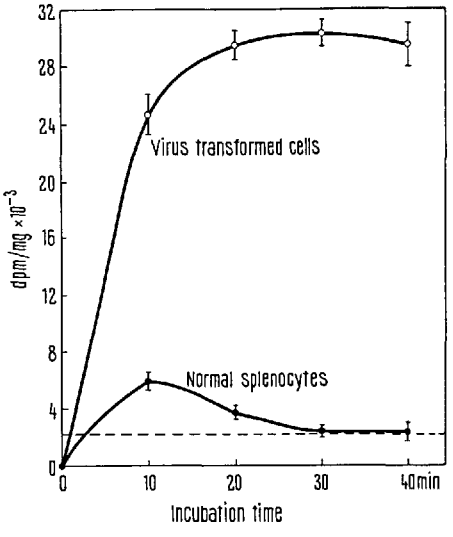
Viral Leukemia: Increased Thymidine Assimilation

Functional alterations in cellular activity stemming from incorporation of a viral genome are of interest from diverse points of view. In the present study, the effect of murine leukemia virus on the translocation of exogenous thymidine into the intracellular acid soluble pool of mouse splenocytes was investigated.

Female SJL/J mice (Jackson Laboratories) were injected with stock Friend virus. Spleens were removed from positive donors (with advanced splenomegaly), and transected. The cells were harvested by several injections of iced culture medium M-199 (5 ml syringe, 20 gauge needle). The resultant brei was filtered through sterile gauze to provide a single cell suspension. Approximately 10^7 leukemia cells were injected i.p. into recipient animals. The experiments to be described were performed on 4th passage spleen cells obtained 12 to 14 days after injection of leukemia cells, or on spleen cells pooled from normal animals.

The techniques for measuring thymidine uptake into the acid soluble pool were similar to those described previously[1]. Cell suspensions were prepared in M-199 from normal or leukemic spleens. 10 ml of a 1:50 cell suspension were incubated in a 50 ml Ehrlenmeyer flask at 37 °C for 4 min. Hydroxyurea (in 0.1 ml) was added to result in a final concentration of $10^{-3}M$. This inhibitor of DNA synthesis was added to circumvent an appreciable decline in medium precursor (via incorporation into DNA) during the incubation period. Incubation was continued for 8 min, at which time 10 μCi tritiated thymidine (^{3}HTdR, specific activity 0.36 Ci/mmole; Schwarz Bio-Research) were added. At intervals, a 1 ml aliquot was withdrawn and placed in a tared 2 ml centrifuge tube. 1 ml of iced phosphate buffered saline was added, and the tube was centrifuged at 250 *g* for 1 min at 0 °C. The cells were washed once with 2 ml iced PBS, the supernatant removed, and the tubes thoroughly wiped dry. The wet weight of the cell pellet was determined. This was followed by the addition of 0.3 ml iced 5% TCA and resuspension of the cell pellet. After 15 min in an ice bath, the tubes were centrifuged. A 0.1 ml aliquot of supernatant was removed and placed in 1.0 ml of soluene in a glass counting vial. After solubilization, 10 ml of scintillation cocktail (toluene system) were added. The ^{3}H activity was determined in a liquid scintillation spectrometer employing an Absolute Activity Analyzer (Packard Inst. Co.) for quench correction.



Thymidine uptake into the acid soluble fraction of leukemic and normal splenocytes as a function of incubation time. Horizontal line indicates concentration of ^{3}HTdR in the medium (dpm/mg).

Thymidine uptake into the acid soluble fraction, is shown in the Figure. Clearly the virus transformed cells have a greatly increased capacity for thymidine assimilation: the maximum distribution ratio for leukemic cells is 13.8, while that for normal splenocytes is 2.7. In addition, the transformed cells are capable of maintaining the gradient for a longer time under these conditions. Since ^{3}HTdR incorporation into cells synthesizing DNA is highly dependent on functional translocation processes[2], it might be predicted, inter alia, that high specific activity ^{3}HTdR (thymidine suicide) would be considerable more effective in killing S phase leukemic cells than normal splenocytes.

While this work was in progress, a report appeared describing increased thymidine uptake in polyoma virus transformed hamster embryo cells[3]. Hence, a marked increase in thymidine translocation may be a general characteristic of a number of virus transformed cells[4].

Résumé. Dans les splénocytes leucémiques le virus de Friend a provoqué l'entré d'une quantité de thymidine dans le pool intracellulaire acide-soluble 5 fois plus grande que celle des splénocytes de souris normale.

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X-Ray Induced Increase in Number of Cysteine-rich Periventricular Glial Cells in the Rat Brain

The brains of a wide variety of animals show the presence of periventricularly and perivascularly localized glial cells containing cytoplasmic granulations very rich in cysteine and cystine[1-3]. These cells were shown to increase in number after a whole body 800 R X-ray irradiation[2,4]. In the experiment reported here 58 adult hooded rats weighing about 200 g were X-ray irradiated under light ether anaesthesia with a Müller I and Müller II therapeutic X-ray machine (250 kv, 15 ma, Thoräus filter, HVL 2.5 mm Cu, dose rate 85 R/min, target distance 50 cm, field 20 × 24 cm). Only the head region was irradiated, the remainder of the body being shielded with lead. Single doses of 1000 R,

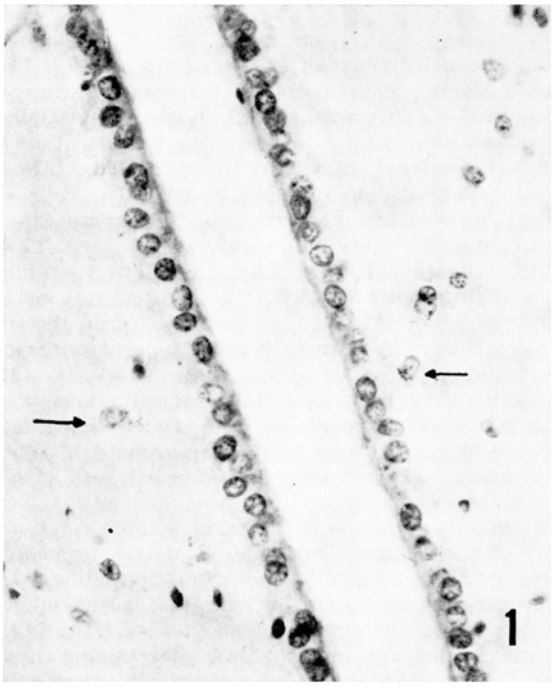

Fig. 1. Cysteine-rich periventricular cells in a control animal. Chrome haematoxylin phloxin. × 500.

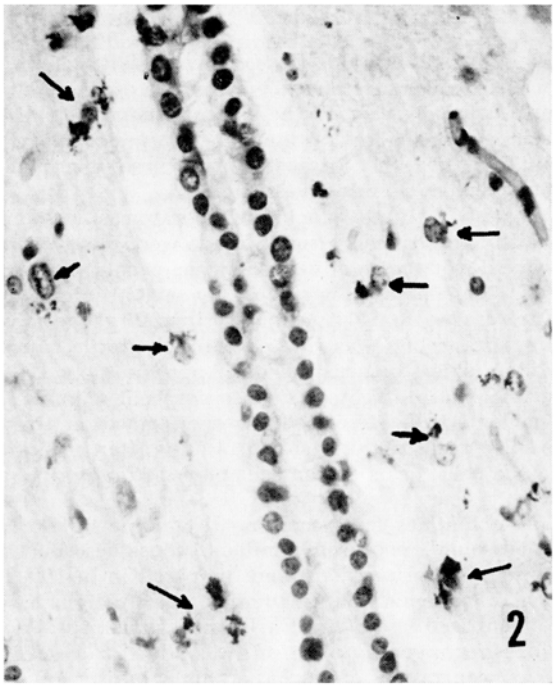

Fig. 2. Cysteine-rich periventricular cells in 4000 R irradiated animal. Chrome haematoxylin phloxin. × 500.

2000 R, 3000 R, and 4000 R were given. 10 normal non-irradiated animals matched for sex and age served as controls. The animals were killed on the 7th, 14th, or 21st post-irradiation days and their brains examined histologically. 7 μm serial sections were prepared from each brain and stained with Gomori's chrome alum haematoxylin-phloxin method, the DDD reaction of BARRNETT and SELIGMAN[5], or the method of ADAMS and SLOPER[6] for cysteine and cystine. The cysteine-rich glial cells were counted in the nucleus arcuatus in an area of 0.087 mm² in each of 25 adjacent serial sections in the left and right half of the brain. The results were analyzed statistically with Student's *t*-test.

The cytoplasmic granulations of the periventricular glial cells are strongly positive both in the DDD reaction and in the performic acid-Alcian blue method of Adams and Sloper thus indicating a high content of cysteine. They were equally positive in the X-ray irradiated and control animals.

High mortality was observed in the 3000 R and 4000 R irradiated groups, 50% of the animals dying before the 7th postirradiation day. The surviving animals of these groups showed considerably higher numbers of the cysteine-rich glial cells in the brains than individuals of the control group (Figures 1–3).

The significance of the presence of cysteine-rich granules-containing glial cells in the brain is unexplained. These cells probably originate from the ependyma, even in adult life[2]. Our recent unpublished histochemical findings[5] show that the cysteine-rich granules of the periventricular glia are negative for acid phosphatase, β-glucosaminidase, and β-glucuronidase. Thus, these cells are neither macrophages nor of microglial type. The cysteine-rich periventricular cells are also negative for alcohol dehydrogenase, lactate dehydrogenase, and NADH diaphorase[7]. A hypothesis was advanced recently[2,4] as to the role of the cysteine-rich glial cells. According to this, they form a protective system of the brain, connected with the blood-brain barrier, trapping blood-borne toxic substances such as heavy metals, oxidants, stable organic free readials, etc. Earlier experiments of WISLOCKI and LEDUC[8] support this hypothesis. These authors showed that prolonged ad-

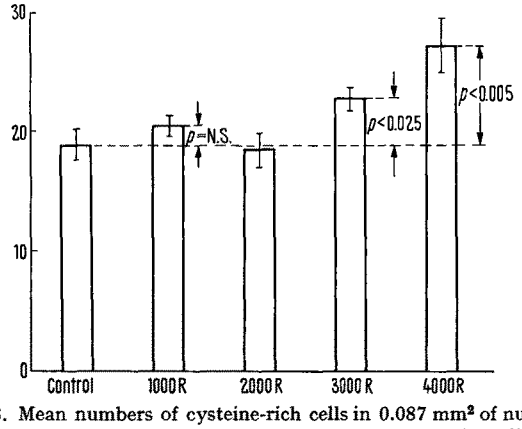

Fig. 3. Mean numbers of cysteine-rich cells in 0.087 mm² of nucleus arcuatus. 7th post-irradiation day. N.S., difference statistically not significant; vertical bar, standard error.

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ministation of silver nitrate causes accumulation of silver granules in the periventricular glial cells. The increase now observed in the number of the cysteine-rich glial cells in X-ray irradiated animals may be due to enhanced synthesis of the cysteine-rich material during the post-irradiation period in response to the presence of toxic substances arizing in consequence of the irradiation. The lack of lysosomal enzymes in the cysteine-rich periventricular glia[5] is against the possibility that the cysteine-rich granulations arise from a phagocytosed material.

Zusammenfassung. Nach einmaliger Röntgen-Kopfbestrahlung bei Ratten mit 3000 und 4000 R wurde eine statistisch gesicherte Zunahme der Zahl cysteinreicher periventrikulärer Gliazellen im Gehirn festgestellt.

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Isolation of a Pharmacologically Active Principle from Ehrlich Carcinoma Tumor Cells

The presence of pharmacologically active substances in tumor tissue has been relatively little investigated. A polypeptide undistinguishable from bradykinin has been isolated from human pulmonary carcinoma[1]. Overproduction of serotonin is an almost uniform finding in patients with carcinoid tumors[2]. Liberation of kalikrein into the blood stream with subsequnet formation of bradykinin has been demonstrated in patients with carcinoid tumor metastases stimulated by epinephrine[3]. GREENBAUM et al.[4] have found kinin forming and destroying enzymes in mice leukemia L 1210 cells.

We report here the isolation of a pharmacologically active principle from mice ascites carcinoma cells. 5×10^8 Ehrlich ascites tumor cells were extracted for each experiment by repeated freezing and thawing in saline. The cell fragments were removed by high speed centrifugation and the supernatant used in the experiments. Similar amounts of Ehrlich ascites tumor cells were extracted with 10% trichloracetic acid. The supernatants were subsequently extracted with ether, the aqueous phase then evaporated to dryness, and the residue redissolved in 10 ml saline and the pH adjusted to 7.2. The saline extracts will

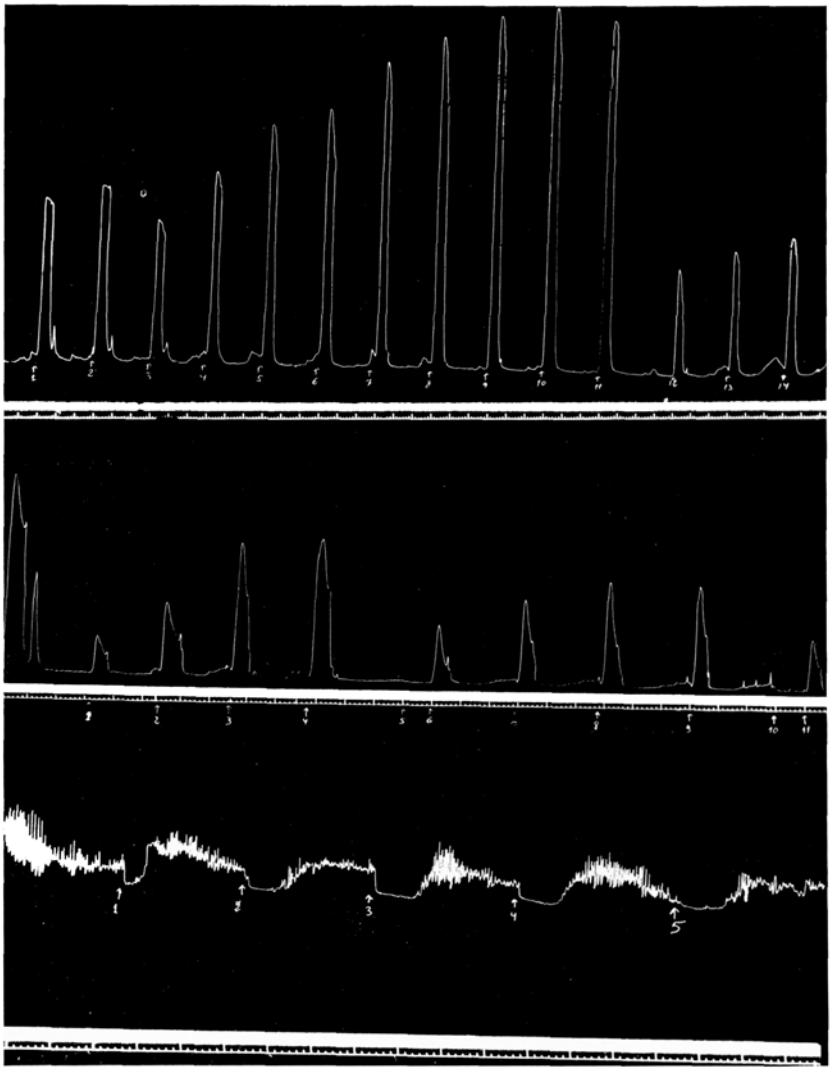

Fig. 1. Effect of Ehrlich ascites carcinoma tumor extract on the isolated guinea-pig ileum. Experiment 1. Isolated guinea-pig ileum suspended in 10 ml of Tyrode solution. Time of contact 1 min. 1 and 2, 0.01 γ acetylcholine; 3, 0.1 ml; 4, 0.15 ml; 5, 0.2 ml; 6 ,0.25 ml; 7, 0.3 ml; 8, 0.4 ml; 9, 0.5 ml; 10, 0.6 ml; 11, 0.8 ml of TCA extract (TE); 12, partial neutralization of extract by 1 γ atropine 0.6 ml extract after 1 γ atropine; 13, 0.6 ml 7 min after 1 γ atropine; 14, 0.6 ml 10 min after 1 γ atropine.
Experiment 2. Isolated guinea-pig ileum suspended in 10 ml of Tyrode solution. Time of contact 1 min. 1, 0.2 ml; 2, 0.4 ml; 3, 6 and 11, 0.8 ml; 4, 1.2 ml of saline extract (SE); 5, 1 γ atropine; 7, 0.8 ml extract 4 min after 1 γ atropine; 8, 0.8 ml extract 7 min after 1 γ atropine; 9, 0.8 ml extract 10 min after 1 γ atropine; 10, 2 γ atropine.
Experiment 3. Effect of Ehrlich ascites carcinoma tumor extract on the isolated mouse ileum suspended in 10 ml of Tyrode solution. Time of contact 1 min. 1, 0.1 ml; 2, 0.2 ml; 3, 0.4 ml; 4, 0.8 ml of extract (TE); 5, 1 γ atropine + 0.4 ml extract.