regarded as significant.

RESULTS

In most of our experiments rats were given nearly twice the LD_{50} of beryllium. For up to 30 hr the animals showed no abnormalities in general behaviour and body temperature as measured in the colon by a thermocouple. Rats allowed to survive longer invariably died within 48–72 hr in poor condition with staring fur, a marked drop of body temperature and terminal jaundice. Livers of such animals were conspicuously damaged.[3]

In most instances the liver was seen to be swollen, pale and yellow with a dull cut surface 24 hr after beryllium. Liver composition is given in Table 1. The slight differences in tissue water and calcium observed in the liver 24 hr after beryllium were not significant, but a significant rise in isocitric-dehydrogenase activity in the plasma was observed at 6 hr already (Table 2).

TABLE 2. PLASMA ISOCITRIC-DEHYDROGENASE ACTIVITY AT DIFFERENT TIMES AFTER BERYLLIUM

Time after beryllium	Plasma isocitric-dehydrogenase activity (mμmole/ml/min)		
	Geometric mean	Log of mean ± S.E.M.	*P*
2 hr Control	1·35 (6)	0·130 ± 0·054	
Beryllium	2·37 (6)	0·375 ± 0·082	<0·10
6 hr Control	1·55 (6)	0·191 ± 0·043	
Beryllium	13·03 (6)	1·115 ± 0·107	<0·01
24 hr Control	1·68 (6)	0·226 ± 0·099	
Beryllium	235·0 (6)	2·371 ± 0·179	<0·01

The results are statistically evaluated as indicated in ref. 11.

Changes in liver glycogen

Earlier investigations had shown that after a 24 hr fast, beryllium-poisoned rats retained more glycogen in their livers than untreated controls. When the fasting period was extended up to 72 hr, the controls increased their liver glycogen progressively

whereas in the poisoned animals it continued to fall.[5] Liver glycogen was therefore determined at different intervals in the period from 0 to 30 hr after beryllium. The rate of disappearance up to 8 hr was comparable to that in fasting controls (Fig. 1). From 8 hr however, the rate of disappearance in beryllium-treated rats decreased and at 30 hr the concentration of the liver glycogen, though small, was ten times higher

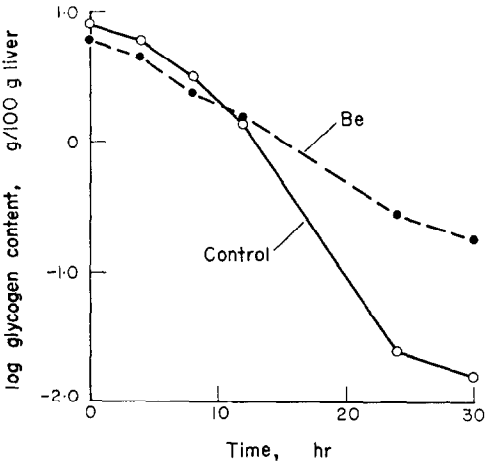

FIG. 1. Liver glycogen at various times after 0·75 mg Be/kg.

than in controls. Poisoned rats, however, could resynthesize glycogen from glucose 6 and 10 hr after beryllium (Table 3).

TABLE 3. RISE IN LIVER GLYCOGEN IN RATS AFTER BERYLLIUM

Time after beryllium when killed (hr)	Time of fasting before injection of glucose	Liver glycogen (g/100 g liver)		
		Controls	Beryllium	*P*
6	29	1·39 ± 0·12 (9)	1·56 ± 0·16 (9)	⩾0·1
10	33	1·72 ± 0·27 (8)	1·45 ± 0·16 (9)	⩾0·1

Liver glycogen was depleted by fasting rats for 24 hr. The animals were then injected with beryllium 0·75 mg/kg or saline. Five or nine hours later the animals were given 1 g of glucose by intraperitoneal injection and killed 60 min later.

Further studies on glycogen metabolism were undertaken using labelled glucose. After different periods of fasting when the animal had different concentrations of glycogen in their livers, [U-^{14}C]-glucose was injected intravenously and the animals killed 20 or 50 min later. The incorporation of the labelled glucose into glycogen was determined. In control animals the counts/min incorporated in 20 min into the glycogen of 1 g of liver was largely independent of the concentration of glycogen in the liver. For 50 min incorporation of [U-^{14}C]-glucose a four-fold increase in incorporation was obtained after prolonged fasting when the glycogen content was 1·5 g/100 g liver or less (Fig. 2). At 6 hr after beryllium and 50 min after administration of [U-^{14}C]-glucose, there was about 60 per cent more labelled glucose in the liver glycogen than

in the corresponding controls (Table 4). This difference increased further with time after treatment with beryllium. Changes earlier than at 6 hr after beryllium were not detected, even when rats were given 5 mg Be/kg, i.e. about ten times the LD_{50}.

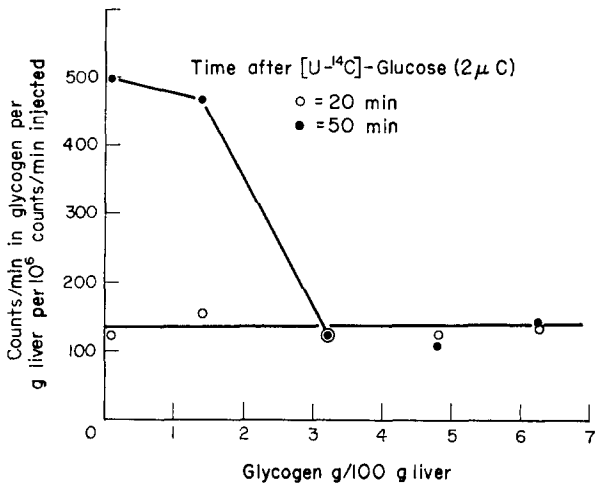

FIG. 2. Incorporation of [U-^{14}C]-glucose into liver glycogen of control rats. Abscissa: g glycogen per 100 g liver, data obtained from the experiments shown in Fig. 1. Points from right to left represent a fasting period from 4, 6, 8, 12 and 24 hr. Ordinate: counts/min per glycogen of 1 g of liver per 10^6 counts/min injected in control rats, animals killed 20 or 50 min after administration of glucose. It is shown that the activity incorporated into the glycogen of 1 g of liver is largely independent of the amount of glycogen present in the liver.

These changes in incorporation of [U-^{14}C]-glucose into liver glycogen could be due to an increased specific activity of the blood glucose in the treated rats but no significant changes in blood sugar level and specific activity of blood glucose have been found 50 min after injection of [U-^{14}C]-glucose 6 and 24 hr after beryllium (Table 5).

TABLE 4. INCORPORATION OF [U-^{14}C]-GLUCOSE INTO LIVER GLYCOGEN AFTER BERYLLIUM

Time after beryllium (hr)	Counts per min in glycogen from 1 g of liver/10^6 counts per min injected		
	Control	Beryllium	
(a) Animals killed 20 min after injection of [U-^{14}C]-glucose			
2	260 (2)	234 (2)	
4	133 (2)	133 (2)	
6	110 (3)	131 (2)	
8	124 (2)	149 (2)	
12	205 (2)	348 (2)	
24	123 (2)	370 (2)	
(b) Animals killed 50 min after injection of [U-^{14}C]-glucose			
2	337 (2)	235 (2)	
4	140 (2)	180 (3)	
6	124 ± 11 (10)	200 ± 26 (11)	$P < 0{\cdot}05$
8	125 ± 12 (9)	312 ± 61 (10)	$P < 0{\cdot}02$
12	470 (2)	890 (2)	
24	520 ± 98 (7)	2090 ± 620 (7)	$P < 0{\cdot}05$

TABLE 5. BLOOD SUGAR LEVEL AND SPECIFIC ACTIVITY OF BLOOD GLUCOSE IN FASTED RATS KILLED 6 AND 24 HOURS AFTER 0·75 mg Be/kg OR SALINE

	6 hr			24 hr		
	Control	Beryllium	*P*	Control	Beryllium	*P*
mg Glucose/100 ml blood	118 ± 6 (6)	104 ± 7 (6)	>0·1	96 ± 11 (6)	96 ± 6 (6)	—
Counts/min per μg glucose per 10^6 counts/min injected	2·4 ± 0·2 (6)	2·7 ± 0·4 (6)	⩾0·1	4·5 ± 0·7 (6)	4·2 ± 0·7 (6)	⩾0·1

These results may therefore reflect a disturbed steady-state between glycogen synthesis and glycogen breakdown, the equilibrium shifted towards an increased synthesis or towards an impaired glycogenolysis.

An alteration of glycogen metabolism after beryllium could possibly be due to an inhibition of phosphoglucomutase since beryllium has been shown to be a very powerful inhibitor of this enzyme *in vitro*.[7] An attempt was therefore made to determine the actual level of glucose-6-phosphate and glucose-1-phosphate in the liver of poisoned rats. In Table 6 are the results given for glucose-6-phosphate. Measurable

TABLE 6. CONCENTRATION OF GLUCOSE-6-PHOSPHATE 6 AND 24 HOURS AFTER BERYLLIUM

	Glucose-6-phosphate (μmole/g liver)		
	Control	Beryllium	*P*
6 hr	0·42 ± 0·03 (7)	0·36 ± 0·04 (7)	<0·4
24 hr	0·15 ± 0·02 (12)	0·29 ± 0·05 (12)	<0·01

amounts of glucose-1-phosphate in normal animals or in rats 2, 5 and 24 hr after beryllium were not found, even though recovery of known amounts of glucose-1-phosphate added *in vitro* to liver homogenate was complete. It is therefore not possible to say whether beryllium does or does not lead to an increased concentration of glucose-1-phosphate in the liver.

Properties of mitochondria from livers of poisoned animals

Since changes in glycogen metabolism and the activity of plasma isocitric dehydrogenase were found in the liver 6 hr after beryllium injection and were pronounced at 24 hr, mitochondria were isolated at these times from the livers of normal and poisoned rats and their respiration determined using a manometric technique. Six hours after poisoning, the same amount of mitochondrial protein was present in the total liver of control and beryllium treated rats. No difference in oxygen uptake per mg mitochondrial protein was found (Table 7). Twenty-four hours after poisoning there was less protein in the mitochondrial fraction in the total liver of normal animals, whereas a similar fraction from beryllium-treated animals was the same as at 6 hr. The specific activity of the mitochondria (μl O_2/min/mg protein) isolated from normal liver was significantly higher after 24 hr fasting than it was after 6 hr; no significant increase in activity was observed in the mitochondrial fraction from beryllium-poisoned livers. No difference could be detected between control and poisoned rats when μl O_2/min/equivalent total liver was compared. Moreover, 30 μM 2,4 dinitrophenol markedly increased oxygen uptake in all four experimental groups (Table 8); taking the degree of increase as a criterion of mitochondrial damage,[22] no evidence for this in beryllium poisoning has been found.

Enzymic activities present in the microsomal fraction from livers of poisoned animals

Inhibition of protein synthesis occurs quite early after intoxication with several hepatotoxins. A slightly decreased capacity to incorporate amino acids into liver protein was detected 24 hr after beryllium, but no significant changes could be found

TABLE 7. ACTIVITY OF MITOCHONDRIA ISOLATED FROM LIVER AFTER BERYLLIUM

	mg Mitochondrial protein/total liver	μl O_2 Uptake/min/mg protein		μl O_2 Uptake/min/total liver	
		Glutamate	Pyruvate/fumarate	Glutamate	Pyruvate/fumarate
Control, 6 hr	245 ± 8	0·332 ± 0·015	0·293 ± 0·012	81·5 ± 5·4	71·8 ± 5·0
Beryllium, 6 hr	237 ± 12	0·380 ± 0·023	0·345 ± 0·032	90·2 ± 7·1	82·0 ± 8·8
	$P \geqslant 0{\cdot}1$	$\geqslant 0{\cdot}1$	$\geqslant 0{\cdot}1$	$\geqslant 0{\cdot}1$	$\geqslant 0{\cdot}1$
Control 24 hr	187 ± 11	0·396 ± 0·015	0·400 ± 0·018	73·6 ± 3·1	75·4 ± 4·5
Beryllium, 24 hr	221 ± 8	0·354 ± 0·013	0·335 ± 0·019	78·4 ± 4·8	74·3 ± 5·6
	$P < 0{\cdot}02$	$< 0{\cdot}1$	$< 0{\cdot}1$	$\geqslant 0{\cdot}1$	$\geqslant 0{\cdot}1$

All results represent the average from six individual experiments. The t-test applied to the comparison between the specific activity of the controls at 6 and 24 hr gives a $P < 0{\cdot}05$ for both substrates.

TABLE 8. PROPERTIES OF MITOCHONDRIA FROM RAT LIVER AFTER BERYLLIUM

	Glutamate as substrate			Pyruvate/fumarate as substrate		
	Geometric mean	log ± S.E.M.	P	Geometric mean	log ± S.E.M.	P
Control, 6 hr	5·74 (6)	0·759 ± 0·019		4·47 (6)	0·650 ± 0·026	
Beryllium, 6 hr	4·61 (6)	0·664 ± 0·049	$P > 0{\cdot}1$	3·52 (6)	0·547 ± 0·053	$P > 0{\cdot}1$
Control, 24 hr	5·43 (6)	0·753 ± 0·010		4·12 (6)	0·615 ± 0·021	
Beryllium, 24 hr	5·78 (6)	0·762 ± 0·018	$P > 0{\cdot}5$	4·04 (6)	0·606 ± 0·027	$P > 0{\cdot}5$

The degree of stimulation of oxygen uptake by the addition of 30 μM 2,4 Dinitrophenol (the ratio O_2 uptake with DNP/O_2 uptake without DNP) was calculated for each liver and the results are given as geometric means with S.E.M., six results being used in each group.

at 2 or 6 hr (Table 9). By 36 hr the decrease in incorporation of amino acids was considerable; at this time the liver was grossly damaged, necrotic and haemorrhagic. Glucose-6-phosphatase in the liver behaved in a similar manner: 6 hr after beryllium, neither the specific activity of the enzyme nor its total activity per liver was different

TABLE 9. INCORPORATION OF [U-^{14}C] LEUCINE INTO LIVER PROTEIN AT DIFFERENT TIMES AFTER BERYLLIUM

Time after beryllium (hr)	dis/min [^{14}C]-leucine/mg liver protein/10^6 dis/min injected		
	Control	Beryllium	*P*
2	54 ± 3 (6)	55 ± 3 (6)	$\geqslant$0·1
6	59 ± 5 (7)	70 ± 8 (7)	$\geqslant$0·1
24	63 ± 1 (8)	56 ± 2 (8)	<0·05
36	95 ± 3 (8)	49 ± 7 (6)	<0·01

from controls (Table 10). After a 24-hr fast, the specific activity was increased in normal rats but remained the same as it had been at 6 hr in the beryllium-poisoned animals. The total activity per liver showed a trend towards increase in the control, whereas in the beryllium animals it had fallen. These findings are in striking contrast to the observed fall of about 50 per cent in the specific activity of the same enzyme only 6 hr after a single dose of CCl_4 (Reynolds[31] and Witschi, unpublished observations).

TABLE 10. GLUCOSE-6-PHOSPHATASE IN RAT LIVER AFTER BERYLLIUM

	Controls	Beryllium	*P*
A.6 hr			
Specific activity	0·035 ± 0·002 (7)	0·032 ± 0·002 (7)	$\geqslant$0·1
Total activity	64·7 ± 5·0 (7)	61·1 ± 2·3 (7)	$\geqslant$0·1
B.24 hr			
Specific activity	0·048 ± 0·003 (6)	0·031 ± 0·001 (6)	<0·01
Total activity	70·0 ± 5·4 (6)	54·4 ± 1·7 (6)	<0·05

Specific activity expressed as μmole P_i liberated/min/mg liver protein and total activity expressed as μmole P_i liberated/min/total liver of glucose-6-phosphatase 6 and 24 hr after beryllium.

Enzymic activities present in the lysosomal fraction from livers of poisoned animals

In the last few years it has also been suggested that lysosomal enzymes might play some part in toxic liver injury.[32] Free and total acid phosphatase per liver was determined 6 and 24 hr after beryllium in a system using pH 5·8 as suggested by Feuer *et al.*[33] the time of assay being limited to 10 min.[32] Six hours after beryllium, no changes were found in the ratio of free to total activity whereas at 24 hr the free activity was considerably increased in the beryllium-treated rats (Table 11). The total activity remained unchanged, in contrast to that described in acute CCl_4 poisoning.[34]

DISCUSSION

The administration of a wide variety of chemicals causes cellular necrosis in the liver of mammalian species (cf. for review McLean *et al.*[1], Rouiller[35]). For some compounds there is considerable evidence that the substance administered is not the

actual toxin[36] and for others it seems likely.[37] Indeed the possibility of such an activation of carbon compounds must always be considered. With this in mind beryllium salts as an experimental tool for the production of liver necrosis would seem to possess an advantage. There are, however, some features of the distribution and concentration of beryllium by the liver which can cause difficulties in the interpretation of any biochemical changes observed in preparations from the whole liver.

TABLE 11. ACID PHOSPHATASE IN RAT LIVER AFTER BERYLLIUM

	Controls	Beryllium	P
A.6 hr			
Total activity	17·3 ± 1·0 (14)	16·8 ± 0·7 (14)	⩾0·1
Free activity in % of total activity	25 ± 2 (14)	28 ± 2 (14)	⩾0·1
B.24 hr			
Total activity	16·6 ± 0·5 (6)	14·6 ± 0·8 (6)	>0·1
Free activity in % of total activity	22 ± 2 (6)	53 ± 4 (6)	<0·01

Results are expressed as μmole P_i liberated/min/total liver.

The liver is damaged by beryllium[3] when beryllium is quickly concentrated in it.[4,5,38] When beryllium is injected into rats as suspensions of particles of different sizes so that it is retained by organs other than the liver, all organs containing such beryllium are damaged (i.e. liver, spleen, lymph glands, bone marrow, lungs and pancreas).[4] Beryllium is therefore known to be toxic to many cells including the subcutaneous tissue and the selectivity of intravenous solutions of beryllium for the liver is due to its localization there. Cheng[4] using histochemical techniques showed that beryllium was first seen in the Kupffer cells and suggested that particles of insoluble beryllium phosphate were taken up by the reticulo-endothelial system of the liver. The original suggestion that beryllium was bound to plasma protein[5] has now been shown to be wrong.[39,40] It now seems likely that beryllium is associated in blood with phosphate.[40,41]

Other evidence indicates a diminished activity of the reticulo-endothelial system after administration of beryllium.[42,43] The simplest hypothesis to explain the above observations is that beryllium salts after injection form a colloidal beryllium phosphate in the blood and this is removed by the reticulo-endothelial cells of the liver. Beryllium is not therefore uniformly distributed in the liver and the distribution will vary with time after injection.[4] It is therefore not possible, when discussing any quantitative biochemical change in the liver, to differentiate between a small depression of the activity of the majority of cells and a large depression or complete removal of the activity of a few cells.

Six hours after the injection of a lethal dose of beryllium sulphate to rats, no large biochemical changes in the liver were found. At even 24 hr, when the concentration of beryllium is highest[3] focal necrosis develops[4] and changes in blood flow secondary to cell damage may be observed,[44] only a few biochemical changes were found. Beryllium therefore differs from other hepatotoxins which produce biochemical changes early after dosing (Table 12). Since beryllium induces a focal type of necrosis[3,4]

TABLE 12. METABOLIC CHANGES IN LIVER AFTER NECROGENIC DOSES OF SOME HEPATOTOXINS

	Protein synthesis (*in vivo*)	Glycogen	Fat accumulation	Glucose-6-phosphatase	Mitochondria	Free acid phosphatase
Carbon tetrachloride	Early failure[50]	Early loss[52]	Pronounced	Fall in specific activity[33]	Changes observed[56]	Increased after 24 hr[45]
Dimethylnitrosamine	Early failure[36]	Early loss[53]	Discrete			Decreased after 24 hr[45]
Pyrrolizidine alkaloids		Early loss[54]	None		Changes observed[57]	
Thioacetamide	Early failure[51]	Early loss[55]	None			Increased after 18 hr[45]
Beryllium	Only slight and late inhibition	Slight loss first, later retention	None	No fall in specific activity	No changes observed up to 24 hr	Increased after 24 hr

The term "early" implies events occurring in the first 6–10 hr after application of the toxic substance.

grossly impaired function in only a small proportion of liver cells would be masked by undamaged cells. If this is true, the onset of biochemical changes due to beryllium in the majority of liver cells occurs later than 24 hr after injection; that is, biochemical lesions due to beryllium do not occur gradually in all or most cells of the liver, but take place rather abruptly in one cell after another. However, the increase of free acid phosphatase 24 hr after beryllium is similar to that induced by carbon tetrachloride, dimethylnitrosamine and thioacetamide in doses causing maximal liver necrosis within 18–24 hr.[45] If an increase of free acid phosphatase is concerned rather with the aftermath of a necrotic process than with its initiation,[34] it remains to be explained why beryllium releases as much of this enzyme as the other compounds well before maximal beryllium necrosis.

The early changes in liver metabolism were a delayed loss of glycogen during fasting and an increased labelling of liver glycogen with glucose. These changes were apparent 6–8 hr after beryllium, when a slight increase in plasma isocitric-dehydrogenase suggested the beginning of liver cell damage. Phosphoglucomutase is involved in glycogen synthesis from glucose,[46] and is also inhibited by low concentrations of beryllium[7]. If phosphoglucomutase were involved in the changes in glycogen metabolism, then the steady state values of glucose-1-phosphate and glucose-6-phosphate should be changed. Attempts to verify this by determinations of glucose-6-phosphate and glucose-1-phosphate have failed. The amount of glucose-6-phosphate was increased 24 hr after beryllium; this could be due either to an inhibition of phosphoglucomutase, or to a diminished activity of glucose-6-phosphatase in the prenecrotic liver. Moreover, an increased incorporation of labelled glucose into liver glycogen has been found after prednisolone administration;[47] our findings reported in Table 4 might therefore not be due to a specific action of beryllium on glycogen pathways.

TABLE 13. SUMMARY OF RESULTS OBTAINED IN FASTED ANIMALS 24 HOURS AFTER BERYLLIUM OR SALINE

	Control %	Beryllium %
Fresh liver weight	75*	85*
Glucose-6-phosphate per liver	29*	72*
mg Mitochondrial protein per total liver	76*	93*
Specific activity of mitochondria:		
glutamate	120*	104
pyruvate/fumarate	136*	93
Protein synthesis† (*in vivo*)	117*	102
Protein synthesis (*in vivo*)	106	80
Specific activity of glucose-6-phosphatase	137*	100
Total activity of glucose-6-phosphatase	108	89

The results are calculated from results in previous tables as percentage of the corresponding value at 6 hr for control and beryllium treated rats respectively. All differences between beryllium and control animals are statistically significant ($P < 0.05$).

* Significantly different from the corresponding value at 6 hr.

† 100 per cent = measurement 2 hr after beryllium.

In some experiments it seemed as if the application of beryllium prevented rats from developing changes that occurred in normal animals during fasting (Table 13). For example the total amount of glucose-6-phosphate and mitochondrial protein

per liver fell far less in beryllium treated rats than in normal ones. On the other hand, normal animals increased quite considerably the specific activity of respiration of the mitochondrial fraction or of glucose-6-phosphatase, changes that were not observed in the beryllium treated ones. Possibly beryllium interferes with some mechanisms of adaptation and/or regulation. The fact that in normal rats the incorporation of [U-^{14}C] leucine into liver protein increases during 2–24 hr fasting, whereas the same is not true for beryllium treated animals (Table 9) is not inconsistent with this possibility.

No critical point of attack of beryllium upon liver metabolism has been detected and a biochemical explanation for its powerful necrogenic action remains to be found. It has been shown[48,49] that even a drastic depression of protein synthesis does not

TABLE 14. NECROSIS, FATTY LIVER AND EARLY INHIBITION OF PROTEIN SYNTHESIS

	Necrosis	Fatty liver	Inhibition of protein synthesis
Carbon tetrachloride[50]	+	+	+
Dimethylnitrosamine[58]	+	−	+
Ethionine[48]	−	+	+
Actinomycine-D[49]	−	−	+
Hydrazine[59]	−	+	−
Beryllium	+	−	−

necessarily lead to cell death and liver necrosis. Our experiments show that beryllium leads to severe liver necrosis without early inhibition of protein synthesis (Table 14).

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