fluency in 37 days (80 cm² flask) while LR had poor growth after 48 days in culture.

Discussion

This method allows the storage of a small skin biopsy from every necropsy in which there is the slightest possibility of a need for a fibroblastic cell culture, for long enough to determine whether culture is required or not. Later, cultures can be established if necessary or samples can be discarded when the need for a culture has been eliminated.

Further experience (data not presented) has shown that the skin biopsy can be left in the transport medium at room temperature for several hours before adding dimethyl sulphoxide and freezing. This procedure has also been used successfully for other tissues such as tumours and calf tendon.

We are conducting further experiments to determine how long biopsies can be kept at -70°C and whether transfer to liquid nitrogen after holding at -70°C overnight will allow long term preservation.

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Active site directed inhibitor used in the production of antibodies against urokinase

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Plasminogen activators are highly specific proteases found in trace quantities in most human tissues. These enzymes play important roles in tissue reorganisation and in the prevention of fibrin deposition. Urine contains a plasminogen activator, urokinase, which is not generally detected in normal tissues. Increased plasminogen activator synthesis is a feature of malignant transformation and may be specifically related to invasive and metastatic prop-

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erties of tumour cells.^{1,2} In a number of instances urokinase antigen has been detected as a malignant cell product, especially from ovarian tumours, and it has been suggested that radioimmune assay for urokinase might play a role in the detection of carcinoma.³

The development of antibodies suitable for immune assay has been difficult owing to the lack of suitably purified plasminogen activators. This report describes a method by which antibodies to urokinase were obtained without prior purification of the antigen.

Material and methods

Glutamyl-glycyl-arginine-chloromethyl ketone (Glu-Gly-Arg-CH₂-Cl) was synthesised by Dr Elliott Shaw, Brookhaven National Laboratory, New York, USA, and kindly supplied by Dr D Pepper, Scottish National Blood Transfusion Service, Edinburgh, and Dr RAG Smith, Beecham Pharmaceuticals, Epsom, UK. This peptide inhibits

urokinase by forming a covalent complex with the active centre histidine residue.^{4,5} The peptide was coupled to cyanogen bromide activated Sepharose-CL-4B (Pharmacia Fine Chemicals, London) to give a calculated substitution concentration of 0.68 µg/mg of Sepharose.

Semipurified urokinase (UK) (360 units/ml, specific activity 12 500 units/mg) was reacted with the Sepharose bound inhibitor (50 µl/mg gel) and the disappearance of enzymatic activity from the solution was measured at intervals on bovine fibrin-agar plates supplemented with plasminogen.

Non-coupled proteins were washed from the Sepharose-Glu-Gly-Arg-CH₂-UK with urea (5 mol/l) containing sodium dodecyl sulphate (20 g/l), and then emulsified with Freund's complete adjuvant. A New Zealand White rabbit was immunised with subcutaneous injection of gel on days 0, 12, and 22 and bled regularly from day 28. IgG was isolated by chromatography in Sepharose bound protein A (Pharmacia Fine Chemicals, London, UK) and readjusted to the original serum volume. Antiurokinase antibodies were detected by mixing equal volumes of IgG fraction with urokinase and measuring the enzyme inhibition produced.

Human uterine tissue plasminogen activator was prepared as described by Rijken *et al.*⁶

Results and discussion

Sepharose-Glu-Gly-Arg-CH-Cl₂ reacts rapidly (t_{1/2} 1–2 min) with urokinase, completely inhibiting >95% of the enzyme after 60 min. A coupling ratio of 15 units/mg gel was obtained, which represents 150 ng urokinase per mg/gel since the specific activity of urokinase is 10⁵ units/mg. Subcutaneous injection of 67 mg gel (equivalent to 10 µg urokinase) provoked a good antibody response by day 28, when 80% of urokinase (50 units/ml) was inhibited (Figure). This IgG fraction did not inhibit human uterine tissue plasminogen activator, nor did it produce precipitin reactions with any protein other than urokinase. Non-immune IgG did not inhibit urokinase.

The present antiurokinase antibodies have been prepared by a novel technique without extensive purification of the antigen. Antisera against plasminogen activators are not easy to prepare owing to the difficulty in purifying these enzymes. This present approach circumvents the problem since the inhibitor bound gel reacts only with active enzyme and the gel itself may enhance the immunogenicity

Immunoenzymic inhibition of urokinase (6 units/ml) and tissue plasminogen activator (10 units/ml) caused by the addition of equal volumes of antiurokinase IgG fraction.

of the protein. This antiserum is now under assessment for immunological assay of urokinase produced by normal and malignant tissues.

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