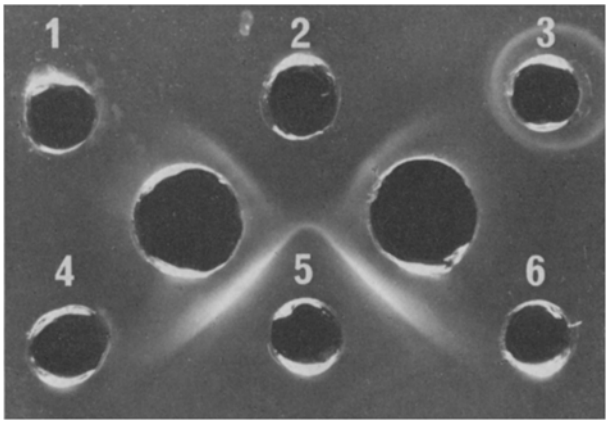
the treatment of acute myelocytic leukaemia, precipitating and complement-binding antibodies were demonstrated[20]. We investigated the cross-reaction between *E. coli* L-asparaginase preparation and L-asparaginases obtained from *Fusarium* species on agar-immuno-diffusion. Anti-*E. coli* L-asparaginase antiserum was raised in rabbits by multiple injection of *E. coli* L-asparaginase (Worthington Biochem. Co.) mixed in Freund's complete adjuvant. As seen in the Figure, *Fusarium* and *Hypomyces* L-asparaginase preparations gave no identical precipitation lines with *E. coli* L-asparaginase. Thus, L-asparaginases from *Fusarium* and *Hypomyces* were antigenically different from *E. coli* L-asparaginase.



Immunoprecipitation of several kinds of L-asparaginases against anti-*E. coli* L-asparaginase antiserum. The antiserum was raised in rabbits by injecting i.v., 2 ml of L-asparaginase from *E. coli* (Worthington, 200 IU/6 mg/2 ml) in physiological saline solution twice 3 days apart. 1 and 3 weeks later, rabbits were boosted s.c. with the same amount of the L-asparaginase emulsified in Freund's complete adjuvant (Difco). The sera were collected 1 week after the last injection. Micro-gel diffusion was carried out on microslides using 1% agar (Difco, Noble) in veronal buffer, 0.06 *M*, pH 8.6. Central wells: 50 µl of anti-*E. coli* L-asparaginase rabbits sera. Peripheral wells: 10 µl of 0.2% of *E. coli* L-asparaginase and 10 µl of 2% of other L-asparaginase preparations: 1. *Fusarium solani*, IPFO 5893; 2. *Escherichia coli* (Kyowa, crystalline); 3. *Fusarium oxysporum*, IFO 9660; 4. *Fusarium oxysporum*, IFO 9331; 5. *Escherichia coli* Worthington; 6. *Hypomyces solani*, IFO 9661.

It may be important to supply various L-asparaginases which differ in the antigenic properties to each other, so as to avoid the problems arising from antigen-antibody reactions such as neutralization of the enzyme activity or anaphylactic shock.

SCHEETZ et al.[21] have pointed out the inability of L-asparaginase from mycelia of *Fusarium tricinctum* to suppress Gardner lymphosarcoma; therefore, we should like to examine the properties and antilymphoma activities of intracellular L-asparaginases of *Fusarium* species studied in the present report.

Résumé. Les champignons de *Fusarium* et ceux qui ont le type fusarium dans leur état asexues sécrétent l'asparaginase. Celle-ci n'a pas l'activité de la glutaminase et elle arrête le developpement de la leucémie chez les souris.

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Equivalence of Continuous Infusion and Single Injection of ^{3}H-Thymidine for Analysis of Intravascular Kinetics of Neutrophilic Granulocytes in the Rat

A quantitative description of the turnover of neutrophilic granulocytes requires the measurement of the mean intravascular lifespan of these cells. In dogs[1] and in man[2], autotransfusion studies after in-vitro labelling with ^{32}P-diisopropylfluorophosphate and subsequent scintillation counting of isolated leukocyte samples have been used to determine this parameter. The method is, however, not applicable to small laboratory animals. A new experimental approach to this problem was therefore developed using continuous infusion of ^{3}H-thymidine and autoradiography to determine the replacement of unlabelled peripheral blood granulocytes by labelled granulocytes coming from the bone marrow. Results for the rat, as well as a discussion of the principles of the method, were published previously[3,4]. Experiments reported here demonstrate that continuous infusion of ^{3}H-thymidine in this experimental system can be replaced by a single injection of the radioactive precursor, resulting in a considerable simplification of the technical procedure.

Materials and methods. ♂ Wistar AF-Han rats (250–350 g) were used in the experiments. For a period of 120 h the animals received a continuous infusion of either ^{3}H-thymidine in 0.9% saline (3 µCi/g body wt. per day) or 0.9% saline following a single i.v. injection of ^{3}H-thymidine (2 µCi/g body wt.). Blood samples were obtained at 12-h-intervals by repeated punctures of the tail artery[5]. The percentage of labelled blood granulocytes was determined by autoradiography of leukocyte-enriched blood smears. Details of the methods employed have been published[4].

Results. The percentage of labelled blood granulocytes obtained at various times after starting a continuous infusion or after giving a single i.v. injection of ^{3}H-thymidine is shown in Figure 1. It can be seen that the replacement of unlabelled blood granulocytes by labelled granulocytes from the marrow is the same for both schedules of

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tracer application. Because of this equivalence of the two labelling procedures, it was justified to use blood granulocyte labelling after one single injection of ^{3}H-thymidine for the determination of the intravascular lifespan of neutrophils. Figure 2 shows the results of such experiments obtained in 8 different rats. A semi-logarithmic plot of the percentage of unlabelled blood granulocytes shows an exponential decrease of these cells beyond 72 h after tracer application with a half-life (T 1/2) of 6.0 h. The corresponding value for the mean intravascular lifespan of neutrophilic granulocytes is 8.7 h (T 1/2 divided by ln 2).

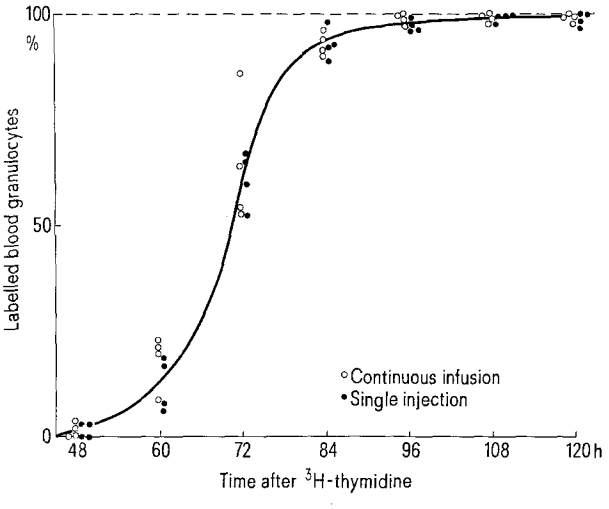

Fig. 1. Percentage of labelled neutrophilic granulocytes in the blood of rats during continuous infusion (open circles) and after single injection (full circles) of ^{3}H-thymidine.

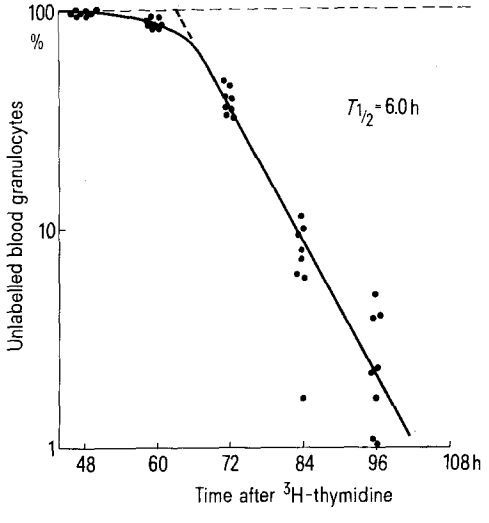

Fig. 2. Disappearance of unlabelled neutrophilic granulocytes from the blood stream of rats after a single injection of ^{3}H-thymidine (semilogarithmic scale).

Discussion. The results presented show that the labelling pattern of neutrophilic blood granulocytes in the rat is identical for continuous infusion and single injection of ^{3}H-thymidine. This finding is at variance with results of previous experiments[4], but the discrepancy can easily be explained. Analysis of the labelling intensity of blood neutrophils shows a progressive increase with time in continuous infusion experiments, a progressive decrease in single injection experiments. In the latter type of experiment, cells containing low levels of radioactivity are easily scored as 'unlabelled' cells in case the exposure time of autoradiograms is insufficient. To exclude this source of error, in the present experiments slides were examined after stepwise prolongation of exposure times until the percentage of labelled cells remained constant. Using this procedure, the percentage of labelled blood granulocytes obtained after a single injection of ^{3}H-thymidine did reach congruence with the values measured during continuous tracer infusion.

Continuous infusion of ^{3}H-thymidine results in labelling of every single cell leaving the proliferating granulocyte precursor pool of the bone marrow. In contrast, a single injection of ^{3}H-thymidine initially labels only that fraction of proliferating precursors engaged in DNA synthesis at the moment of tracer injection, due to the short availability time of the nucleoside. Cells in G_1, G_2 and M remain unlabelled, so that a mixture of labelled and unlabelled cells is expected to leave the proliferating precursor pool. From this point of view, the identical labelling pattern of blood granulocytes observed in our experiments after the two different schedules of tracer application remains unexplained. It must be kept in mind, however, that re-utilization of DNA occurs in the bone marrow of rats[6]. Indeed, further experiments have shown that the results obtained are dependent on re-utilization of labelled DNA breakdown products[7]. This phenomenon leads to continuous availability of radioactive DNA precursors in the bone marrow of rats, even after 'pulse' labelling with ^{3}H-thymidine, and can be successfully utilized to analyse intravascular granulocyte kinetics, as demonstrated by the experiments reported here. The intravascular half-life of 6.0 h for neutrophils obtained after 1 single injection of ^{3}H-thymidine is in excellent agreement with the figure of 5.7 h reported for conditions of continuous application of the radioactive precursor[4].

Zusammenfassung. Einmalige und kontinuierliche Applikation von ^{3}H-Thymidin führen bei der Ratte zu identischen Markierungskurven der Blutgranulocyten. Eine Einzelinjektion des radioaktiven Vorläufers ermöglicht deshalb die autoradiographische Analyse der intravaskulären Granulocytenkinetik.

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