considered reproducible. These calculations illustrate the importance of high specific activity ^{55}Fe for cytocidal studies. They also emphasize the necessity of exact analysis of iron content in samples which conventionally may be designated as 'carrier-free'.

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2 M.G. Berger, National Bureau of Standards, Internal Report, p. 73107. Washington, D. C. 1973.
3 U. Reincke, H. Burlington, E.P. Cronkite, M. Hillman and J.A. Laissue, Blood *45*, 801 (1975).
4 U. Reincke, D. Brookoff, H. Burlington, E.P. Cronkite, N. Pappas and E. Zanjani, Radiat. Res. *74*, 66 (1978).
5 U.R. Reincke, D. Brookhoff, H. Burlington and E.P. Cronkite, Blood Cells, in press.
6 J.A. Laissue, H. Burlington, E.P. Cronkite and U. Reincke, Cancer Res. *37*, 3545 (1977).
7 J.A. Laissue, H. Burlington, E.P. Cronkite, B. Heldman and U. Reincke, in preparation.
8 S.T. Taketa, A.L. Carsten, S.H. Cohn, H.L. Atkins and V.P. Bond, Life Sci. *9*, 169 (1970).
9 W.D. Noyes, T.H. Bothwell and L.A. Finch, Br. J. Haemat. *6*, 4355 (1960).
10 M. Pollycove, Sem. Hemat. *3*, 235 (1966).
11 B.A. Cheney, K. Lathe, E.H. Morgan, S.K. Sood and C.A. Finch, Am. J. Physiol. *212*, 376 (1967).
12 I. Cavill and G. Ricketts, in: Iron in Biochemistry and Medicine, p. 614. Ed. A. Jacobs and M. Wotwood. Acad. Press, New York 1974.
13 E.H. Belcher, E.B. Harris and L.F. Lamerton, Br. J. Haemat. *4*, 390 (1958).
14 J. Fletcher, Clin. Sci. *41*, 395 (1971).
15 A.M. Ganzoni, B. Baviera and D. Hahn, Experientia *30*, 32 (1974).
16 P. Pootrakul, A. Christensen, B. Josephson and C.A. Finch, Blood *49*, 957 (1977).
17 E.H. Morgan and C.A. Finch, Biol. Med. *122*, 92 (1966).
18 N. Biewald and J. Billmeier, Experientia *34*, 412 (1978).

A new method for measuring radioactivity in select areas of intact tissue sections[1]

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Summary. A new technique is described for measuring radioactivity in select areas of intact tissue sections. The method, which makes use of a liquid scintillation counter, also makes possible identification of the studied zones by prestaining.

Incorporation of labelled compounds into different areas of tissue sections may be assessed by 2 common techniques. 1 of these utilizes a liquid scintillation counter and requires cutting off samples from the sections[3]. The other is the prolonged method of autoradiography[4]. This report describes a new scintillation technique for determining the level of radioactivity in select areas of intact sections.

Materials and methods. Male albino rats were injected into the left brain ventricle with 100 µCi ^{35}S-methionine. Injected brains were removed 30 min later and coronal sections of 8, 16, 32, 64 and 128 µm thickness were sliced in cryostat, mounted on pieces of glass slide and then fixed in Carnoy's fluid (3 parts ethyl alcohol + 1 part acetic acid).

A) For counting the radioactivity in select areas, slides bearing sections 64 µm thick were covered with transparent paper and the select areas were marked off by pencil. This paper was then fastened onto aluminium foil and apertures of 0.5 mm diameter were bored with appropriately sharpened syringe needle at the points indicated on the paper. The perforated foil was now placed on the section-bearing slide with the apertures occurring at the pre-selected areas. The back of the slide was covered by a continuation of the same piece of foil. To expose only 1 select area through 1 aperture in the foil, a solid aluminium plate with a single aperture was used (figure 1). This assembly, comprising the slide, section, foil and plate all held together by an ordinary paper clip, was transferred into a Tri-Carb liquid scintillation spectrometer (Packard), vial filled with scintillator (Insta-Gel, Packard). To measure radioactivity in other regions of the section, the aluminium plate was shifted each time to expose a new area for counting. Identification of histological structures in the specimen was made possible by staining with hematoxylin-eosin, Feulgen, Giemsa or other stains.

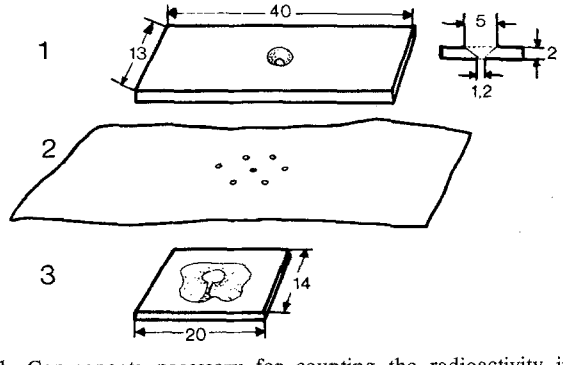

Fig. 1. Components necessary for counting the radioactivity in select areas of the tissue sections by liquid scintillation spectrometer (sizes in mm). *1* Aluminium plate. *2* Aluminium foil. *3* Section on glass slide.

B) Absorption of radioactivity by sections of different thickness was evaluated by comparing the radiation level of the intact section with that following its solubilization. The former was determined by use of a vial containing 4 ml of scintillator and 1 ml of tissue solubilizer (Soluene 350, Packard); the solubilizer, although inactive at such a ratio, was added to yield the same concentration of scintillator molecules as in the section undergoing solubilization. Solubilization of the sections was effected by breaking the slides and immersing the resulting fragments for 3 days at 37 °C in 0.9 ml of tissue solubilizer and 0.1 ml distilled water. The vials were shaken throughout to enable penetration of the solution between the broken pieces and the release of air bubbles. At the end of the incubation, 4 ml of scintillator were added to each vial. For the various thicknesses of sections, the ratio of radioactivity level of the intact section to that of the solubilized one was expressed in percent.

Results. For purposes of illustration of the technique, the values of the radioactivity measured in some areas of the rat brain are presented in the table. As figure 2a indicates, the correlation between the radioactivity of the solubilized

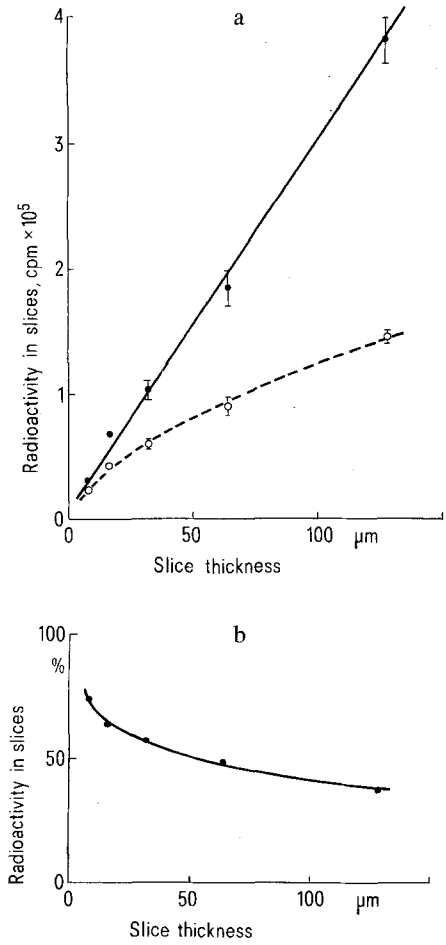

Fig. 2. *a* Radioactivity counts of the whole intact sections (●——●) and radioactivity of the same sections following their solubilization (○- - - -○) against their thickness; means of measurements ±SEM for 3–5 sections. *b* Ratios of the radioactivity levels before and after solubilization of the sections (in %) against their thickness.

Radioactivity of a number of brain structures after injection of ^{35}S-methionine into the left lateral brain ventricle; counts for section of 64 µm thickness through apertures of aluminium foil. Corrections on the basis of figure 2b

Structure	Counts (cpm)	Corrected values of the counts
Hippocampus	344	688
Medial thalamic nucleus	260	520
Plexus choroideus in the 3rd ventricle	178	356
Paraventricular nucleus, hypothalamus	37	-
Background	35	-

sections and their thickness is linear. However, the radioactivity of the nonsolubilized intact sections is not proportional to the increase in their thickness (figure 2a). Figure 2b shows that the ratio of 'surface' radioactivity of intact, nonsolubilized sections to that after their dissolving changes from about 75% for 8-µm thick sections to about 40% for sections of 124 µm thickness.

The data from the table can be corrected with the aid of figure 2b to yield the real values of radioactivity extent under the aperture in the foil. For sections of 64 µm thickness, the increment is in the order of 50% (table). This calibration curve is valid only for brain tissue, since other tissues obviously have their own characteristic absorption of radioactivity. However, the method itself is simple, rapid and reproducible, and may be applied for investigation of various tissues.

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3 K.B. Eik-Nes and K.R. Brizzee, Biochim. biophys. Acta *97*, 320 (1964).
4 A.W. Rogers, Techniques of autoradiography. Elsevier Publishing Comp., New York 1969.

A simple technique for testing the in vitro response of rabbit lymphocytes to PHA and allogeneic cells[1]

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Summary. Lymphocytes of rabbits can be separated from small quantities of heparinized whole blood using a simple density gradient of Ficoll-Ronpacon 1.09. This separation technique yields a pure suspension of viable cells allowing reproducible results from cultures stimulated either with PHA or allogeneic lymphocytes isolated by the same technique.

Histocompatibility studies and immunological function tests rely on reproducible lymphocyte cultures in animal models and in man. Numerous techniques for rabbit lymphocyte cultures have been described[2-6]. However, important differences in lymphocyte separation technique made a direct comparison impossible with methods used in human lymphocyte studies where reproducibility from laboratory to laboratory is good.

For our studies on bone marrow transplantation, we needed a simple test system for the rabbit allowing repeated cultures from the same animal, in order to assess histocompatibility by mixed lymphocyte culture (MLC).

We used a slight modification of a sedimentation gradient with Ficoll-Ronpacon employed routinely in human tests. Performing the sedimentation on a higher density gradient allowed lymphocyte cultures with reproducible stimulation by PHA and allogeneic cells with small amounts of peripheral blood obtained from living animals.

Materials and methods. 1. Animals. Normal outbred rabbits of 2 different strains, Burgundy and Dutch, were used as lymphocyte donors. Mixed lymphocyte cultures were performed between the 2 strains, which were supposed to be histo-incompatible.

2. Lymphocyte separation. 8–10 ml of heparinized blood (1000 IU/10 ml Liquemin®) was drawn from the artery of the ear under sterile conditions and diluted with 10 ml phosphate buffered saline (PBS). 4 ml of diluted blood was layered on 2 ml Ficoll-Ronpacon with a density of 1.09 and