formation utilizing these systems may facilitate understanding of specific heart antiheart antibodies and clarify the effects such antiserum might have on the development of the heart as well as general embryonic development.

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PATHOLOGY

Quantitative Technique for Uniform Tumour Implantation by Trocar in Gel-foam

Tumour transplantation in animals by injection of tumour fragments or tissue mince does not offer a quantitative method of implanting a known number of viable cells. Cell suspension techniques give the quantitative data but do not afford a uniform growth in a single implantation site. Often the injected fluid suspension spreads in the tissue and yields more than one tumour or a 'spread-out' tumour, the size of which is not a reflexion of cell count only.

The following method was devised to yield both the known cell count as well as single site of implantation with resulting uniformity of tumour growth.

Sterile methods are used in all procedures. Solid or ascites tumours are obtained from donor mice. Solid tumours are gently homogenized in a glass Dounce homogenizer or a cyto-sieve[1] with a 10-fold volume of sterile Locke's or other physiological diluent (1 g tumour plus 10 ml. diluent) containing 500 units penicillin and 50 units streptomycin per ml. The mechanical homogenization is accomplished by hand, using only six full strokes of the plunger with a coarse fitting Dounce homogenizer with a clearance of about 0·005 in. The resulting homogenate is allowed to sediment by gravity in an ice water bath for 10 min. The supernatant is removed with a syringe and 13-gauge trocar and transferred to a sterile vial. Aliquots are removed and treated with an equal volume of 0·5 per cent solution of eosin according to the method of Schrek[2]. Counts of the viable (non-stained) cells are made on a hæmocytometer. A minimum of 3 aliquots are counted, and if uniform, subsequent dilutions of the homogenate are prepared to yield the desired viable cell concentration. The final dilution is recounted to assure quantitative precision.

Sterile absorbable gelatine sponge (kindly supplied by the Upjohn Co., Kalamazoo. Mich.), size No. 12–7 mm thick, was cut into 5-mm strips. The gel-foam strip was further cut with sterile fine scissors into 2-mm pieces yielding cubes 2 × 5 × 7 mm. The desired volume of the counted cell suspension was then added drop-wise to each cube with a 0·25 ml. syringe. The cell containing fragment was then taken into a 13-gauge trocar and implanted subcutaneously by the usual method utilized with tumour fragments. Microscopic examination of a fixed stained preparation of the cell suspension impregnated gel-foam fragment reveals the presence of tumour cells in the interstices of the gel-foam.

This technique is at present used by us for tumour propagation and has been used in several experiments in which a uniform single site implantation of a quantitated tumour cell inoculum was desired.

An experiment adopting the foregoing method was performed with 144 *C3H* mice using the mammary adenocarcinoma *H*2712. A cell suspension containing 50,000 viable cells per 0·1 ml. was prepared as here, absorbed on to the sterile gel-foam cubes and implanted by trocar subcutaneously in the supra-scapular area. A uniform tumour growth pattern resulted in the implanted mice. The latent period was 12–15 days from time of implant and the tumours, uniform in contour, achieved approximately equivalent sizes in all mice as tumour growth progressed.

This method adopting gel-foam impregnation has also been used by us for implantation of chemical carcinogens, as well as tumour cells, subcutaneously, intra-vaginally and in other anatomical sites.

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Suppression of Experimental Allergic Encephalomyelitis by Cytotoxic Drugs

The action of cytotoxic drugs in experimental allergic encephalomyelitis is of interest in the examination of the mechanism of this disease in animals and may have therapeutic implications for auto-immune disease in man. Hoyer *et al.*[1] have shown that 6-mercaptopurine can suppress experimental allergic encephalomyelitis in rabbits and guinea pigs, but Field[2] has reported negative findings in the latter species.

In the observations described here, two compounds were tested for activity against experimental allergic encephalomyelitis and hæmolysin formation in the guinea pig—*cyclo*phosphamide ('Endoxana'), an alkylating agent, and 4-amino-*N*-methyl-pteroylglutamic acid ('Methotrexate'), a folic acid antimetabolite.

Guinea pigs weighing 200–500 g were sensitized with a single intradermal injection of homologous whole brain suspension, emulsified with an equal volume of complete Freund adjuvant (Difco). Each injection (0·1 ml.) contained 1 mg guinea pig brain (dry weight) and 0·1 mg M butyricum. *Cyclo*phosphamide 20 mg/kg/day and 'Methotrexate' 5 mg/kg/day were given intraperitoneally, while control animals received 1 ml. isotonic saline each day by the same route. The interval between sensitization and treatment, and the duration of drug administration, were varied as indicated in Table 1. In addition, those animals having prolonged treatment (1–24 days) were given 1 ml. of a 20 per cent suspension of sheep red cells subcutaneously on the second day, to assess hæmolysin production.

All animals were examined daily for clinical evidence of experimental allergic encephalomyelitis (weight loss, fæcal incontinence, weakness, paralysis, convulsions and coma). Because of the possibility of drug toxicity, only the presence of definite paralysis or histological lesions were accepted as unequivocal evidence of the disease. Paralysed animals were killed within 2 days of onset, and