*Lethality tests.* In this test, (−)(S)-warfarin was 8·5 times as active as (+)(R)-warfarin, as shown in Fig. 3. The $LD_{50}$ concentration determined at 10 days for (−)(S)-warfarin was found to be 2 mg/kg cornmeal ration. The $LD_{50}$ concentration for (+)(R)-warfarin was found to be 17·7 mg/kg cornmeal ration.

The results found here are in general agreement with those of earlier studies in this laboratory.[9,10] The isomeric ratio of 5–8:1 found here and the average human maintenance dose of the racemic mixture of this compound (5–10 mg/day) puts this compound in the predicted range given by Pfeiffer[11] in his generalized scheme for the relationship between isomeric ratios and drug potency.

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# Studies of the relationship between chemical structure and porphyria-inducing activity—II

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THE overproduction of porphyrins and porphyrin precursors in the livers of animals fed porphyria-inducing drugs results from an enhanced synthesis of the first enzyme in the porphyrin biosynthetic pathway, viz. δ-aminolaevulic acid synthetase.[1,2] Recent studies of the structure–activity relationships of porphyria-inducing compounds in chick embryo liver cells lead to the conclusion that steric rather than chemical factors are important for activity.[3] The most active compounds appear to have a planar portion with a side chain out of the plane.[3,4] This conclusion is difficult to reconcile with the emphasis placed by previous workers[5–7] on the importance of a free allyl group for porphyria-inducing activity in intact animals and chick embryos. For this reason a group of compounds previously tested in whole animals has been reinvestigated in chick embryo liver cells in order to evaluate the importance of the free allyl group for activity.

## MATERIALS AND METHODS

The method of Granick[2, 3] was used for evaluating the activity of the porphyria-inducing compounds: a mixture of crystallized and lyophilized trypsin (100 mg) and Pangestin* (30 mg; Difco) in calcium and magnesium free Earle's medium (6 ml) was used to dissociate the liver cells of two chick embryos, 16–17 days old. About 6·5–7 × $10^5$ cells of the resulting suspension were added to vials (18 × 60 mm) containing a 16-mm coverslip. Each vial contained Eagle's basal medium (1 ml) supplemented with 10% fetal bovine serum, 1% glutamine, and the antibiotics penicillin, streptomycin, and mycostatin. After the cells were incubated for 24 hr in an atmosphere of 5% $CO_2$ in air, forming a monolayer on the cover slip, the medium was renewed, drugs added in ethanol (1 μl), and the vials re-incubated for 24 hr. The coverslip was then removed and examined in the fluorescence microscope (Table 1). The cultures of chick embryo cells to which no porphyria-inducing compounds were added were found to contain a trace of porphyrin (Table 1).

TABLE 1. PORPHYRIN ACCUMULATION IN CHICK EMBRYO LIVER CELLS, INDUCED BY VARIOUS DRUGS AND MEASURED BY FLUORESCENCE MICROSCOPY

| Compounds tested for porphyria-inducing activity in chick-liver parenchyma cells | Concentration M × $10^{-5}$ | Fluorescence intensity* observed 24 hr after addition of compound |
|---|---|---|
| No addition | | trace |
| Alcohol (1 μl) | | trace |
| 2-Allyl-2-isopropylacetamide | 71 | +3 |
| | 14·2 | +2·5 |
| | 3·5 | +1·5 |
| | 0·7 | +1 |
| 2-Propyl-2-isopropylacetamide | 70 | +3 |
| | 14·0 | +2·5 |
| | 3·5 | +2 |
| | 0·7 | +1 |
| 2-Isopropylpent-4-enoylurea | 54·3 | +2·5 |
| | 27·2 | +1 |
| | 2·7 | trace |
| 2-Propylpentanoylurea | 27 | +2 |
| | 2·7 | +1 |
| Diallylbarbituric acid | 48·1 | +1 |
| | 9·6 | trace |
| Seconal | 38·5 | +2·5 |
| | 1·9 | +1 |
| Isoniazid | 73·1 | +1 |
| | 36·5 | trace |
| δ-Allylmalonamide | 70·4 | trace |

* Fluorescence intensity was scored as follows: +3, most colonies fluoresce intensely; +2, most colonies fluoresce partially; +1, some colonies fluoresce partially (cf. Granick[2, 3]).

The 2-allyl-2-isopropylacetamide and 2-propyl-2-isopropylacetamide were obtained from Prof. C. Rimington, and the isoniazid and 2-propylpentanoylurea were obtained from Drs. R. F. Labbe and M. L. Cowger. Sedormid (2-isopropylpent-4-enoylurea) was supplied through the courtesy of Hoffman-La Roche Inc., Nutley, N.J.; Seconal by Eli Lilly and Co., Indianapolis, Ind.; diallylbarbituric acid by Ciba Pharmaceutical Products, Summit, N.J.; and δ-allylmalonamide by Parke Davis & Co., Detroit, Mich.

## RESULTS AND DISCUSSION

A comparison of the porphyria-inducing activities of 2-allyl-2-isopropylacetamide and 2-propyl-2-isopropylacetamide is of interest since the only structural difference between these two compounds is the replacement of the allyl group by a propyl group. The activity of these compounds is similar in the system *in vitro* (Table 1), whereas in the intact animal 2-allyl-2-isopropylacetamide was reported

* A preparation of the enzymes of the pancreas, principally amylopsin, trypsin, and steapsin.

to be active and 2-propyl-2-isopropylacetamide devoid of activity.[7] Thus the free allyl group does not appear to be required for activity in the system *in vitro*, and this conclusion is supported by the comparable activities (Table 1) of 2-propylpentanoylurea and 2-isopropylpent-4-enoylurea. Moreover, it is of interest that Granick[3] has shown that phenobarbital (which lacks an allyl group) exhibits approximately the same activity as diallylbarbituric acid in the system *in vitro*. On the basis of these results it appears that the free allyl group endows the molecule with suitable physicochemical properties, enabling it to achieve a significant concentration at the liver cell when administered to whole animals.

Talman *et al.*[5] compared the porphyria-inducing activities of a variety of drugs related to Sedormid by injecting them into the yolk sac of 8-day-old chick embryos, and measuring the porphyrin concentration of the allantoic fluid. It was of interest to compare the activities, reported for some of these compounds by Talman *et al.*, with their activity in the chick embryo liver cell system. The results obtained by the two methods were found to be similar. Thus 2-allyl-2-isopropylacetamide, 2-isopropylpent-4-enoylurea, and Seconal were active; diallylbarbituric acid and Isoniazid weakly active; and δ-allylmalonamide inactive (Table 1). An important difference was the finding that 2-propylpentanoylurea (di-*n*-propylacetylcarbamide) was active in the chick embryo liver cell system, but inactive when injected into the chick embryo. The chick embryo liver cell method offers several important advantages over previous methods of evaluating porphyria-inducing activity. It is more suited for the interpretation of pharmacological action on a molecular level, and the laborious analysis of porphyrins and/or porphyrin precursors is avoided.

Granick[3] has recently suggested that the porphyrin biosynthetic pathway is involved in drug detoxication mechanisms and that the heme formed may activate $O_2$ for hydroxylation reactions. It is of interest that in the active analogues of 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-2,4,6-trimethylpyridine there exists a steric hindrance to the hydrolysis of the amide and ester groups respectively.[4] It is possible that the drugs cannot be solubilized by enzymic hydrolysis and that the porphyrin biosynthetic pathway is activated to participate in alternative mechanisms for solubilizing the drugs.

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