Hypercholesterolaemia and Liver Enlargement in Experimental Hepatic Porphyrria

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Schwartz (1955) was the first to describe a disturbance of lipid metabolism in experimental porphyria: he noted a significant increase of liver lipid phosphorus and total lipids in rats treated with Sedormid (2-isopropylpent-4-enoylurea). Labbe, Hanawa & Lottsfeldt (1961) later reported that the synthesis of fatty acids by the liver of rats injected with the related drug 2-allyl-2-isopropylacetamide was increased more than twofold and postulated the existence of a relationship between haem and lipid metabolism in the liver of porphyric animals. Taddeini, Nordstrom & Watson (1964) have found that the administration of 2-allyl-2-isopropylacetamide and also of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine, another drug that induces porphyria, results in a marked elevation of serum cholesterol, total lipids and phospholipids in the rabbit. A considerable increase in liver size was also noted in their animals, a finding already reported by several other authors in experimental hepatic porphyrias caused by various drugs. The finding of hypercholesterolaemia together with increased porphyrin formation in rabbits made porphyric by two unrelated drugs and also in patients with hepatic porphyria (Taddeini *et al.* 1964) suggested the existence of a close relationship between porphyrin and lipid metabolism in porphyria.

Granick (1963) has shown that the drugs that cause experimental porphyria induce the formation of increased amounts of the liver enzyme δ -amino-laevulate synthetase, but the exact mechanism involved in this biochemical effect and the primary site of action of these drugs are not known. The association of other metabolic changes with porphyria may indicate that the synthesis of several liver enzymes is stimulated by these drugs and that porphyria is only one aspect of a more basic alteration involving liver protein biosynthesis (De Matteis & Gray, 1965). The main purpose of the present work was to establish whether porphyria, hypercholesterolaemia and liver enlargement, as well as the increased activity of certain liver enzymes (see below), are closely related phenomena and can be considered as effects resulting from one single action of these drugs.

Griseofulvin (7-chloro-2',4,6-trimethoxy-6'-

methylgris-2'-ene-3,4'-dione) causes a very marked hepatic and erythropoietic porphyria in mice (De Matteis & Rimington, 1963). In this work two compounds structurally related to griseofulvin, the isomer 7-chloro-4,4',6-trimethoxy-6'-methylgris-3'-ene-2',3-dione (isogriseofulvin) and the 2'- β -hydroxyethyl thioether analogue of griseofulvin (HET-griseofulvin) have been tested for the production of porphyria and other metabolic changes in male albino mice and rats.

In the mouse experiments the drugs were finely powdered and incorporated into diet M.R.C. 41B at a concentration of 1%. Animals were treated for 5-8 days. Faecal protoporphyrin was estimated during the treatment and liver protoporphyrin after the animals had been killed. Heparin-treated plasma from pooled blood of several animals was analysed for total cholesterol by either the method of Zlatkis, Zak & Boyle (1953) or that of Abell, Levy, Brodie & Kendall (1952), and also for lipid phosphorus concentration (Fiske & Subbarow, 1925). After 8 days of treatment mice were given access to normal diet for 24 hr. and then injected intraperitoneally with 150 mg. of hexobarbitone sodium/kg. body wt.: their 'sleeping time' was compared with that of control mice to assess the activity of the drug-metabolizing microsomal enzymes in the oxidation of the barbiturate *in vivo*.

It was found (Table 1) that isogriseofulvin was much more active than griseofulvin in inducing porphyria, whereas HET-griseofulvin was practically inactive. However, liver enlargement, elevation of plasma cholesterol and lipid phosphorus concentration and decrease of the hexobarbitone 'sleeping time' were observed after treatment with all three drugs. A marked decrease of the hexobarbitone 'sleeping time' was also observed in mice injected subcutaneously with 2-allyl-2-isopropylacetamide (200 mg./kg. body wt./day) for 3 days.

In starved male albino rats, a considerable increase in liver size and a significant hypercholesterolaemia were observed after the oral administration of griseofulvin or either of its two analogues (2 g./kg. body wt./day) or after subcutaneous injection of 2-allyl-2-isopropylacetamide (300 mg./kg. body wt./day) for 3 days. 2-Isopropyl-2-propylacetamide and chlortone (1,1,1-trichloro-2-methylpropan-2-ol) also produced liver enlargement and hypercholesterolaemia, but to a smaller extent. The

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Table 1. *Effect of the administration of griseofulvin, isogriseofulvin or HET-griseofulvin (1% in the diet) on liver size and protoporphyrin content, plasma cholesterol and lipid phosphorus concentrations and hexobarbitone 'sleeping time' of male albino mice*

The values are the means $\pm$ S.E.M. of the numbers of observations in parentheses. * $P < 0.025$, ** $P < 0.01$, *** $P < 0.001$, where P (obtained by Student's t test) is the probability that the observed differences from control values could have arisen by chance.

	Liver wt. (g./100 g. body wt.)	Liver protoporphyrin (μ g./g. wet wt. of liver)	Plasma cholesterol (mg./100 ml.)	Plasma lipid phosphorus (mg./100 ml.)	Hexobarbitone 'sleeping time' (min.)
Control	5.31 $\pm$ 0.27 (10)	< 0.6† (6)	125.0 $\pm$ 8.8 (9)	6.73 $\pm$ 0.1 (9)	65.4 $\pm$ 4.2 (12)
Griseofulvin	7.10 $\pm$ 0.56 (6)*	23.6 $\pm$ 11.6 (6)***	204.9 $\pm$ 29.9 (4)*	11.77 $\pm$ 2.3 (4)*	35.8 $\pm$ 5.3 (6)***
Isogriseofulvin	9.36 $\pm$ 0.66 (6)**	311.8 $\pm$ 61.2 (5)***	188.4 $\pm$ 15.8 (4)*	10.89 $\pm$ 0.9 (4)**	26.0 $\pm$ 4.5 (12)***
HET-griseofulvin	9.29 $\pm$ 0.16 (9)***	< 0.6† (6)	194.0 $\pm$ 16.5 (10)**	9.49 $\pm$ 0.9 (9)*	28.4 $\pm$ 2.8 (13)***

† The value of 0.6 μ g./g. wet wt. of liver is the lowest concentration of protoporphyrin that can be determined with accuracy in the method employed.

last two drugs and HET-griseofulvin did not, however, cause any appreciable increase in urinary porphobilinogen excretion.

Some additional investigations were carried out in rats made porphyric by 2-allyl-2-isopropylacetamide treatment. A 30–50% increase in the activity of the liver glucose 6-phosphate dehydrogenase and UDP-glucose dehydrogenase (per mg. of protein) was observed after treatment with the drug. Treated animals also showed an approx. 50% increase in the size of the adrenals. Actinomycin D injected at the dose of 250 μ g./kg. body wt./day together with 2-allyl-2-isopropylacetamide produced a considerable inhibition of porphyria, but did not influence the degree of liver enlargement due to the drug and caused a further increase in the concentration of blood cholesterol. This is similar to the results obtained for the increased L-ascorbic acid excretion after 2-allyl-2-isopropylacetamide treatment, which can also be dissociated from porphyria by treatment with actinomycin D (De Matteis, 1964). It was also found that the administration of actinomycin D alone produced a considerable hypercholesterolaemia and a significant enlargement of the adrenals, but no increase in liver size.

These results show that a complex metabolic reaction follows the administration of several chemically unrelated drugs and that one single drug, such as 2-allyl-2-isopropylacetamide, can cause a rise in the activity of several different liver enzymes. The present findings also suggest that liver enlargement and elevation of plasma lipids are not closely related to the disturbance of porphyrin metabolism and may result from different actions of the drugs; both these changes are also produced by drugs that

do not cause porphyria and their association with increased porphyrin formation should probably be regarded as coincidental. The same is likely to be true for the increased activity of drug-metabolizing enzymes and for the enhanced excretion of L-ascorbic acid (De Matteis, 1964) that also occur in experimental porphyria.

In contrast with these metabolic changes produced by many chemically unrelated drugs, porphyria appears to be a more specific effect caused only by some drugs, probably those possessing some particular structural configuration. Studies carried out with compounds chemically related to 2-allyl-2-isopropylacetamide (Goldberg & Rimington, 1955) and to 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (Marks, Hunter, Turner & Schneek, 1965) have shown the existence within both groups of a structural requirement for the induction of porphyria. The results of the present work show that this is also the case for the griseofulvin group of drugs.

The mechanism involved in the production of hypercholesterolaemia by these drugs is not known. Increased hepatic synthesis of cholesterol and other lipids may result from increased activity of liver enzymes concerned in their biosynthetic pathways.

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