at a rate of 10 ml/min for 15 minutes, the endoscope was removed and the patient remained resting for a further 15 minutes. The patients were then asked whether they had experienced any symptoms similar to their normal abdominal pain. Because of the lack of sedation the procedure proved too unpleasant for several patients who needed sedation before the endoscope was passed or during the procedure. These subjects were not included in the trial. Informed consent was obtained from all patients.

*Result*

None of the 8 patients experienced a pain in any way similar to his normal pain. The ulcer areas became a little hyperæmic during the procedure but there was no other real change in appearance.

*Discussion*

It is difficult to account for the conflict between the results of this very simple experiment and those of other workers, notably Rhodes *et al.* One possible reason is that while the symptoms experienced in gastric ulcer and duodenal ulcer are indistinguishable the mechanism of production of symptoms may be different in the two conditions. Duodenal ulcers are intermittently bathed in acid and Rhodes' experiments suggest that when a rapid fall in pH occurs in the duodenum, pain results; perhaps prepyloric ulcers should be regarded as part of the same group as duodenal ulcers. By contrast hyperacidity is not a feature in patients with gastric ulcer in the body of the stomach. Thus it may be that in the upper part of the stomach where acidity is low and changes of intraluminal pressure are high during pain, it is these changes which are responsible for the production of the pain rather than local hyperacidity. Conversely, in the duodenum and prepyloric region changes in acidity and local hyperacidity may be responsible for the production of pain.

*Acknowledgments:* We are grateful to Mr G Qvist for suggesting this project and for his support and encouragement.

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# Growth of Human Tumours in Immune-suppressed Mice

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In these investigations, human tumour xenograft growth has been studied. As a preliminary, three immune-deficient mouse systems were considered, using the growth of HeLa tumour implants to assess the most effective system, in which tumours from patients with primary cancer were subsequently studied. Each mouse under test was inoculated with $2 \times 10^5$ HeLa cells in a 0.2 ml inoculum, by the subcutaneous route. The 3 groups were: (1) Mice treated with antilymphocyte serum (ALS). (2) Thymectomized, irradiated, and reconstituted mice (T− B+). (3) Congenitally athymic mice (Nu Nu).

All the mice, apart from those in Group 3, were obtained from the specific pathogen free colony at the National Institute for Medical Research, in order to reduce to a minimum the complications that arose from using mice inherently infected with bacteria, viruses or helminths. Group 3 mice were obtained from the Medical Research Council Laboratory Animals Centre, Carshalton. All the mice studied were females.

In Group 1, the ALS was prepared by the method of Levey & Medawar (1966). Each

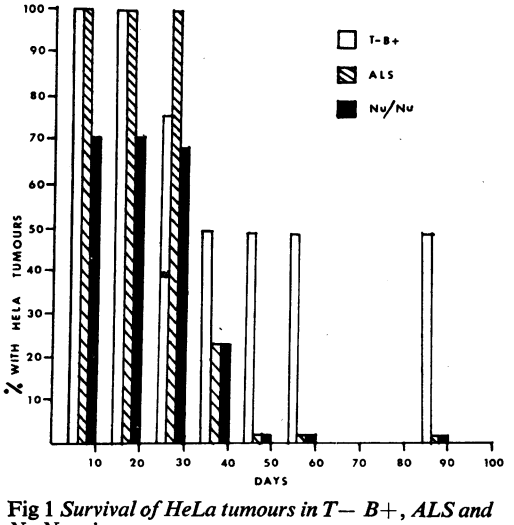


Fig 1 *Survival of HeLa tumours in T− B+, ALS and Nu Nu mice*

*Table 1*

**Tumour growth in 13 primary tumour types**

| *Type of tumour* | *Patient donor takes* | *Mice takes* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *1 week* | *4 weeks* | *8 weeks* | *12 weeks* | *16 weeks* | *20 weeks* |
| Breast | 8/10 | 37/41 | 34/41 | 19/41 | 17/41 | 13/41 | 9/41 |
| Colon | 11/11 | 42/45 | 22/45 | 7/45 | 7/45 | 3/45 | 2/45 |
| Stomach | 7/7 | 33/33 | 17/33 | 6/33 | 3/33 | 2/33 | 2/33 |
| Rectum | 9/9 | 30/45 | 15/45 | 3/45 | 3/45 | 3/45 | 3/45 |
| Cæcum | 2/2 | 14/16 | 7/16 | 6/16 | 5/16 | 3/16 | 2/16 |
| Melanoma | 6/6 | 15/28 | 6/28 | 2/28 | 1/28 | 1/28 | 1/28 |
| Anus | 1/1 | 17/20 | 4/20 | 2/20 | 1/20 | 1/20 | 1/20 |
| Squamous cell carcinoma | 4/4 | 15/15 | 7/15 | 4/15 | 2/15 | 1/15 | 1/15 |
| Tongue | 3/3 | 9/9 | 5/9 | 4/9 | 2/9 | | |
| Pancreas | 1/1 | 8/8 | 1/8 | | | | |
| Bronchus | 1/1 | 7/7 | | | | | |
| Œsophagus | 1/1 | 4/4 | 4/4 | 3/4 | 2/4 | 1/4 | 1/4 |
| Bladder | 2/2 | 4/4 | 2/4 | 2/4 | 2/4 | 2/4 | 1/4 |

mouse received 0.25 ml of ALS on the day before giving HeLa cells (Day −1), on the day HeLa cells were given (Day 0) and then on Days 1, 3, 9, and 16 only. In previous studies (Stanbridge & Perkins 1969, Franks, Curtis & Perkins 1973) HeLa tumour growth has been very variable. The controlling factor has always been the efficacy of the ALS, which cannot be predicted prior to *in vivo* use, because there is no *in vitro* test available at the present time. The immunosuppressive activity of different batches of ALS vary, even though the method of preparation is the same.

In Group 2, the mice were thymectomized by the sternal approach (Miller 1961) at four weeks, whole body irradiated with a lethal dose (900 rad) from a cobalt source two weeks later, and then reconstituted using $1 \times 10^7$ syngeneic bone marrow cells, within four hours of irradiation. In this and previous investigations (Franks, Perkins & Holmes 1973) more bone marrow cells have been used than was originally proposed by Davies *et al.* (1969).

Mice in Group 3 proved to be very difficult to keep alive. Lacking immune competence, they were susceptible to infection. Although rotating antibiotics have been put forward as a means of keeping these animals healthy, this has not been investigated in these studies.

HeLa tumour survival was initially assessed by direct palpation of the nodule, followed by histological analysis of samples of random tumours. Fig 1 shows a comparison of HeLa tumour survival in the three groups of mice. Although regression of tumours rapidly occurs from Day 30 in the ALS and Nu Nu mice, in the T− B+ mice the percentage tumour survival is maintained at about 50% from Day 35 onwards. In view of this, the variability of different ALS samples, and the susceptibility of Nu Nu mice to infection, the T− B+ system was selected as a viable system in which to study tumours from patients with primary cancer.

The tumours were obtained at operation and transported to the laboratory in Eagle's minimal essential medium, containing 2% calf serum, 200 units of penicillin, and 100 $\mu g ml^{-1}$ streptomycin. Tumour pieces of about 2 $mm^3$ were implanted subcutaneously in a ventrolateral site over the hip-joint. This was the most suitable site for implantation, in view of the large tumour growths obtained, often of the order of 2–3 cm. To date, 13 primary tumour types have been grown in the T− B+ mouse (*see* Table 1). The results in Table 1 refer only to direct palpation of the implant. It can be seen, that, in 10 of the tumour types, there is still evidence of continued growth 20 weeks after the tumour has been

Fig 2 *Human melanoma 67 days after subcutaneous implantation in T− B+ mouse*

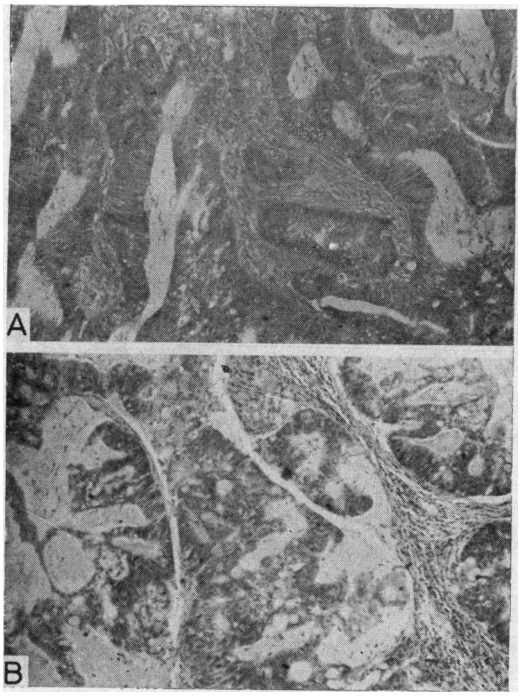


Fig 3 *Photomicrographs.* A, *carcinoma of cæcum at operation.* × *80.* B, *57 days after subcutaneous implantation in T— B+ mouse.* × *80*

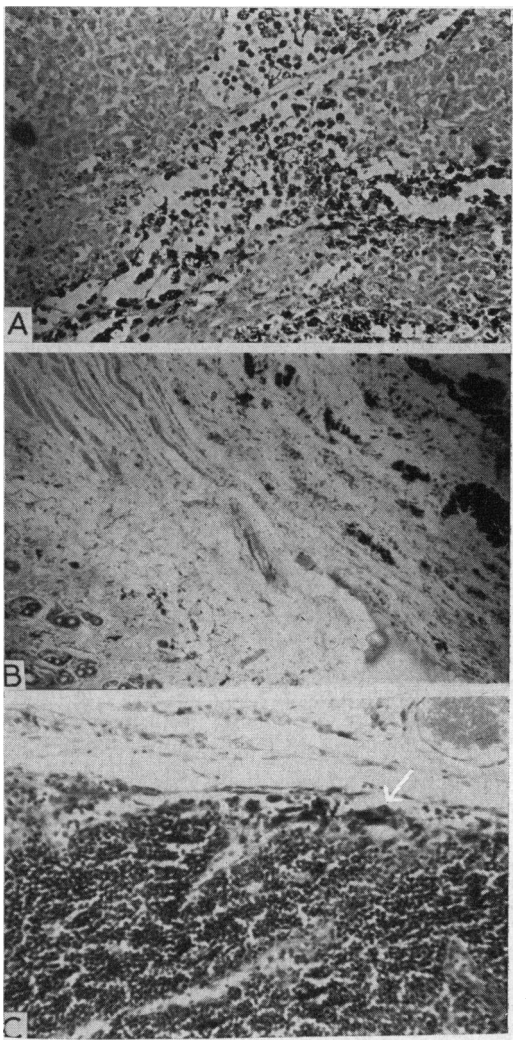


Fig 4 *Photomicrographs.* A, *melanoma at operation.* × *80.* B, *65 days after subcutaneous implantation in T— B+ mouse.* × *80.* C, *mouse para-aortic node showing metastic growth.* × *200*

removed from the patient. Most surprising of all is the fact that tumours of breast origin appear to survive quite well, using this system. Fig 2 shows a large human melanoma 67 days after subcutaneous implantation. As has already been mentioned, tumour growth of this size has been obtained with other tumour types.

At the end of the experimental period (or sooner if the tumours show signs of regression), the mice are sacrificed and submitted to a comprehensive post-mortem examination. In particular, the tumour implant is histologically examined, and compared with the original tumour from the patient. Only if there is an exact histological match between the original tumour and the implant is a 'take' considered to be positive. Fig 3A shows a photomicrograph of a tumour of cæcum origin at the time of operation. Fig 3B shows the same tumour 57 days after implantation in the mouse. Fig 4A shows a melanoma at the time of operation. Fig 4B shows the same tumour 65 days after implantation in the mouse. Fig 4C shows a para-aortic node from a recipient mouse in which metastatic growth of the implanted melanoma has occurred. Metastatic growth has not been reported before.

In these studies, it has been shown that many different human tumour xenografts can be grown by subcutaneous implantations in the T— B+ mouse. Tumour growth is easily assessed by direct palpation, and the mouse system used can be reproduced accurately, and therefore standardized. In view of the wide spectrum of tumour growths, it is suggested that this technique could possibly have clinical applications. For example, this could form the basis for a comparative chemotherapy study of the sensitivity of patients' tumours to chemotherapeutic agents. It also gives an opportunity to maintain *in vivo* tumours which will not readily grow *in vitro*. It is our opinion that this technique is under-used.

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## The Current Status of Sub-zero Organ Preservation [*Summary*]

by Professor K E F Hobbs ChM FRCS
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In the field of organ preservation prior to transplantation there are many problems. Current methods involving cooling with or without perfusion have succeeded in preserving organs for 24–36 hours only (Pegg 1973). Ideally this period needs to be longer. Theoretically the lower the temperature of the organ, the slower the metabolism and therefore the longer the storage period. So investigators have been looking into the possibility of preservation at temperatures below freezing point (Hobbs & Ellis 1973).

When unprotected living cells are frozen they are invariably destroyed. This is secondary to changing solute concentrations resultant upon the formation of ice in the tissue. These damaging effects of freezing can be prevented or modified by the use of a group of compounds called 'cryoprotectants'. Using the technique of prior exposure of cells to cryoprotectants, freeze preservation techniques have been applied successfully to red blood cells, spermatozoa, white blood cells, cells in tissue culture, single cell suspensions and thin layers of tissue such as cornea and skin. However, it has not yet been possible to cryopreserve an intact solid organ in a fully viable state.

To preserve a solid organ, every constituent cell must be exposed to cryoprotectant by perfusion. However, these compounds are toxic at 37°C, so in order to reduce this it is necessary to cool the organ while perfusing with a steadily increasing concentration of cryoprotectant.

The author's modified Langendorff technique of rat heart perfusion (Hobbs & Ellis 1973) uses equipment which permits programmed cooling and re-warming of the organ. After any preservation technique the heart is perfused with dilute rat blood at 37°C, and any damage assessed by observing its ability to beat spontaneously, by recording right ventricular pressures, electrocardiogram, intracellular enzyme release into the perfusate, and finally histological and histochemical examination of the heart.

Encouraging results are being obtained using this technique and other workers are reporting some success (Kubota *et al.* 1974). However, the problem of intact solid organ cryopreservation is still unsolved.

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The following papers were also read:

**Value of Frusemide in the Transplanted Kidney**
Mr J P Hopewell and Mr O Fernando
(*Royal Free Hospital, London NW3*)

**A Case of Constipation**
Mr G Haig
(*Royal Free Hospital, London NW3*)

**Surgical Practice in a Developing Country**
Mr A Lewis
(*Royal Free Hospital, London NW3*)

A minisymposium on **Obstructive Jaundice** was held. Topics discussed were: diagnosis by duodenography; endoscopic retrograde cholangiopancreatography; gallium scanning; treatment of biliary infection and Gram-negative septicæmia. The speakers were Professor Sheila Sherlock, Dr E Elias, Dr R Dick, Dr P Scheuer and Professor W Brumfitt.