thalamic area on a median line. The method allows a large number of transplantations (about 50 per h) and a prompt, complete recovery of the animals. The animals were observed for 40 days, at the end of which time the survivors were killed and their brains macroscopically and microscopically examined. The T_8 uterine epithelioma was the tumour yielding more reproducible results. As shown in the Table, all the animals died within two weeks. There is a very small time variation and the analysis of the variance demonstrates that in 6 successive transplantations the survival times of the various experimental groups were not statistically different. The tumour was always recognizable as a well-delineated spheric mass or having a tendency to infiltrate the cerebral tissue. Histologically the tumour was different from one grown subcutaneously, since connective tissue was not observed. The T_8 uterine epithelioma growing within the brain could be successfully transplanted into other rats, either subcutaneously or intracerebrally. A line of T_8 uterine epithelioma with cerebral localization is being developed. Flexner-Jobling and Walker tumours required a longer time to kill the rats, as is shown in the Table, while D 117 osteosarcoma did not kill the animals during a period of 40 days after the transplantation. In order to establish whether death occurring after intracerebral injection of T_8 tumour was related to non-specific factors, homogenates of liver, kidney and lung or plasma of normal rats were also injected intracerebrally.

There was no mortality and, after sacrifice, the animals did not show brain lesions.

Studies are in progress to establish the behaviour and the sensitivity of the T_8 uterine epithelioma transplanted in the brain to known antitumoral drugs[4].

Tissue homogenate	No. of rats died/injected	Average survival (days)	Take %	No. of groups
T_8 Guèrin	59/59	14.2 ± 0.3	100	6
Flexner-Jobling	27/27	23.4 ± 0.6	100	1
Walker	22/23	18.2 ± 0.7	96.5	1
D 117 osteosarcoma	2/24	–	0	1
Liver	0/17	–	0	2
Kidney	1/13	–	0	2
Lung	1/ 7	–	0	1
Plasma	0/10	–	0	1

Surviving animals were observed for 40 days.

Riassunto. Si descrive una semplice e rapida tecnica per trapiantare tumori sperimentali nel cervello di ratto, in modo non traumatizzante. Tra i tumori saggiati, l'epitelioma T_8 uterino di Guèrin, si sviluppa nella totalità dei casi, con maggior rapidità rispetto ad altri tumori (Flexner-Jobling, Walker) ed in un tempo sensibilmente costante. Questo modello sperimentale viene utilizzato per studi di chemioterapia antitumorale.

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[4] The technical help of Miss A. Guaitani is gratefully acknowledged. – This study was financed by Euratom (contract No. 040-65-1 B101).

Labelling of Corticotropin with Iodine-125

One of the problems in the determination of corticotropin by radioimmunochemical methods is the labelling of corticotropin. Felber[1] and Yalow et al.[2], using the method of Hunter and Greenwood[3], employed iodine-131 to label corticotropin in their immunochemical methods. Felber used an Amberlite IRA-400 column to purify the labelled material, but it was not possible to remove the 'damaged' corticotropin by this technique. Yalow et al. employed their purification method developed for labelling insulin, but the yield from the cellulose column was low.

This paper describes a modification of the method of Hunter and Greenwood, based on the observations of Sanfelippo and Surak[4] and Jørgensen[5], by which a greater purification of more efficiently labelled corticotropin may be achieved.

Sanfelippo and Surak observed a $K\check{D} = 0.3$ for corticotropin on a Sephadex G-50 column with a $0.02M$ acetate buffer (pH 5.5) containing $0.3M$ KCl as eluent. As shown in Figure 1 I found the same $K\check{D}$ on a Sephadex G-50 column, while the corticotropin was completely excluded from the gel particles on a column of G-25 ($K\check{D} = 0$). There seemed to be a better separation of the peak of labelled corticotropin from the $Na^{125}I$ on the G-25 column.

It is essential for successful labelling that the iodine solution is completely free from thiosulphate. To prevent oxidation during transport, sulphite can be used as reducing agent. Before labelling, this can be removed by the method of Jørgensen described below.

Iodine-125 is preferable to iodine-131 as a labelling material since with iodine-125 radiation damage is much smaller during the labelling procedure and subsequent storage. (γ-Energy for iodine-125 is 0.035 MeV and for iodine-131 0.36 (80%) MeV.) The long half-life of iodine-125 (60 days against iodine-131: 8.01 days) permits the use of one iodination lot for months using the radioimmunochemical method of Felber.

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[2] R. S. Yalow, S. M. Glick, J. Roth, and S. A. Berson, J. clin. Endocrin. *24*, 1219 (1964).
[3] W. M. Hunter and F. C. Greenwood, Nature *194*, 495 (1962).
[4] P. M. Sanfelippo and J. G. Surak, J. Chromatogr. *13*, 148 (1964).
[5] K. Jørgensen, unpublished.

The following routine procedure was adopted: 3–5 mC carrier-free Na^{125}I (Nuclear Science and Engineering Corporation, Pittsburgh, USA) in NaOH-Na_2SO_3 solution, with a specific activity of about 100 mC/ml, is placed in a conical test tube. 50 μl of 0.1 N HCl, 5 μl of 0.1 M $NaHSO_3$ and 500 μl of H_2O are added, after which the volume is reduced to about 50 μl under vacuum. In order to bring the pH to about 7.0, 50 μl of 0.1 M $NaHCO_3$ are added. Immediately thereafter 5–10 μg of corticotropin in 10 μl of phosphate buffer (ionic strength 0.04, pH 7.4) are added while mixing, followed by 200 μg of chloramine-T (in 25 μl of the above mentioned phosphate buffer – freshly prepared). After 1 min, 480 μg of $Na_2S_2O_5$ and 2 mg of KI, both in phosphate buffer, are added, and after mixing the material is transferred to a Sephadex column.

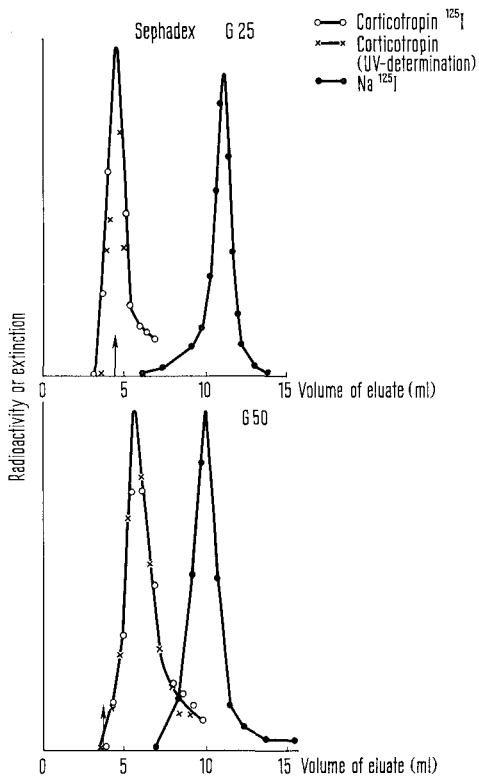

Fig. 1. Elution pattern on Sephadex G-25 and G-50 of corticotropin, corticotropin-^{125}I and ^{125}I. The arrows indicate void volumes. Unlabelled corticotropin was determined in a Beckman DU spectrophotometer at 280 nm.

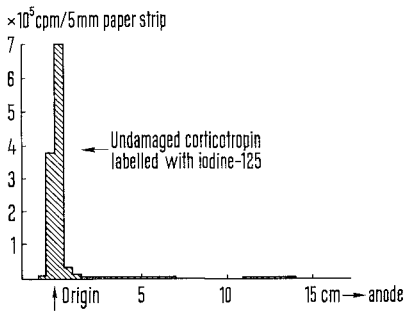

Fig. 2. Paper electrophoresis of corticotropin-^{125}I.

The column dimensions are 20 · 0.75 cm. The column is equilibrated with 0.02 M acetate buffer (pH 5.5) with 0.3 M KCl and presaturated with 20 mg of bovine plasma albumin (Armour Pharmaceutical Company Ltd., fraction V) in 1 ml of the same buffer. Elution is made with about 25 ml acetate buffer as described by GREENWOOD et al.[6].

The elution of the radioactive mixture is carried out with the acetate buffer. Using Sephadex G-25 the first 3 ml of the eluates are discarded. The next 2.5 ml fraction containing the labelled corticotropin is collected in 2.5 ml of phosphate buffer containing 25 mg of bovine plasma albumin, mixed, divided into 30 μl portions and immediately frozen.

The yield of labelled corticotropin was about 50–60% and the specific activity about 350–600 mC/mg corticotropin. Paper electrophoresis (Schleicher and Schüll No. 2043 bmgl) in veronal buffer (ionic strength 0.1, pH 8.6) gave the pattern shown in Figure 2. The paper was cut into strips of 5 mm. The radioactivity of each strip was measured in a liquid scintillation spectrometer (Packard TRI-CARB, model 3003).

With this labelled corticotropin, 80% binding to guinea-pig anticorticotropin serum can be obtained, and the material is well suited for autoradiography[7].

Zusammenfassung. Es wird eine Routinemethode zur Markierung des Corticotropins mit Jod-125 und Reinigung des markierten Produktes auf einer Sephadex-G-25-Säule beschrieben. Die erreichte spezifische Aktivität beträgt 350–600 mC/mg Corticotropin. Das markierte Hormon ist für radioimmunochemische Methoden zur Bestimmung des Corticotropins und für autoradiographische Zwecke besonders geeignet.

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[6] F. C. GREENWOOD, W. M. HUNTER, and J. S. GLOVER, Biochem. J. *89*, 114 (1963).

[7] Acknowledgments: Thanks are due to K. JØRGENSEN (Novo, Denmark) for his great help and advice, and to Ciba (Basle), Endopancrine (France), Ferring (Sweden) and Organon (Holland) for supplies of corticotropin. – This work was supported in part by grants from Statens almindelige videnskabsfond, Fonden til lægevidenskabens fremme, Novo-fonden, Løvens kemiske fabriks legat, F. L. Smidth & Co. A/S's jubilæumsfond and Frk. P. A. Brandts legat.