A needle of 0·6 mm diameter enables only detached cells to be aspirated; these can be studied only cytologically and not by the usual histological methods. Thus the information gained on the general state of the liver is not so comprehensive as from a tissue sample taken by the usual coarse-needle technique. Nevertheless, much information can be obtained (Søderstrøm, 1966), and fine-needle aspirates are usually adequate for diagnosing malignancy. The use of a thin needle also allows specimens for biopsy to be obtained from a greater depth (we have aspirated material from a depth of 15 cm).

It seems from these preliminary results in a small series of patients that ultrasonically-controlled fine-needle aspiration of material from the liver for biopsy is more accurate than the Menghini method in the diagnosis of liver metastases. There is no reason why the ultrasonically-controlled method should not be applied in other cases of focal liver disease, especially primary cancer of the liver.

References

Conn, H. O., and Yesmer, R. (1963). *Annals of Internal Medicine*, **59**, 53.
Holm, H. H. (1971). *British Journal of Radiology*, **44**, 24.
Holm, H. H., Kristensen, J. K., Rasmussen, S. N., Northeved, A., and Barlebo, H. (1972). *Ultrasonics*. **10**, 83.
Holmes, J. H. (1966). In *Diagnostic Ultrasound*, ed. C. C. Grossman, J. H. Holmes, C. Joyner, and E. W. Purnell, p. 249. New York, Plenum Press.
Holmes, J. H., and Howry, D. H. (1963). *American Journal of Digestive Diseases*, **8**, 12.
McCarthy, C. F., *et al.* (1970). *British Journal of Radiology*, **43**, 100.
Sherlock, S. (1968). *Diseases of the Liver and Biliary System*, 4th edn., p. 673. Oxford, Blackwell Scientific.
Søderstrøm, N. (1966). *Fine Needle Aspiration Biopsy*, p. 122. Stockholm, Almquist and Wiksell.

Hepatic Homing of Labelled Lymphocytes in Man

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Summary

The homing of human lymphocytes has been studied in man by means of chromium labelling and external organ counting. The method was applied to the study of lymphocyte alteration induced by antilymphocyte globulin (A.L.G.). At non-cytotoxic concentrations A.L.G. has an opsonizing effect on lymphocytes, increasing selectively their homing to the liver. No correlation could be defined between in-vitro cytotoxicity or rosette-inhibiting activity and hepatic fixation. These results suggest that A.L.G. may act by promoting lymphocyte trapping by the reticuloendothelial cells.

Introduction

In animals lymphocyte circulation has been well documented in several studies using labelled lymphocytes (generally with ^{51}Cr or tritiated thymidine) (Zatz and Lance, 1970; Parrott and De Sousa, 1971). In man, on the other hand, much less information is available for lymphocytes (Hersey, 1971) although many studies have been done with other types of labelled blood cells—for example, red cells, platelets, and leucocytes (Eyre *et al.*, 1970). We have labelled purified human peripheral lymphocytes in vitro and studied their homing after intravenous injection into the donor. We have studied antilymphocyte globulin (A.L.G.) activity by incubating labelled lymphocytes with A.L.G. before their injection. This work was based on work in mice and rats by several investigators (Taub, and Lance 1968; Martin, 1969a), who reported that lymphocyte opsonization with A.L.G. was correlated with immunosuppressive potency.

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Materials and Methods

Subjects.—Eight normal subjects and six patients with mild renal failure (creatininaemia less than 50 mg/100 ml) were investigated. Thirty-six measurements were made.

Lymphocyte Preparation and Labelling.—Lymphocytes were isolated from peripheral blood (25 ml collected in heparin) according to the method of Harris and Ukaejiofo (1969) by using a Ficoll-Triosil mixture. They were then labelled with sodium chromate-51 (C.E.A., Saclay: average specific radioactivity 300 $\mu Ci/\mu g$). The labelling was performed according to a modification of the technique of McCall *et al.* (1955). Lymphocytes ($10\text{-}30 \times 10^6$) were incubated in 0·4 ml of Hanks's medium with ^{51}Cr (10 $\mu Ci/10^6$ lymphocytes) for 45 minutes at 37°C and then washed twice. The criteria for quality and stability described by Bunting *et al.* (1963) were verified.

A.L.G.—Three horse antihuman A.L.G.s were used. Details of their preparation and activities in vitro are given in the Table. Methods used for cytotoxicity and rosette inhibition were those reported by Bach *et al.* (1969). All A.L.G. batches were heated at 56°C for 30 minutes before use. A.L.G. or normal serum was incubated at 37°C for 45 minutes with lymphocytes (average 20×10^6 in 0·4 ml). The concentration of A.L.G. was generally 1/1,000 for antilymphocyte sera 1 and 3. For A.L.G. 2 several concentrations were used (1/500, 1/1,000, 1/2,000, and 1/4,000). Normal horse serum was tested at a concentration of 1/1,000. Lymphocyte viability was evaluated by the trypan blue exclusion test before the injection and preparations with more than 10% dead cells were discarded. An average of 3×10^6 lymphocytes labelled with about 3 μCi of ^{51}Cr were injected.

Preparation and In-vitro Activities of Normal Serum and A.L.G.

	Origin	Antigen	Cytotoxicity Titre	Rosette Inhibition Titre
A.L.G. 1 (LH 023)	Horse	Human blood lymphocytes	1/512	1/8,000
A.L.G. 2 (90623)	Horse	Human blood lymphocytes	1/4,000	1/16,000
A.L.G. 3 (H 329 B)	Horse	Human blood lymphocytes	1/1,000	1/32,000
Normal serum	Horse		<1/100	<1/100

Detection of Radioactivity.—Radioactivity was measured with a 20 by 10 cm INa (T1) crystal scintillator equipped with a cylindrical collimator (8 cm internal diameter). Data were analysed by a 400-channel analyser (Intertechnique) which gave the value of peak activity of ^{51}Cr. Radioactivity was counted over the liver, spleen, precordium, and thigh. Each area was given precise geometric values. Counts were taken before injection and at 4 hours, 12 hours, and on the following seven days.

Homing Index.—We postulate that homing radioactivity in the counted organ after background substraction is illustrated by

the difference between organ counting minus thigh counting. Homing index is calculated by dividing this difference by the amount of injected microcuries (c.p.m./1 μCi).

Results

Activity over the precordium and thigh was almost constant from day to day (30 to 90 c.p.m./μCi injected) and these values were thus used as references. Hepatic activity increased during the six hours after the injection of labelled lymphocytes. A plateau was reached after 12 hours and the activity began to decrease after 24 hours (7-10%/day). The peak hepatic activity was always 10 times more than thigh or precordium activity. Splenic activity varied greatly but was never three times higher than the reference activity. The activity in one subject who received labelled lymphocytes incubated respectively with normal serum and with A.L.G. 1 is shown in Fig. 1. Homing index measured at the 24th hour was selected to compare the experiments.

Incubation of lymphocytes with A.L.G. immediately before injection modified their migration by increasing their homing to the liver. Whereas the homing index was 104 ± 9 (S.D.) after incubation with normal serum (concentration 1/1,000) it became 234 ± 51 with A.L.G. 1, 164 ± 37 with A.L.G. 2, and 167 ± 19 with A.L.G. 3 (concentration 1/1,000). The difference between normal serum and all batches of A.L.G.

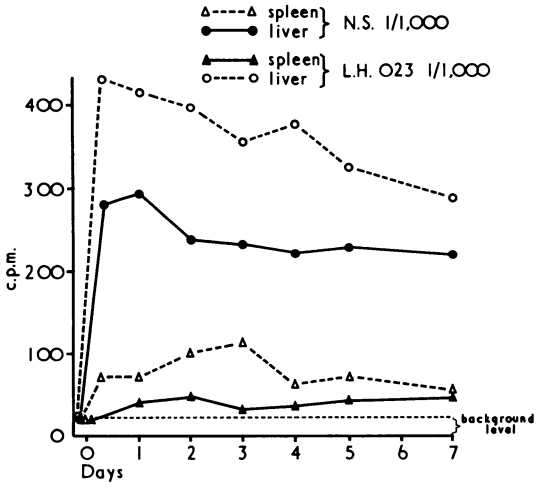

FIG. 1—Hepatic and splenic homing in normal subject after injection of 3 μCi of chromium-labelled lymphocytes incubated with normal serum (N.S.) or A.L.G.

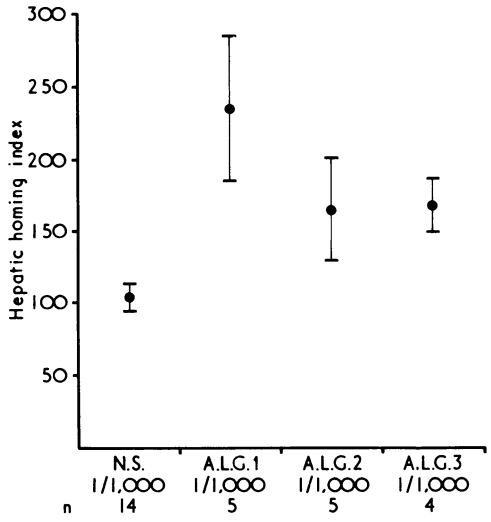

FIG. 2—Hepatic homing index of labelled lymphocytes incubated with normal serum (N.S.) or A.L.G. (mean value ± S.D.).

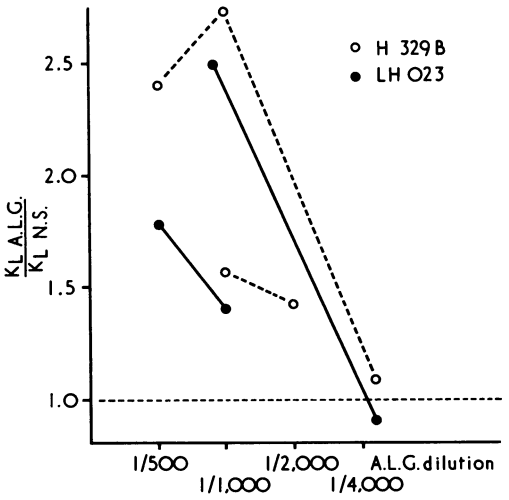

FIG. 3—Hepatic homing index ratio between A.L.G. and normal serum (N.S.) in the same subject versus A.L.G. dilution.

(Fig. 2) is highly significant ($P < 0.001$). This index depended on the concentration of A.L.G. used for the incubation and on the A.L.G. batch. For graded concentrations of A.L.G. studied in four subjects (Fig. 3) only higher concentrations of A.L.G. (1/500, 1/1,000) induced selective homing to the liver. Since A.L.G. can induce sensitization against horse protein all of the subjects tested received only a single batch of A.L.G.

The effect of normal serum and the three batches of A.L.G. were compared at the concentration 1/1,000. The first batch was tested on five subjects, the second on five subjects, and the third on four subjects. A.L.G. 1 induced a significantly greater migration to the liver than A.L.G. 2 and A.L.G. 3. There was no correlation between the opsonizing activity as defined by liver migration and in-vitro activities shown in the Table, since the best opsonizing serum was the least cytotoxic and not the best rosette inhibitor.

Discussion

This study of lymphocyte homing in man was performed with a sensitive detector. The mean dose of radioactivity injected, 3 μCi, presents no risk for the subject and repeat injections can be given. Such small amounts of radioactivity make it difficult to define the areas corresponding to each organ, especially when the organ is not perfectly localized, as in the case of the spleen in the absence of splenomegaly or without previous scintigraphic detection. After injection of single chromium-labelled lymphocytes the activity over the spleen is just above background and not very different from the activity counted over the thighs or the precordium. Under our technical conditions lymphocyte migration can be studied only by their accumulation in the liver, where precise geometric criteria are available. Hepatic homing, as measured by the index defined above, is related to the percentage of injected lymphocytes going to the liver, since chromium which is eluted from dead cells is not electively trapped in the liver and is not taken up by unlabelled lymphocytes (Mertz, 1969).

Our study shows that A.L.G. in man has the same opsonizing activity as in the mouse (Greaves *et al.*, 1969); the influence of A.L.G. on lymphocyte homing in the liver is probably linked to phagocytosis, as has been shown in rodents (Harris *et al.*, 1971; Zatz and Lance, 1971). In the mouse A.L.G. decreases the clearance of carbon by the Kupfer cells (Sheagren *et al.*, 1969). The similarities with the rodents, however, cannot be discussed further because of technical limitations in accurate evaluation of total organ radioactivity and the impossibility of detecting activity in lymph nodes. This limitation may explain the lack of correlation with immunosuppressive potency (Martin, 1969b). The absence of correlation with cytotoxicity strongly suggests

that the elective migration to the liver is probably not due to a cytotoxic effect of antilymphocyte globulin occurring in vivo after introduction of the lymphocytes coated with A.L.G., but rather to an opsonizing effect involving different mechanisms and the action on the first four components of complement (Gigli and Nelson, 1968).

We thank Dr. J. Dormont for his valuable advice about the choice of A.L.G. and Mrs. M. Brami for technical help. Dr. M. Raynaud, of the Institut Pasteur, Paris, supplied the house antihuman A.L.G.s.

References

Bach, J. F., Dormont, J., Dardenne, M., and Balner, H. (1969). *Transplantation*, **8**, 265.
Bunting, W. L., Kiely, J. M., and Owen, A., jun. (1963). *Proceedings of the Society for Experimental Biology and Medicine*, **113**, 370.
Eyre, H. J., Rosen, P. J., and Perry, S. (1970). *Blood*, **36**, 250.
Gigli, I., and Nelson, R. A. (1968). *Experimental Cell Research*, **51**, 45.
Greaves, M. F., *et al.*, (1969). *Lancet* **1**, 68.
Harris, N. S., Merino, G. E., and Najarian, J. S. (1971). *Transplantation Proceedings*, **3**, 797.
Harris, R., and Ukaejiofo, E. O. (1969). *Lancet*, **2**, 327.
Hersey, P. (1971). *Blood*, **38**, 360.
McCall, M. S., Sutherland, D. A., Eisentraut, A. M., and Lanz, H. (1955). *Journal of Laboratory and Clinical Medicine*, **45**, 717.
Martin, J. W. (1969a). *Journal of Immunology*, **103**, 979.
Martin, J. W. (1969b). *Journal of Immunology*, **103**, 1000.
Mertz, W. (1969). *Physiological Reviews*, **49**, 163.
Parrott, D. M. V., and De Sousa, M. (1971). *Clinical and Experimental Immunology*, **8**, 663.
Sheagren, J. N., Barth, R. F., Edelin, J. B., and Malmgren, R. A. (1969). *Lancet*, **2**, 297.
Taub, R. N., and Lance, E. M. (1968). *Immunology*, **15**, 633.
Zatz, M. M., and Lance, E. M. (1970). *Cell Immunology*, **1**, 3.
Zatz, M. M., and Lance, E. M. (1971). *Journal of Experimental Medicine*, **134**, 224.

MEDICAL MEMORANDA

Danger of Skin Burns from Thiomersal

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Thiomersal has been in common use as a preoperative skin preparation for several years in this hospital. A standard solution of 1:1,000 thiomersal in 50% alcohol is used. A case is reported here in which skin burns resulted from the chemical interaction of thiomersal (Merthiolate) and aluminium.

Case Report

On 28 June 1971 preparation was made to perform an abdominal hysterectomy and salpingo-oophorectomy. The patient was placed on a standard rippled surface rubber mattress on the operating table and a paper-backed aluminium foil diathermy electrode 20 by 6 in (51 by 15 cm) was arranged under her buttocks. This was connected to a surgical diathermy set by a genitourinary connector having four press studs fixed through the foil.

The vagina was cleaned with a single application of thiomersal and two applications were made to the abdominal skin to clean the site of the incision.

A normal amount of diathermy was used to coagulate bleeding vessels during the opening of the abdomen. The operation was uneventful.

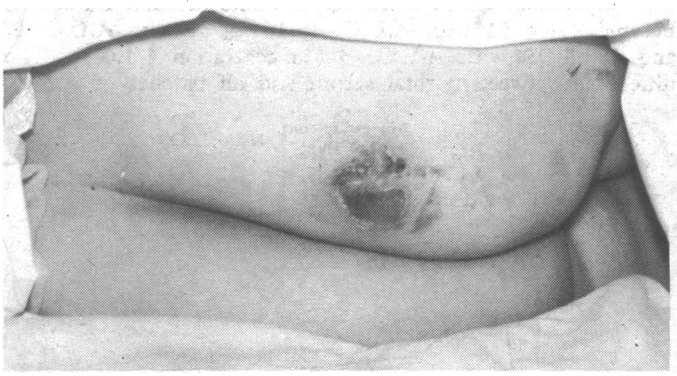
The next day the patient complained of pain at the buttocks. On examination a blister 2 in (5 cm) in diameter which had burst was found on the right buttock. There was a surrounding area of erythema (see Fig.).

The site of the lesion corresponded to the point of contact of the aluminium diathermy electrode. Because of the possibility of the burn being due to the use of the diathermy the advice of the Department of Health and Social Security was sought.

The design and use of the diathermy were considered to have conformed to accepted practice and electrical burning was not likely to have occurred.

A number of tests were made for chemical interaction between thiomersal and aluminium.

Patch Test.—This was carried out on a safety officer from the Department of Health and Social Security, who volunteered. A 2 in (5 cm) square of six thicknesses of open woven bandage was soaked in thiomersal, placed in the centre of a 3 in (7·5 cm) square of aluminium foil, and fixed to the chest with adhesive plaster. Surgical diathermy was not used. After about five minutes, when the thiomersal had largely evaporated or been absorbed, a series of burning sensations were felt along two of the ½ in (1·3 cm) wide strips where the aluminium was in direct contact with the skin. The centre of the electrode felt warm. More thiomersal was added, and this resulted after a short delay in further sensations of heat burns. After about 20 minutes the reactions died down and the patch was removed. The aluminium foil had largely disappeared, leaving a pink and white powder. Six hours or so later about eight small blisters appeared in an L formation where the aluminium had been in contact with the skin.



Burst blister on buttock surrounded by erythema.

Patch Test for Electrolytic Action.—This test was also performed on the safety officer from the Department of Health. A patch of foil about 2 in (5 cm) square was applied to the skin close to a second patch consisting of a 3 in (7·5 cm) square of bandage soaked in thiomersal with an aluminium electrode 2 in (5 cm) square placed above it which was connected to the first electrode by a microammeter. A current of 4 μA was registered. No lesions were produced.

Chemical Reaction Test.—About 1 ml of thiomersal was poured on to each of three different types of aluminium patient electrode and observed. When the liquid had almost all evaporated chemical action started, and by the time the liquid had completely evaporated a number of pillars of aluminium oxide had formed on the two thicker foils and a loose oxide powder on the thinnest paper-backed foil. (A similar reaction has been observed when thiomersal is allowed to stand in disposable aluminium foil galley-pots used for skin preparation agents).

Heat Generation Test.—(1) A piece of paper-backed aluminium foil 4 by 2 in (10 by 5 cm) was folded into a trough, paper inside, filled with about 0·5 ml of thiomersal, and wrapped around the bulb of a mercury-in-glass thermometer 0-110°C. Outside the foil

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