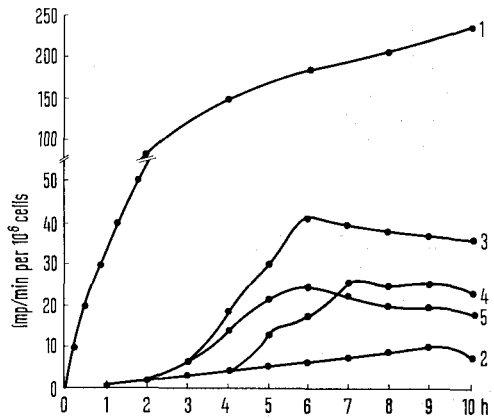
for the study of the inhibition of the synthesis of viral RNA by various compounds.

The present study illustrates the application of this method to the synthesis of RNA of Newcastle disease virus (NDV).

A monolayer culture of chick embryo fibroblasts was suspended in 0.5% lactalbumin hydrolysate with 20 μM/ml of L-glutamine. Each flask contained $5 \cdot 10^7$ cells. Actinomycin D, 10 μg/ml, was added to the culture 20 min before or 40 min after infection. The culture was infected with NDV, 180 ID 50 per cell, and in 40 min C^{14}-uridine, 4.5 μCu/ml, was added. All procedures were carried out at a temperature of 37°C. The incorporation of C^{14}-uridine was arrested by the addition of 0.1 initial volume of 5% solution of sodium dodecyl sulphate and then by the addition of an equal volume of 20% cold trichloroacetic acid. The suspension was passed through a millipore filter, RUFS type, and washed by 5% cold trichloroacetic acid. The filters were placed on cardboard and radioactivity was determined with a counter of the Geiger-Mueller type.



Synthesis of RNA in chick embryo fibroblasts infected with NDV and treated with inhibitors of protein synthesis as measured by the incorporation of C^{14}-uridine. 1, normal cells; 2, cells treated with actinomycin D, 10 μg/ml; 3, cells treated with actinomycin D and infected with NDV, 180 ID 50 per cell; 4, experiment (2) with addition of D,L-hexafluorovaline $7 \cdot 10^{-4} M$; 5, experiment (2) with addition of L-ethionine, $3 \cdot 10^{-3} M$. Amino acid analogues were added 60 min before the inoculation.

In some experiments total RNA was isolated after SCHERRER and DARNELL[4].

The results of the study are shown in the Figure. It is evident that the rate of synthesis of cellular RNA as measured by incorporation of C^{14}-uridine is strongly inhibited by actinomycin D. In virus-infected cells the synthesis of viral RNA begins 2–3 h after infection and is not inhibited by the antibiotic. The virus-specific RNA synthesis is about 20% of that of the cellular RNA in non-infected cells.

Two compounds, D,L-hexafluorovaline and L-ethionine[5,6], inhibit the synthesis of viral RNA, although the time and the rate of inhibition for the two compounds are different. While L-ethionine decreases the rate of C^{14}-uridine incorporation by not more than 45–50%, D,L-hexafluorovaline also prolongs the latent period. Supposedly similar inhibitor action may be achieved by the use of *p*-fluorophenylalanine. This action of the inhibitors of proteinsynthesis may be connected with the alteration of the synthesis of early virus-specific proteins which are necessary for the replication of viral RNA.

Zusammenfassung. In mit Actinomycin D behandelten und mit dem Pseudogeflügelpest-Virus (NDV) infizierten Zellen gelingt der Nachweis einer ausgesprochenen Phase der Virus-RNS-Synthese. Mit dieser Methode lässt sich ebenfalls die Wirkung verschiedener Präparate auf die Virusnukleinsäuresynthese erfassen.

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A Model of a Cerebral Tumour for Studies in Cancer Chemotherapy

This note is intended to describe a simple and reproducible method for studying the effect of antitumoral agents on tumours growing within the brain. The methods previously employed require traumatic procedures and animal species, such as hamsters and rabbits, which are not frequently used in cancer chemotherapy[1]. Male Sprague-Dawley rats, average weight 140–180 g, were used throughout all experiments. Flexner-Jobling carcinoma, Walker 256 carcinosarcoma, T_8 uterine epithelioma or D 117 osteosarcoma transplanted subcutaneously 15 days before the experiment, were excised, cleaned of necrotic material and minced under conditions of sterility. The fragments were homogenized in a stainless steel homogenizer[2] previously sterilized. The homogenate was diluted with a phosphate buffer at pH 6.9 (1:5 w/v) and then centrifuged for 2 min at 800 rpm. The supernatant transferred into a sterile tube was used for the cerebral implantation. The intracerebral injection was performed according to the method described by VALZELLI[3]. A No. 20 needle covered with a plastic muffle 11 mm from the tip was inserted into the squamo-petrosal fissure of animals submitted to a light ether anaesthesia. The amount injected with a Hamilton syringe (0.1 ml) was always 0.02 ml. By using this technique, the cells are implanted in the

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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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