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THE EFFECTS OF ADJUVANT-INDUCED ARTHRITIS ON THE LIVER METABOLISM OF DRUGS IN RATS

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Abstract—During a period of 15 days following induction of adjuvant arthritis in rats, the liver microsomal *N*-demethylase and $NADPH_2$-oxidase enzyme activity and levels of cytochrome P-450 are greatly reduced. This implies a reduced capacity for the oxidative metabolism of steroids and foreign compounds in adjuvant arthritic rats which should influence the toxicity and half-life of any administered compound. The ability of the liver to form β-glucuronide conjugates is also reduced. These effects on microsomal metabolism are associated principally with the onset of adjuvant arthritis rather than with delayed hypersensitivity to the injected tuberculin which occurs at the same time.

THE PRODUCTION of adjuvant-induced arthritis in rats was first described by Stoerck, Bielinski and Budzilovich[1] and Pearson.[2]

This polyarthritic syndrome, induced by sub-plantar injection of killed mycobacteria in liquid paraffin, is widely used in the routine testing of potential anti-inflammatory compounds.[3]

A number of studies have been concerned with immunological components of the syndrome,[2, 4, 5] while others have studied serum protein changes[6, 7] and the inflammatory response.[8, 9]

The present paper describes changes in liver microsomal drug-metabolising enzyme systems during the development of arthritic lesions in rats, together with histochemical and histological examination of the liver. Measurements have been made of the level and activity of components of microsomal oxidative electron transport systems and the ability of the liver to form glucuronide conjugates using *N*-acetyl-*p*-aminophenol (paracetamol). *N*-acetyl-*p*-aminophenol is conjugated by the liver at the hydroxyl group and excreted in the urine as sulphuric and glucuronic acid conjugates.

MATERIALS AND METHODS

Materials

Glucose-6-phosphate, NAD, NADP and $NADPH_2$ were obtained from The Sigma Chemical Company.

Animals

Young, adult, male rats of the Lodgemoor Wistar strain weighing between 150 and 170g were used in groups of six per cage and were maintained on Oxoid diet 41B and water *ad lib.*

Induction of arthritis

The arthritis was produced by a subcutaneous injection of 0·05 ml of a fine suspension of dead tubercle bacilli in liquid paraffin (5 mg/ml) into the right hind foot of the rats.

Injections into lymph nodes

Injections of 0·1 ml of an emulsion, prepared by homogenising dead tubercle bacilli in saline with an equal volume of liquid paraffin to give a final concentration of bacilli of 5 mg/ml, were made into the right cervical lymph node. The node was exposed by a ventral incision in animals anaesthetised with sodium methohexobarbitone. Control animals received similar injections of sodium methohexobarbitone.

Cotton pellet granuloma test

The granuloma was produced by a modification of the method of Winter and Porter.[10] Cellulose pellets weighing 10 mg were sterilised in an autoclave for 30 min at 15 lb pressure. Twelve pellets were inserted subcutaneously in the ventral region, six on either side, in each rat under light ether anaesthesia. Control animals were placed under similar ether anaesthesia.

Liver enzyme induction

Some of the adjuvant-induced arthritic and control rats received i.p. injections of sodium phenobarbitone (100 mg/kg) on days 8 and 13 of the test, before being killed on days 10 and 15 respectively.

Preparation of tissues

The animals were killed by cervical fracture on days 5, 10 or 15 after induction of adjuvant arthritis and blood samples were removed by cardiac puncture. For one of the experiments (see Fig. 1) the animals were killed on days 4, 8, 12 and 16 after

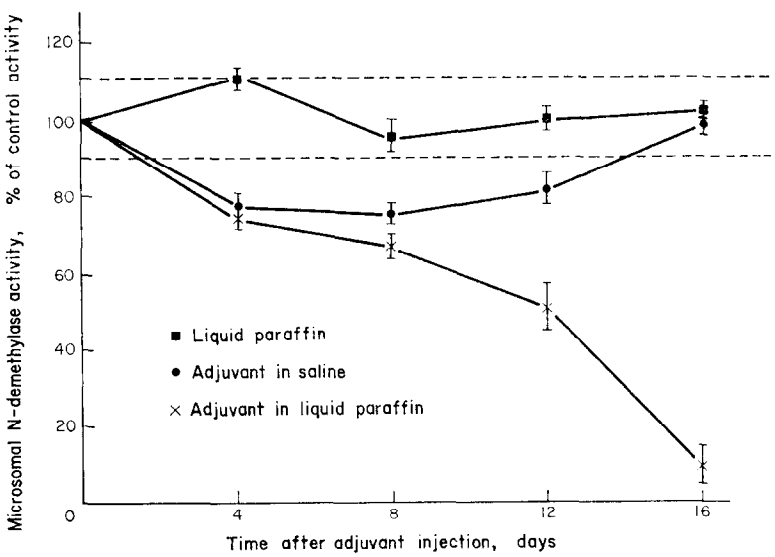

FIG. 1. Effect of adjuvant injection on the *N*-demethylase activity of rat liver microsomes.

induction of arthritis. The livers were immediately removed and samples for histochemical examination were frozen in a carbon dioxide–isopentane mixture at −70°. Sections were cut on a cryostat at 20° and glucose-6-phosphatase and alkaline phosphatase demonstrated by the method of Wachstein and Meisal[11] and Burstone[12] respectively. Liver glycogen was demonstrated by the Periodic acid–Schiff reaction described by Lillie,[13] succinic dehydrogenase by the Tetrazolium method, Nachlas[14] and liver RNA by the method described by Trevan and Sharrock.[15] The remaining liver was homogenised with two volumes of ice-cold 1·15% w/v potassium chloride solution in a Waring blender and centrifuged at 10,000*g* for 30 min at 0°. The supernatant, containing microsomes and the soluble fraction, was used in the incubation experiments.

The microsomal fraction was prepared by centrifugation of the 10,000*g* supernatant at 105,000*g* for 1 hr at 0° in an MSE Super Speed 40 refrigerated centrifuge. The microsomal fraction was resuspended in the original volume of ice-cold 1·15% w/v KCl in an all-glass Potter–Elvehjem homogeniser. Microsomal protein was determined by the method of Lowry *et al.*[16]

Assay of NADPH oxidase

$NADPH_2$ oxidase activity of the rat liver microsomes was obtained by measuring the rate of disappearance of substrate absorbing at 340 mμ on a Unicam SP800 spectrophotometer. The reaction was started by adding 0·2 ml $NADPH_2$ solution (2·4 μmoles/ml) to a cuvette (maintained at 37° by a water-jacket) containing 2·1 ml of 0·05 M tris HCl buffer pH 7·4 and 0·2 ml of microsomal suspension. The blank cuvette contained 0·2 ml of buffer in place of the $NADPH_2$ solution. Extinctions were measured automatically and the rate of oxidation, determined as a change in extinction in a given time, was calculated from the initial linear decrease in extinction. Results were expressed as μmoles of $NADPH_2$ oxidised per minute per milligram of microsomal protein.

Estimation of cytochrome P-450

Cytochrome P-450 levels of the rat liver microsomes were determined by a modification of the method described by Schenkman *et al.*[17] Two ml of liver microsomal suspension were added to 6 ml of 0·1 M phosphate buffer pH 7·0, a few mg of sodium dithionite were added to reduce the cytochrome P-450 and the suspension was divided between two cuvettes. A baseline extinction was obtained by recording a continuous u.v. spectrum from 400 mμ to 500 mμ on a Unicam SP700 spectrophotometer. Carbon monoxide was then bubbled through the sample cuvette for 30 sec, the spectrum repeated and the difference in extinction between 450 mμ and 500 mμ used to estimate the level of cytochrome P-450 present.

A molar extinction coefficient for cytochrome P-450 of 91 cm^{-1} mM^{-1} was assumed.[18]

Incubation experiments

The incubation experiments were carried out in 25 ml Ehrlenmeyer flasks each containing 1·0 μmole NADP, 0·5 μmole NAD, 20 μmoles glucose-6-phosphate, 60 μmoles magnesium chloride, 10·0 μmoles of *d*-propoxyphene hydrochloride all dissolved in phosphate buffer (0·1M) pH 7·5 (5·0 ml) and 2·0 ml of liver homogenate

to give a total volume of 7·0 ml. The incubations were performed in a Mickle shaking incubator in air at 37° (± 0·5°) for 30 min. In control flasks the substrate was omitted.

Demethylation studies in vitro

The demethylation studies were carried out in the incubation medium plus 70 μmoles semicarbazide in the final volume of 7·0 ml. The resulting incubate was assayed for formaldehyde by the method of Cochin *et al.* [19]

Serum analysis

Serum albumin, total protein, glutamate-pyruvate transaminase and cholesterol levels were determined by Technicon Autoanalyzer Methods N.15.C, N.14.B, N.44 and N.24.A respectively.

Paracetamol excretion studies

Free and total paracetamol excreted in urine following oral administration of paracetamol (500 mg/kg) to arthritic and control rats, were determined by the method described by Brodie *et al.*[20]

Phenobarbitone toxicity test

Adjuvant arthritic rats, in nine groups of five received oral doses of 150, 225 and 300 mg/kg sodium phenobarbitone in distilled water on days 5, 10 or 15 after induction of the arthritis. The toxicity of the phenobarbitone was shown by the number of rats surviving 24 hr after the injection.

RESULTS

During 15 and 16 days following adjuvant injection, the *in vitro* liver microsomal *N*-demethylase and $NADPH_2$-oxidase enzyme activity and levels of microsomal cytochrome P-450 were greatly reduced (Table 1, Fig. 1). It should be emphasized

TABLE 1. EFFECT OF ADJUVANT-INDUCED ARTHRITIS ON THE *IN VITRO* ACTIVITIES OF LIVER $NADPH_2$-LINKED ELECTRON TRANSPORT SYSTEMS AND *N*-DEMETHYLASE ENZYM IN RATS*

Treatment (six animals per group)	Days after adjuvant injection	Mean *N*-demethylase activity		Mean cytochrome P-450		Mean $NADPH_2$-oxidase activity	
		Formaldehyde produced in 30 min per g of liver (μmoles)	% of control	mμmoles per mg of microsomal protein	% of control	mμmoles oxidised per mg microsomal protein	% of control
Adjuvant rats	5	0·83	85	0·37	79	5·7	76
Control rats		0·98	—	0·47	—	7·4	—
Adjuvant rats	10	0·62	51	0·26	65	4·1	70
Control rats		1·23	—	0·40	—	5·9	—
Adjuvant rats	15	0·08	7	0·07	16	2·5	43
Control rats		1·10	—	0·45	—	5·7	—

*The results represent mean values from bulked liver homogenates from pairs of rats (three pairs in each group).

that since the data on cytochrome P-450 and $NADPH_2$-oxidase activity are expressed as specific amounts or activity (amount/mg microsomal protein), they are independent of differences in microsomal protein content per gram of liver.

The reduction in *N*-demethylase activity occurred after injection of killed tubercle bacilli suspended in liquid paraffin and for a short time after injection of tubercle bacilli in saline, but not after liquid paraffin alone (Fig. 1).

No significant reduction in liver microsomal enzyme activity was observed following injection of a saline-liquid paraffin emulsion of tubercle bacilli into rat cervical lymph nodes, or after subcutaneous implantation of cellulose pellets (Table 2).

TABLE 2. EFFECT OF ADJUVANT INJECTION INTO LYMPH NODES AND CELLULOSE PELLET IMPLANTATION ON THE LEVELS OF LIVER CYTOCHROME P-450 AND *IN VITRO* *N*-DEMETHYLASE ACTIVITY IN RATS*

Treatment (four animals per group)	Days after start of test	Mean *N*-demethylase activity		Mean cytochrome P-450	
		formaldehyde produced in 30 min per g of liver (μmoles)	% of control	mμmoles per mg of microsomal protein	% of control
Lymph adjuvant rats	5	0·89	110	0·53	104
Control rats		0·81	—	0·51	—
Lymph adjuvant rats	10	1·03	97	0·46	100
Control rats		1·06	—	0·46	—
Lymph adjuvant rats	15	1·16	102	0·43	96
Control rats		1·14	—	0·45	—
Cellulose pellet rats	5	0·91	90	0·46	98
Control rats		1·01	—	0·47	—
Cellulose pellet rats	10	0·83	86	0·37	92
Control rats		0·97	—	0·40	—
Cellulose pellet rats	15	0·97	88	0·42	91
Control rats		1·10	—	0·46	—

*The results represent mean values from the bulked liver homogenates of pairs of rats (two pairs in each group).

The microsomal *N*-demethylase activity and levels of cytochrome P-450 were markedly increased in adjuvant arthritic rats 48 hr after phenobarbitone was administered on the day 8 but phenobarbitone on the day 13 did not cause induction of the enzymes (Table 3).

Following oral administration of paracetamol to arthritic rats, 10 and 15 days after injection of tubercle bacilli, reduced amounts of urinary paracetamol β-glucuronide and sulphate conjugates and increased amounts of unconjugated paracetamol were observed (Table 4).

The maximum tolerated oral dose of phenobarbitone was found to be lower in rats 15 days after injection of tubercle bacilli than in control rats (Table 5).

Whilst normal histological techniques failed to show major changes in the rat

TABLE 3. EFFECT OF PHENOBARBITONE (100 mg/kg) ON THE LEVELS OF LIVER CYTOCHROME P-450 AND *N*-DEMETHYLASE ACTIVITY DURING DEVELOPMENT OF ARTHRITIS IN ADJUVANT TREATED RATS*

Treatment (four animals per group)	Days after adjuvant injection	Mean *N*-demethylase activity: Formaldehyde produced in 30 min per g of liver (μmoles)	Mean *N*-demethylase activity: % of control	Mean cytochrome P-450: mμmoles per mg of microsomal protein	Mean cytochrome P-450: % of control
Adjuvant rats	5	0·83	85	0·37	79
Control rats		0·98	—	0·47	—
Adjuvant rats	10	0·63	51	0·26	65
Adjuvant rats dosed with phenobarbitone (day 8)		1·41	115	0·43	108
Control rats		1·23	—	0·40	—
Control rats dosed with phenobarbitone		2·66	216	0·70	180
Adjuvant rats	15	0·08	7	0·07	16
Adjuvant rats dosed with phenobarbitone (day 13)		0·13	12	0·10	22
Control rats		1·10	—	0·45	—
Control rats dosed with phenobarbitone		2·05	186	0·74	164

*The results represent mean values from bulked liver homogenates from pairs of rats (two pairs in each group).

TABLE 4. UNCONJUGATED PARACETAMOL EXPRESSED AS A MEAN PERCENTAGE OF THE TOTAL PARACETAMOL EXCRETED IN URINE (FREE AND CONJUGATED METABOLITES) FOLLOWING ORAL ADMINISTRATION OF PARACETAMOL (500 mg/kg) TO RATS

Treatment (six animals per group)	Sample time after paracetamol administration (hr)	Mean percentage of unconjugated paracetamol in urine ± standard error of mean*: Days after adjuvant injection 5	10	15
Adjuvant rats	0–3	26·4 ± 2·4	22·9 ± 3·6	27·9 ± 4·4
	3–6	33·2 ± 2·9	21·6 ± 1·7	15·8 ± 3·1
	6–24	9·8 ± 1·4	12·3 ± 1·9	8·8 ± 1·0
Control rats	0–3	13·9 ± 1·6	12·3 ± 1·9	15·6 ± 2·7
	3–6	11·2 ± 0·9	6·1 ± 1·7	7·0 ± 1·5
	6–24	3·7 ± 1·2	6·8 ± 0·9	4·7 ± 0·8

*The results represent mean values from bulked urine of pairs of rats (three pairs in each group).

livers, histochemical methods carried out mainly on the left lateral lobes, show decreases in glucose-6-phosphatase and succinic-dehydrogenase activities and also in glycogen levels, together with increases in alkaline phosphatase activity and RNA levels (Table 6). Similar changes were observed in the other liver lobes. Throughout the development of the arthritic syndrome no changes were noted in the level of total serum protein, but decreases occurred in serum albumin levels (Table 7).

TABLE 5. EFFECT OF ADJUVANT-INDUCED ARTHRITIS ON THE TOXICITY OF ORAL DOSES OF PHENOBARBITONE IN RATS

Dose of phenobarbitone administered (mg/kg)	Number of animals dead within 24 hr period following administration of phenobarbitone*		
	Days after adjuvant injection		
	0	10	15
150	0/5	0/5	0/5
225	0/5	1/5	3/5
300	1/5	1/5	5/5

*Each group contained five rats.

TABLE 6. SEMI-QUANTITATIVE HISTOCHEMICAL MEASUREMENT OF LIVER ENZYME ACTIVITY AND CONTENTS IN ADJUVANT ARTHRITIC RATS WHEN COMPARED WITH CONTROL RATS

Histochemical assay	Days after adjuvant injection		
	5	10	15
Glucose-6-phosphatase	−	−−	−−−
Alkaline phosphatase	+++	+++	+++
Liver RNA	+	0	++
Liver glycogen	−	−−	−−
Succinic dehydrogenase	0	0	−−

+ or −, slight increase or decrease respectively.
++ or −−, moderate increase or decrease respectively.
+++ or −−−, large increase or decrease respectively.

TABLE 7. EFFECT OF ADJUVANT ARTHRITIS ON SOME BIOCHEMICAL PARAMETERS OF RAT SERUM AT 5-DAY INTERVALS AFTER INJECTION OF KILLED *M. TUBERCULOSIS**

Assay	Days after adjuvant injection					
	Control rats			Adjuvant-arthritic rats		
	5	10	15	5	10	15
Total plasma protein (g/100 ml)	6·50	6·70	6·25	6·45	6·50	6·50
Total plasma albumin (g/100 ml)	3·95	4·05	2·90	2·75	2·30	1·55
Albumin/Globulin ratio	1·61	1·61	1·37	0·77	0·55	0·32
Glutamate-pyruvate transaminase (Karmen units/ml)	29	41	36	28	35	36
Cholesterol (mg/100 ml)	—	84	76	—	59	64

*The results represent mean values from blood samples from four rats in each group.

DISCUSSION

The majority of foreign compounds are metabolised by the microsomal enzymes of the liver and drug metabolism is slowed in animals with liver disease.[21] The activity of these enzyme systems is affected by species, strain, sex, age, nutrition, partial hepatectomy, hepatic tumours and obstructive jaundice.[22] In addition, Levi *et al.*[23] have demonstrated that the plasma half-life of phenylbutazone, a drug metabolised by a liver microsomal enzyme system, is prolonged in humans with liver disease provided that these patients have not been pretreated with liver enzyme inducing drugs such as phenobarbitone.

In the present experiments induction of adjuvant arthritis in rats decreases the levels and activity of components in the liver microsomal reduced NADP-linked electron transport system. The ability of the liver to form β-glucuronide conjugates of administered *N*-acetyl-*p*-aminophenol was also reduced and the metabolism of administered doses of phenobarbitone impaired, thus increasing its toxicity. These results indicate a reduced capacity for oxidative metabolism of foreign compounds in adjuvant arthritic rats, influencing the toxicity and half-life of any compound under test, unless the compound induces liver microsomal enzymes or inhibits the arthritic syndrome.

The absence of enzyme induction after administration of phenobarbitone at day 13, but not at day 8, shows that the mechanism for microsomal enzyme synthesis had been seriously disturbed during the formation of the secondary lesions in the arthritic rats. Many previous studies have indicated that the stimulant effects of phenobarbitone and other inducers on microsomal enzyme activity are associated with increased synthesis of enzyme protein.[24]

In agreement with earlier findings,[6] no major microscopic histological changes were observed in the liver of the adjuvant arthritic rats. However, histochemical examination showed decreases in glucose-6-phosphatase and succinic dehydrogenase enzyme activity and glycogen levels together with increases in alkaline-phosphatase activity and RNA levels. These changes are indicative of primary liver damage. Previous studies have shown that a consistent fall in the activity of liver microsomal glucose-6-phosphatase can be induced by a variety of hepatoxic agents.[28]

The decreases in microsomal activity were not observed when an emulsion of tubercle bacilli was injected into rat lymph nodes. This route of injection does not induce adjuvant arthritis but has been shown to cause delayed hypersensitivity to tuberculin.[25] It is clear therefore that the effects on metabolism observed in adjuvant arthritic rats were associated principally with the development of arthritic lesions rather than with delayed hypersensitivity to tuberculin which occurs at the same time. Production of an inflammatory reaction in rats by subcutaneous introduction of numbers of cellulose pellets caused only slight changes in the activity of the liver microsomal enzymes. This inflammatory response, however, remains localised and is less severe than the inflammatory response in the arthritic rats. Release of toxic components from the inflammatory sites in the arthritic rats may reach a sufficiently high level to cause a marked reduction in liver microsomal enzyme activity.

The changes observed in the serum proteins of the arthritic rats were similar to those previously seen in arthritic rats[6, 7, 9] and to those in various phases of rheumatic disease in man.[26, 27] These changes may be due to altered liver function as a result of the severe inflammatory response.

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