

Letters to the Editor

Acute Intermittent Porphyrria Studies with Fibroblasts

In this disease, inherited as an autosomal dominant, large amounts of porphobilinogen (PBG) and δ -amino levulinic acid (δ ALA) appear in the urine, at least during the acute episode, and the enzyme δ -amino levulinic acid synthetase is increased in the liver (Tschudy *et al*, 1965): however, no direct toxicity of the excreted compounds has been demonstrated (Jarrett *et al*, 1954), and, while defective control of ALA synthetase synthesis is postulated as the basic defect, the mechanism by which the clinical signs and symptoms are produced is not yet well understood (Tschudy, 1968). Animal models for the study of the disease have been produced by the administration of a wide variety of drugs to rabbits, guinea pigs, and to embryo liver cells in tissue culture (de Matteis, 1967).

In some inherited diseases, eg, mucopolysaccharidoses, homocystinuria, etc, fibroblasts in culture have shown the metabolic defect characteristic of the disease, even sometimes in the heterozygotes in diseases with recessive inheritance. The present study was undertaken to determine whether differences could be demonstrated between fibroblasts from persons with acute intermittent porphyria and those from normal persons.

Skin biopsies were obtained from two women, who had had previous proven attacks of acute intermittent porphyria, who were asymptomatic at the time of the biopsy, and from three healthy doctors (D.B., F.L., and L.W.). The urine of one patient (J.E.), one and one-half years after her last attack, gave a strongly positive Watson-Schwarz reaction; that of the other (I.M.), 16 years after her last attack, gave a barely detectable pink colour.

Biopsies, embedded in chicken embryo extract and chicken plasma, were maintained in Eagle's medium containing 20% human serum; subcultures were grown in Eagle's medium containing 10% calf serum. (All media contain small amounts of penicillin and streptomycin.) Cell growth was estimated by protein determination, using the Lowry method, as described by Oyama and Eagle (1956), in which the cells are initially dissolved in an alkaline solution (pH approximately 11). (δ -Amino levulinic acid + PBG are known to be stable in solutions up to at least pH 9, Bossenmaier, 1968). An aliquot of this

cell solution, after immediate acidification with a drop of 40% HCl, was tested for Ehrlich's reactivity with the highly sensitive Ehrlich's mercury reagent, both before and after treatment, to convert any δ ALA present to its aminoketone derivative (Falk, 1964). Attempts to measure Ehrlich's reactivity in the media were unsuccessful, due to the problems of maintaining correct pH for growth when media contained no phenol red. Haematoxylin and eosin-stained cells were examined by light microscopy. Cells were examined for porphyrin fluorescence in collaboration with Dr A. Jarrett (Department of Dermatology, University College Hospital Medical School).

There was no difference in the appearance of fibroblasts whichever person had been the source, and whether or not the cells had been grown in medium containing 2 mg/100 ml or 20 mg/100 ml thiopental, or in normal medium; nor was any porphyrin fluorescence detected. A slight amount of Ehrlich-reacting material, not increasing with exposure of the cells to thiopental, was detected in all the cells without difference between normal and 'porphyric' ones.

Growth studies are summarized in Figure 1. Aliquots of cells were seeded into a series of 60 ml bottles, and maintained in normal or in drug-containing media for four or five days, after which the total protein content of cells in each bottle was determined.

This preliminary study, therefore, has failed to demonstrate any differences between fibroblasts from normal persons and from persons with acute intermittent porphyria. Further to establish this negative point, studies using cells in cul-

ture from several different patients would be necessary, as would be a search for δ ALA and PBG in the medium, and an attempt to measure levels of δ ALA synthetase in the fibroblasts maintained in culture may not be of assistance either in the detection of latent acute intermittent porphyria in an individual with normal urine who has a family history of the disease, or in the investigation of the metabolic error involved.

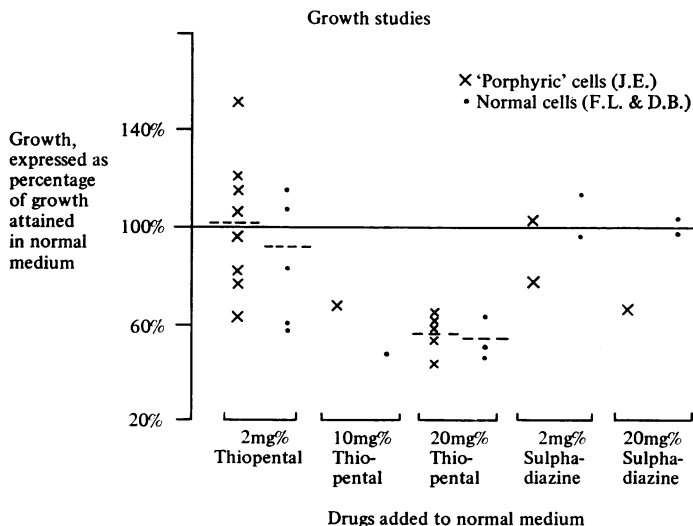
The skin biopsies were obtained from patients of Professor C. E. Dent, who are followed in his metabolic clinic at University College Hospital, and the studies were done in the Galton Laboratory, University College London under the supervision of Professor H. Harris and Dr David Brenton. The author was in receipt of a Medical Research Council of Canada fellowship.

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(Range of actual protein determination: 80-1,000 μ g, as total for cells contained in single 60ml bottle)