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SHORT COMMUNICATIONS

The effect of chlorphenoxyisobutyrate ('Atromid-S') on the biliary excretion and distribution of thyroxine in the rat

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THE mechanism whereby ethyl α-*p*-chlorphenoxyisobutyrate (C.P.I.B. or Atromid-S) brings about a lowering of serum cholesterol and triglycerides in experimental animals[1] and in man[2] is not yet understood.

One possible mechanism suggested[3] is that, since 'Atromid-S' is metabolised to an organic acid, it might exert its effects by displacing certain hormones (and, in particular, androsterone and thyroxine which are known to influence cholesterol and lipid metabolism) from the plasma proteins to which they are bound in circulating blood. It has been shown,[4] for example, that salicylates and dinitrophenol (DNP) reduce the plasma protein-bound iodine and the plasma concentration of thyroxine and that these effects are associated with displacement of thyroxine from thyroxine-binding protein (TBP) and with alteration in thyrotropin secretion. In association with these effects, salicylates and DNP produce definite, though transitory, lowering of serum cholesterol and lipids.

In contrast, 2,4-dichlorphenoxyacetic acid (2,4-D), a compound structurally related to 'Atromid-S', alters the physiological distribution of thyroxine,[5] but leaves the concentration of 'free' thyroxine in the plasma and the output of pituitary thyrotropin essentially unchanged, producing these effects principally by an action on the liver. For example, rats pre-treated with 'Atromid-S' or with 2,4-D and given tracer doses of thyroxine labelled with ^{131}I were found[6] to give identical values for the ratio:

$$R = \frac{\text{(Conc. thyroxine in serum/conc. thyroxine in liver) for treated group}}{\text{(Conc. thyroxine in serum/conc. thyroxine in liver) for control group}}$$

The value less than 1·0 for this ratio R, found for each drug, was taken to indicate an accumulation of thyroxine in the liver (relative to serum concentration) greater in the treated group than the control group.[6]

The following experiments were undertaken to see whether the action of 'Atromid-S' in relation to thyroxine was primarily intrahepatic or whether there was also evidence of displacement of thyroxine from TBP in plasma.

RESULTS

The effect of 'Atromid-S' on the distribution of thryoxine (alluded to above) was confirmed in the following experiment.

Three pairs of albino rats weighing between 220 and 280 g were pre-treated by i.p. injection of a sterile solution of the water-soluble sodium ester of Atromid-S in doses respectively of 25, 50 or 100 mg/kg per pair of rats. Three hours later, one rat of each pair was anaesthetized with ether and a tracer dose (0·02 μg) of thyroxine labelled with ^{131}I was injected into the femoral vein. One hour later, the animal was killed with chloroform, a sample of liver tissue was taken into a weighed bottle and blood withdrawn from the aorta.

The second rat of each pair, three hours after the administration of 'Atromid-S' was similarly anaesthetized with ether. The bile-duct was exposed and cannulated with fine polyvinyl tubing which was secured with ligatures. The incision was then closed. A similar tracer dose of thyroxine labelled with ^{131}I was administered into one femoral vein. Immediately following this, the excreted bile was collected during 20-min periods in small glass vials which had been weighed previously. After an appropriate period of collection (40–60 min) the animal was killed and samples of liver tissue and blood collected as previously.

Six rats not given 'Atromid-S' but otherwise treated identically served as controls.

The weight of bile or liver-tissue was determined, the volume of serum measured and the radioactivity in each sample measured in a scintillation-counter. The errors involved in deriving biliary clearance-rates of thyroxine by this technique have been determined and discussed previously.[7, 10]

It will be seen from the table that the mean value for the ratio of concentration of thyroxine in serum/concentration of thyroxine in liver was significantly lower in the treated group than in the corresponding control group. The value for R of 0·75 obtained in this experiment agrees well with values previously obtained for 2,4-D[5] and for 'Atromid-S'.[6]

TABLE 1. COMPARISON OF DISTRIBUTION AND BILIARY EXCRETION OF ^{131}I-THYROXINE BETWEEN RATS TREATED WITH 'ATROMID-S' AND CONTROLS

Rats treated with 'Atromid-S'				Control rats		
Serial No.	Dose mg/kg	Ratio T_4 serum T_4 liver	Bile output (counts/g/sec)	Serial No.	Ratio T_4 serum T_4 liver	Bile output (counts/g/sec)
1	25·0	1·11	—	4	0·94	—
7		1·19	345	5	1·61	354
2	50·0	0·78	—	6	0·99	—
8		0·83	316	9	0·95	300
3	100·0	0·66	—	10	1·49	—
12		1·03	372	11	1·49	374
Mean:		0·93	344		1·25	343
S.E. of mean		±0·17	±28		±0·35	±38

$$\text{Ratio R} = \frac{\text{(Conc. thyroxine serum/conc. thyroxine liver) for treated group}}{\text{(Conc. thyroxine serum/conc. thyroxine liver) for control group}} = \frac{0{\cdot}93}{1{\cdot}25} = 0{\cdot}75$$

On the other hand, the output of radioactive thyroxine (and its metabolites) in the bile was not significantly different between the treated and control rats (Table). This latter result was confirmed and extended when the experimental design was reversed as follows:

4 male albino rats weighing between 350 and 600 g were anaesthetized with nembutal. Following exposure and cannulation of the bile-duct, the tracer dose of radioactive thyroxine was administered and bile-collection instituted. After a further interval (60–100 min) the solution of 'Atromid-S' was administered and bile-collection continued for another 75–100 min. The 4 rats received respectively 25, 33, 143 and 277 mg/kg of 'Atromid-S'.

As before, the weight of bile was determined and radioactivity measured. Counts, corrected for background, when plotted on semi-logarithmic paper showed a steady exponential decline as shown in the accompanying figure. The rate of decline was uninfluenced by the administration of 'Atromid-S' over the range of dosage employed. In all experiments the rate of biliary excretion remained approximately constant over the duration of each experiment.

Finally, serum from treated and control animals was submitted to paper electrophoresis and the paper-strips scanned for radioactivity as previously described.[8] No evidence was found of displacement of radioactive thyroxine from thyroxine-binding protein by this method.

DISCUSSION

The biliary excretion rate of thyroxine labelled with ^{131}I has been shown to be influenced by its binding to the plasma proteins. As the plasma concentration of thyroxine is raised and binding-sites on thyroxine-binding protein (TBP) become saturated, an increasing proportion of the total plasma thyroxine becomes bound to other plasma proteins with lower binding affinities. This loosely-bound fraction is readily removed from the circulation by the liver and excreted in the bile.[7] Salicylates and DNP have been shown to influence this mechanism by displacing thyroxine from TBP and other binding-proteins,[8] thereby increasing the biliary excretion of thyroxine in the rat.[4]

On the other hand, 2,4-D apparently specifically enhances liver retention of thyroxine. The effect of 2,4-D on biliary secretion of thyroxine was not studied but, by other methods, no increase of 'free' thyroxine was found in the blood.[5]

The present results with 'Atromid-S' confirm that it resembles 2,4-D in enhancing liver retention of thyroxine. But there was no evidence that the rate of biliary excretion of radioactive thyroxine was influenced by the drug. This, and the absence of any direct evidence (by paper electrophoresis studies) of displacement of radioactive thyroxine from TBP, in our opinion fail to support the hypothesis, in this species, that 'Atromid-S' might resemble salicylates and DNP in producing certain pharmacological effects by the displacement of thyroxine from its binding-sites on plasma proteins. In this connection, it may be of significance that little or no alteration of binding of tri-iodothyronine by red cells is detectable in the blood of humans treated with 'Atromid-S'.[9]

The primary effect of 'Atromid-S' on the liver may be reflected in the increase of serum glutamic oxaloacetic and serum glutamic pyruvic transaminases which occurs with continued dosage in experimental animals[11] and in man.[12] It has also been observed[13] that changes in the structure of the liver cell, consisting of an increase of mitochondria and lysosomes occurs in rodents given the drug. Whether these functional and structural changes arise as a result of a continuing effect on the metabolism of the liver cell from the increased concentration of thyroxine in the organ brought about by the drug (with a largely incidental effect on the turnover of lipids) or whether Atromid also exerts a direct effect on enzyme systems concerned with intrahepatic lipid metabolism cannot be established from the evidence at present available.

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Dept. of Experimental Pathology,
University of Birmingham,
Birmingham, 15.

C. Osorio
K. W. Walton
C. H. W. Browne
D. West
P. Whystock

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The effect of vitamin B_6 deficiency and of isoniazid on the pyridoxal phosphate content of rat brain

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Convulsions in animals fed vitamin B_6-deficient diets have been thought to be caused by a deficiency of the coenzyme, pyridoxal phosphate (PALP),[1, 2] in the brain. The object of the present study was