the presence of the hypogammaglobulinaemia gene on the X chromosome. The X is a long chromosome, with about 150 cross-over units, and linkage cannot be detected between genes approaching 50 cross-over units apart. The appearance of close linkage must probably be understood as a matter of chance, but this family can nevertheless be used as an illustration of how informative a real close linkage with a marker gene could be (Fig. 4). II 2 is heterozygous Xg^aXg , for she is $Xg(a+)$ and has $Xg(a-)$ children. To her two sons, III 3 and III 4, she has given Xg gene and her hypogammaglobulinaemia gene; but to her daughter, III 2, she has given her Xg^a gene and, barring crossing over, a gene not determining hypogammaglobulinaemia. III 2 should therefore not be a carrier: that she appears not to be, by having two normal sons, is probably a matter of chance; for the close linkage invoked in this demonstration, we must repeat, in all probability does not exist.

Summary

Two adult brothers, aged 25 and 27, were found to have "congenital" hypogammaglobulinaemia associated with steatorrhoea. Their clinical state has responded to parenteral gammaglobulin replacement, but steatorrhoea persists in mild degree and is not responsive to a gluten-free regime.

Both brothers have had severe intermittent anaphylactic-like reactions to the injected gammaglobulin.

Members of the immediate family were found to have hypergammaglobulinaemia.

The classification of these cases is discussed, and the incidence of steatorrhoea in hypogammaglobulinaemia reviewed. A genetic model of a possible inheritance pattern using the Xg blood-group system is constructed.

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Preliminary Communications

Drug Assays on Organ Cultures of Biopsies from Human Tumours

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In previous communications (Ambrose, Andrews, Easty, Field, and Wylie, 1962; Easty and Wylie, 1963) a method was described for the preparation of monolayer tissue cultures from human biopsy specimens. The method was applied to the quantitative analysis of the effect of chemotherapeutic agents on human tumour specimens. Monolayer cultures have distinct advantages for studying direct effects on individual cells, but it has been known for some time that organ cultures provide conditions which resemble *in vivo* conditions more closely. Fell and Mellanby (1953) described effects due to vitamin A in organ culture which resemble *in vivo* effects. Lasnitzki (1951) described morphological changes in the presence of carcinogens, and Algard (1961) observed a response to hormones in organ culture. We have therefore developed a simple organ-culture method for the study of the response of human tumours to potential anti-tumour agents, antisera, hormones, etc.

Lens paper (Green 105) is treated with a solution of water-soluble silicone ($10^{-3}M$ Drisil supplied by Hopkins and

Williams) after washing three times with ether and twelve times with distilled water. Previous to silicone treatment small holes 1 mm. in diameter or less may be punched in the paper, using the ground end of a hypodermic needle. A medium containing Burroughs Wellcome Eagles HeLa medium with added penicillin, insulin, *p*-aminobenzoic acid, glucose, biotin, and ascorbic acid with 10% Burroughs Wellcome calf serum is used for culture. 5 ml. of medium is placed in a disposable plastic dish. Siliconed paper is floated on the medium and a small square of unsiliconed paper placed over the hole. Fragments of biopsy tissue are cut to a size of 1-2 c.mm. and placed on the squares of paper (see Diagram). In some cases the perforation in the siliconed paper may be less than 1 mm. in diameter or may be omitted altogether, provided that the upper surface of the paper is wetted with a drop of medium. Conditions should be arranged so that the specimens are just moist with medium. An adequate gas exchange is essential for success in organ culture (Trowell, 1959). The plastic dishes are placed in a desiccator, Trowell chamber, or plastic box. We have used a picnic box with stopcocks sealed on the lid for gassing. The lid is sealed with silicone grease. A gas mixture containing 5% CO_2 in air is passed through the apparatus.

To obtain useful data with potential anti-tumour agents it is necessary to measure cell growth or synthesis. In organ culture it is not easy to count cell numbers. We have therefore

estimated the uptake of P^{32} -labelled phosphate by the cells. This method has been used successfully by Ruhenstroth and Gostomzyk (private communication) for bone-marrow cultures and by Wrba and Rabes (1962) for liver cultures. We have modified the method by using an extraction procedure which gives a measure of the incorporation of labelled phosphate into nucleic acid by the cells. This synthesis of nucleic acid should provide a useful measure of cell growth.

For assay purposes control cultures are set up, and cultures treated with a series of log dilutions of the drug, or other agent, are set up in parallel. Cultures are maintained for 42 hours. Labelled phosphate is then introduced to a final concentration of about $0.1 \mu\text{C./ml.}$ and the cultures are incubated for six

Mounting of organ cultures. (a) Plan. (b) Section. (c) Enlarged section. LS=Siliconed lens paper. L=Small square of non-siliconed paper. S=Specimen. M=Medium.

hours. The samples are washed with saline, extracted with warm ethanol, ether, chloroform as a 2:2:1 mixture. They are then weighed on a microbalance or torsion balance. Each specimen weighs about 0.1 mg. The specimens are then extracted with cold trichloroacetic acid (T.C.A.), dried on planchettes, and counted. These solvents remove effectively all lipids and material of small molecular weight from the tissue. Nucleic acids and protein are left in the specimen. As protein does not take up labelled phosphates, the counts obtained after extraction are a measure of the incorporation of phosphate into the nucleic acids.

The method has been applied successfully to the culture of human foetal tissues as shown in Table I. The T.C.A.-extracted specimens give a measure of nucleic acid synthesis. Results of a drug test on a human tumour (myxofibrosarcoma of the thigh) are given in Table II.

It is known from the work of Wolff and Wolff (1961) that the culture of human tumours can be much improved by culture in the presence of chick embryonic mesonephros. By a modifi-

TABLE I.—Organ Cultures of Foetal Tissues Maintained for 48 Hours

Tissue	Sample	Counts/Minute/10 $\mu\text{g.}$ of Sample. Sample after Cold T.C.A. Extraction
Foetal lung	1	28
	2	42
	3	28
	4	35
Foetal kidney	1	45
	2	35
	3	35
	4	35

TABLE II.—Drug Test on Myxofibrosarcoma of Thigh Maintained for 48 Hours in Organ Culture

Tissue	Sample	Counts/Minute/10 $\mu\text{g.}$ of Sample. Sample after Cold T.C.A. Extraction
Control	1	13
	2	50
	3	12
	4	25
2.5×10^{-6} g. methotrexate/ml.	1	9
	2	7
2.5×10^{-5} g. methotrexate/ml.	1	4
	2	0
2.5×10^{-6} g. melphalan/ml.	1	4
	2	4
2.5×10^{-5} g. melphalan/ml.	1	3
	2	4

cation of the lens-paper method an attempt is now being made to apply the quantitative analytical method described in this article to the Wolff method of culture. This should extend the range of tumours which can be examined and the length of maintenance in culture very considerably. As human foetal tissues can be maintained successfully, a systematic study of the effect of anti-tumour agents on both normal and tumour tissues of human origin can now be undertaken.

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