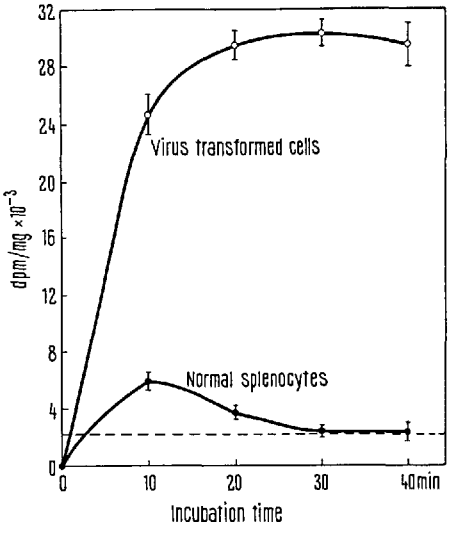
Viral Leukemia: Increased Thymidine Assimilation

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 At intervals, a 1 ml aliquot was withdrawn and placed in a tared 2 ml centrifuge tube. 1 ml of iced phosphate buffered saline was added, and the tube was centrifuged at 250 *g* for 1 min at 0 °C. The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.

R. F. Hagemann

Cellular and Radiation Biology Laboratories, Department of Radiology, Allegheny General Hospital, 320 East North Avenue, Pittsburgh (Pennsylvania 15212, USA), 4 March 1971.

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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,