make a new adjustment. To overcome these problems, we have designed an original type of holder for Ralph knives 25 mm long and 3 mm thick, to be used on a Leitz 1512 microtome. We prefer standard window glass, scored with a diamond and hand-broken. We present here detailed plans which can be used by any workshop. The dimensions were calculated for the Leitz 1512 microtome. The adaptation of the holder for other microtomes like the Minot rotary type is also possible by adjusting the dimensions.

The Ralph knife is held by 2 lateral posterior clamps which are firmly tightened by 2 anterior butterfly nuts. The backing bar is placed in the microtome steel-knife holder. Such a mounting does not require removal of the holder when the knife has to be changed. Alignment and approach are not modified. These plans for an original holder for hand-broken Ralph glass knives may seem a little difficult or confused to a histologist, and we apologize in advance; the plans were designed by a professional industrial draughtsman for direct use by the workshop.

1 Acknowledgments. The authors are greatly indebted to Mr G. Grange who designed and made the prototype. They wish to thank Miss C. Monzy for her help in preparing the manuscript.

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A microquantitative method for investigating the interaction between labeled lectins and the surface membranes of human lymphocytes using a semi-automatic harvesting machine

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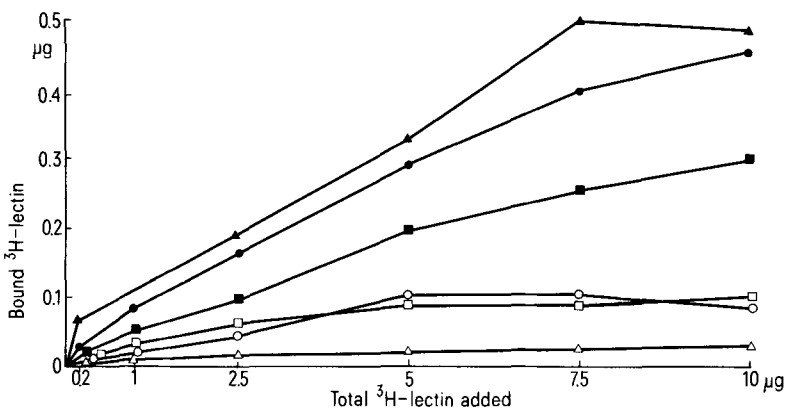
Summary. We describe a simple, rapid and economical method for the study of the interaction of labeled lectins and the surface membranes of human lymphocytes using a semi-automatic harvesting machine (Titertek Multiple Cell Harvester). The procedure requires both small numbers of cells and small amounts of lectin, moreover it reduces the number of experimental steps required.

Lectins are bi-or multivalent proteins which are found predominantly in the seeds of some plants and also occur in invertebrates and mammals[2,3]. These lectins bind to specific cell surface saccharide determinants and induce lymphocyte stimulation and cell agglutination[2]. It is generally accepted that the effects of lectins on the cell presuppose a prior binding to the glycoprotein receptor sites located on the cell surface[4]. Because of their particular properties, lectins have been found to be useful molecular probes for investigating the structure, topology and mobility of plasma membrane saccharide-bearing components[5,6]. Inhibition of the lectin-cell surface interaction with saccharides and cell surface components has been used to investigate the saccharide specificity of lectins and also to evaluate the lectin receptor activity of cell macromolecules. Such studies have been done using lectins labeled with radioactive compounds, and involve large amounts of both lectin and cells. Moreover, a long and repetitive centrifugation procedure is required[7,8]. In the present communication we present a simple, rapid and economical procedure for studying the interaction of labeled lectins and the cell surfaces of human lymphocytes using a semiautomatic harvesting machine (Titertek Multiple Cell Harvester).

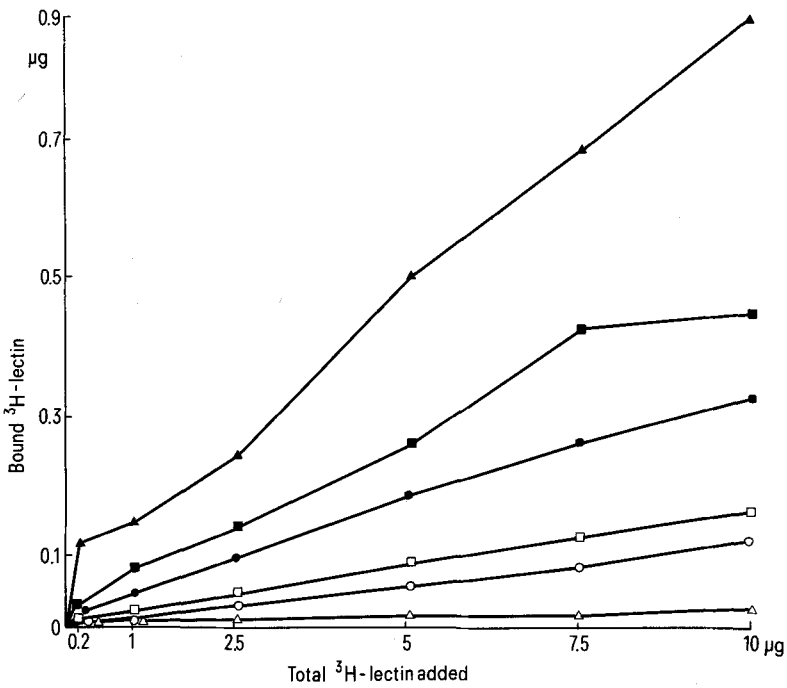
Materials and methods. 1. Lectins. Concanavalin A (grade III) was obtained commercially from Sigma Chemical Co.; Robinia lectin, *Ricinus communis* lectin (Var. sanguineus agglutinin, RCA 120) and anti-Robinia lectin serum were prepared by methods described elswere[9,11].

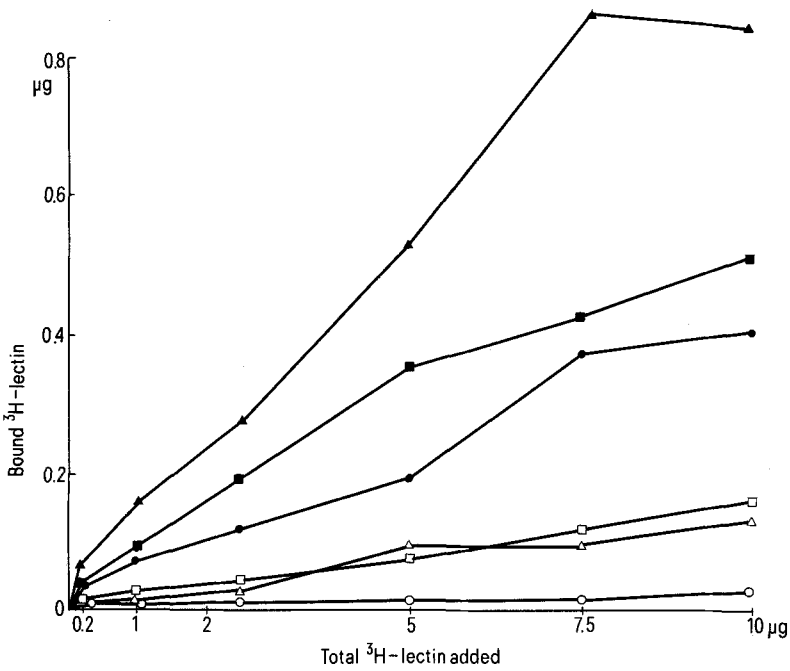


Labeled lectins were prepared according to a modification of the method of Miller and Great[12] using ^{3}H-acetic anhydride (sp. act. 100 mCi/mmole, Amersham). The sp. act. of the labeled lectins were 3610 cpm/µg, 9996 cpm/µg and 3145 cpm/µg for Robinia, Concanavalin A and RCA 120, respectively.

2. Lymphocyte suspensions. Fresh normal lymphocytes. A pellet of human leukocytes was suspended in a small volume of Hank's solution, layered on the surface of 10 ml of a solution of Ficoll Telebrix as described by Boyum[13] and centrifuged for 30 min at 500×g. The resulting lymphocyte ring was washed 3 times in saline solution. Continuous cultured cells. The establishment of the 2 types of lymphoid cultured cells used, LHN_{13} derived from normal blood cells and Reh_6 derived from leukemic blood cells, has been described elsewhere[14].

3. Micromethod of binding. Quantitative binding studies were performed in microtitration plates (Linbro IS.FB 96-TC). In each well 50 µl of cell suspension (2×10^7 cells/ml) were mixed with various concentrations of tritiated lectin (0.2–10 µg) in 50 µl of saline and 100 µl of bovine serum albumin solution (0.5% in saline). (This was to prevent the cells from adhering to the plastic.) After incubation for 30 min, the cells were harvested with a semi-automatic





Figures 1–3. Binding and inhibition of the binding of labeled RCA 120, Concanavalin A and Robinia lectin to the surface membranes of fresh normal human lymphocytes (1), human normal (2) and leukaemic cultured cells (3). ▲—▲, Labeled RCA 120; ●—●, labeled Concanavalin A; ■—■, labeled Robinia lectin, △—△, labeled RCA 120+lactose; ○—○, labeled Concanavalin A+α-methylmannoside; □—□, labeled Robinia lectin+anti-Robinia serum.

harvesting machine (Titertek Multiple Cell Harvester, Flow Laboratories) into glass fiber discs (Filter mat Cat.No.72-10505, Flow Laboratories). The discs were allowed to dry for 1 h at 100 °C and put into plastic counting vials (Beckman mini-vials, Biovials with plastic caps). 100 µl scintillator (ACS) were added to each vial according to Sharif et al[15] and the radioactivity counted in a liquid scintillation counter (Intertechnique).

The quantity of fixed lectin (fixed radioactivity/specific activity of the lectin in counts per min) was determined for each concentration of lectin.

4. Binding inhibition assays. For RCA 120, a solution of 0.4 M lactose in saline was pre-incubated with an equal volume of the agglutinin solution (400 µg/ml) for 30 min at 37 °C. The mixture was then added to each well containing bovine serum albumin and the cell suspensions[11]. For Concanavalin A, a solution of 0.4 M α-methylmannoside was used[16] while for a 700 µg/ml solution of Robinia lectin, twice the volume of antiserum needed to be added[10].

Results. Figures 1-3 show the amounts of labeled lectin bound to the surfaces of the fresh normal human lymphocytes and the normal and leukemic cultured cells in the presence and in the absence of inhibitor. The 3 lectins used were RCA 120, Concanavalin A and Robinia lectin. For the binding inhibition tests, lactose was added to the RCA 120, α-methylmannoside to Concanavalin A and antiserum to the Robinia lectin. In setting up the microfixation technique, increasing concentration of lectin were used: 0.2, 1, 2.5, 5, 7.5 and 10 µg in a volume of 50 µl, and each concentration was tested 3 times. The results appearing in the figures are in each case the mean of 5 experiments. Figure 1 shows the binding of the lectins to fresh normal human lymphocytes, Figures 2 and 3 show the results obtained with the microfixation technique using normal (fig. 2) and leukemic (fig. 3) cultured cells.

As can be seen in the 3 figures, significant and specific binding was observed for all the concentrations of lectin tested and for 10^6 cells. In all 3 cases and for all 3 lectins, binding increased with lectin concentration. Binding was almost identical for the normal (fig. 2) and the leukemic (fig. 3) cultured cells. Smaller amounts of RCA 120 and Robinia lectin bound to the fresh normal lymphocytes than to the cultured cells.

For the 3 types of cell, prior incubation of the lectins with their inhibitors decreased the amount of binding, i.e. by 62-71% in the case of Robinia lectin, 64-78% in the case of Concanavalin A and 88-99% in the case of RCA 120.

Discussion. Until now, the methods used to study the interaction of lectins to to receptor sites have necessitated using large amounts of labeled lectin, and several successive experimental steps. With the semi-automatic machine used in the technique described here, the amount of biological material is reduced by a factor of 10. The number of cells is reduced to 10^6 and the quantities of lectin and inhibitor to 0.2-10 µg. In addition, fewer experimental steps are required. The centrifugation formerly needed after the incubation of the cells with the labeled lectins is no longer necessary, since with the semi-automatic machine the radiactive cell pellet is collected on glass fiber filter discs thus discarding the supernatant containing the unbound labeled lectins. The bound radioactivity can then easily be counted once the discs have been soaked in 100 µl scintillator, as described previously[15]. The fact that the binding of all 3 lectins (Concanavalin A, Robinia lectin and RCA 120) was significantly decreased (62-99%) in the presence of inhibitor (α-methylmannoside, anti-Robinia serum and lactose) clearly shows that the binding is specific and can easily be quantified. The higher percentage of inhibition of RCA 120 is explained by the fact that unlike the other 2 lectins, this agglutinin was repurified on Sephadex G 200 equilibrated in 0.01 M sodium bicarbonate, 0.15 M NaCl, pH 7.4 after being labeled with the tritiated acetic anhydride.

A comparative study of the binding capacity of 3 lectins (Robinia, Concanavalin A and RCA 120) to fresh normal human lymphocytes was performed, and similar data were obtained with the present and with the classic method. The slight decrease of bound lectin observed using the classic method could be related to the loss of some cells during the repetitive centrifugation procedure (fig. 4).

1 The authors gratefully acknowledge Dr C. Rosenfeld (Institut de Cancérologie et d'Immunogénétique Villejuif - France) for suppling the continuous cultured cells and Miss J. Font for providing Robinia lectin. A. S., Chargé de Recherches INSERM. To whom reprint requests should be addressed.

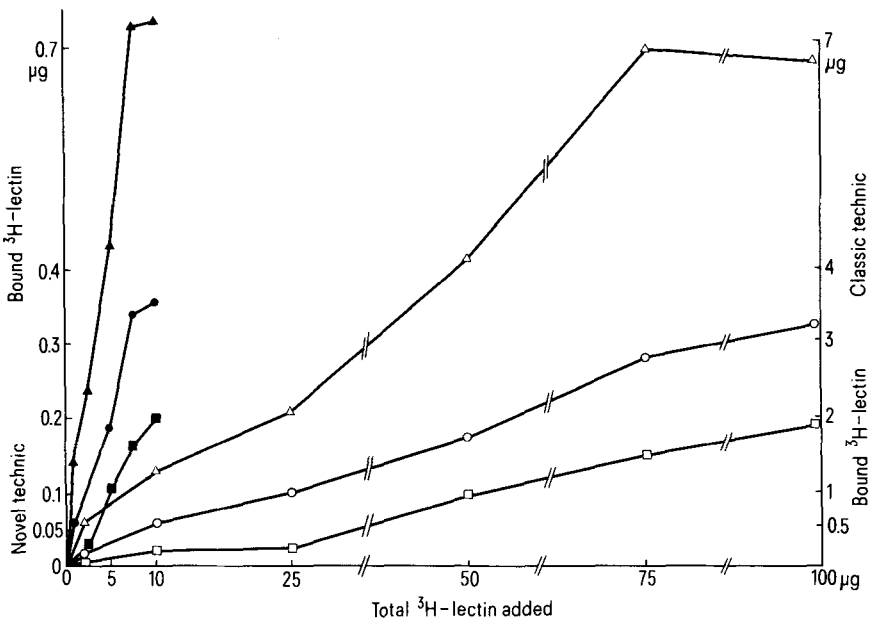


Figure 4. Specific binding of labeled RCA 120, Concanavalin A and Robinia lectin to the surface membranes of fresh normal human lymphocytes by the present and classic methods. Novel method: ▲—▲, labeled RCA 120; ●—●, labeled Concanavalin A; ■—■, labeled Robinia lectin. Classical method: △—△, labeled RCA 120; ○—○, labeled Concanavalin A; □—□, labeled Robinia lectin.

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Announcements

Federal Republic of Germany

3rd international symposium on invertebrate reproduction

Tübingen, August 22–27, 1983

The symposium is organized by the International Society of Invertebrate Reproduction (ISIR), with 5 sessions with invited speakers covering: 1. Cellular differentiation and cellular events in invertebrate reproduction, especially gametogenesis and fertilization; 2. endocrine control of invertebrate reproduction; 3. environmental adaptations of invertebrate reproduction; 4. population dynamics, reproductive strategies of invertebrate reproduction and their genetical background; 5. manipulation and control of invertebrate reproduction.
Further information by Prof. Dr W. Engels, LS Entwicklungsphysiologie, Auf der Morgenstelle 28, D-7400 Tübingen/FRG.

XIIth international pigment cell conference (IPCC)

Giessen, September 18–23, 1983

The program will cover aspects related to all kinds of pigment cells and color changes in the animal kingdom. Information by XIIth IPCC Secretariat, Genetisches Institut der Justus-Liebig-Universität, Heinrich Buff-Ring 58–62, D-6300 Giessen/BRD.

Honors

Marcel Benoit Prize 1982

It was announced last month that Professor Karl Illmensee has been awarded the 1982 Marcel Benoit Prize. On behalf of all Experientia editors, I wish to congratulate our Advisory Board Member for being chosen to accept this honor.

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