Results and discussion. Table I illustrates the results of 11 individual experiments. In the 1050 gradient interphase a BL enriched population is collected: there are 70.52% EAC rosettes forming cells with 0% E rosettes. On the contrary, in the 1068 interphase, there is an enrichment of TL, with 68% E rosettes and 8.55% EAC rosettes. When these proportions are compared with the total lymphoid population obtained in a 1074 density gradient (Table II) a 187% enrichment of BL at 1050 and a 36% enrichment of TL at 1068 densities were appreciated. Viability of all cells collected from gradients was over 95%.

These results show that it is possible to obtain populations enriched for E and EAC rosette forming lymphocytes (TL and BL respectively). The discontinuous FH gradients used offer the advantage of being a simple and quick method for the separation of 2 types of lymphocytes and requiring small volumes of blood.

Moreover, as no ligand is attached to the cell membrane receptors during the whole procedure, these receptors remain unchanged, a property which may be extremely useful when the function of these subpopulations are to be studied subsequently.

A Simple Method for Blood Exchange in Mice[1]

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Summary. Ease of transfusion and high long-term survival rate were obtained when whole blood was administered via the corpus cavernosum of the penis and removed from the orbital sinus of male C57Bl mice. When 2 volumes of blood are replaced approximately 14% of pre-transfusion red cells remain after hematocrit corrections are made. The post-transfusion hematocrit levels dropped 19%, probably the result of leakage, which is difficult to avoid.

Because of technical difficulties, blood transfusions are rarely attempted in small laboratory animals such as mice. However, if a simple technique for blood transfusion were available, it could have wide application in many small animal studies. A method has been developed in this laboratory in which whole blood can be injected into male mice via the corpus cavernosum of the penis while at the same time blood is removed from the ophthalmic plexus. The latter route has, in the past, been used successfully to obtain blood from mammals[3–5] and frogs[6]. The corpus cavernosum of the penis, although not to our knowledge referenced in the literature, is a route commonly utilized for injecting material i.v. in many laboratories[7]. Although injection via the tail vein is possible, the marked decrease in tail blood flow that occurs in anesthetized mice presents major problems. To demonstrate the procedure's practical application, male C57Bl mice pre-injected with ^{59}Fe-labelled red cells were transfused with whole, unlabelled blood to determine the efficiency of blood replacement and survival in the transfused animals.

Mature C57Bl male mice (27–30 g) were anesthetized by injection of 10 mg of chloral hydrate i.p. This dose was sufficient to keep most animals anesthetized for the duration of the experiment ($\sim$ 2 h). However, on occasion, animals required an additional 3–6 mg. The experimental protocol was as follows: 1. Washed, ^{59}Fe-labelled isologous red cells were suspended in sufficient 0.9% saline to maintain normal hematocrit levels and 0.1 ml was injected via the corpus cavernosum of the penis; 2. The erythrocytes were allowed to equilibrate for 15 min and a 20 μl aliquot of tail blood was collected into a pre-heparinized capillary tube; 3. fresh, whole heparinized blood was injected into the corpus cavernosum of the penis. The penis was exposed as shown in the photograph (Figure) and a 25 gauge needle attached to a 5 ml syringe was inserted into the corpus cavernosum for transfusion. Simultaneously, blood was removed into a graduated centrifuge tube via capillary tube inserted into the orbital sinus (Figure). The procedure required approximately 5 min to replace 2 volumes of the mouse's blood (4.2 ml/30 g). Technically, it was easier to insert the capillary tube into the orbital sinus first and as a result usually 0.2–0.3 ml of blood was removed prior to beginning the corpus cavernosum injection. Once the injection was begun, it proceeded at a more rapid rate than removal until 0.2 ml more blood had been injected than removed. This differential was maintained until the 2 volumes of blood were administered at which time both the capillary tube and the injection needle were removed simultaneously. The extra 0.2 ml was to allow for the leakage that generally occured both from the orbit and the corpus cavernosum following transfusion; 4. the transfused blood was then allowed to equilibrate for approximately 1 h and a second 20 μl sample was taken. The samples were centrifuged and the hematocrit percents determined. Following transfusion, surviving mice were replaced in their cages. Some have been subsequently maintained for 3 months.

Extent of replacement and hematocrit changes following blood exchange

Period	cpm/20 μl Blood[a]	Initial level (%)	Hematocrit	Initial level (%)
Pre	2566 $\pm$ 228	100	48 $\pm$ 3	100
Post	363 $\pm$ 85	14	39 $\pm$ 4	81

[a] Values expressed as mean $\pm$ standard deviation. Post-replacement values are corrected to initial hematocrit.

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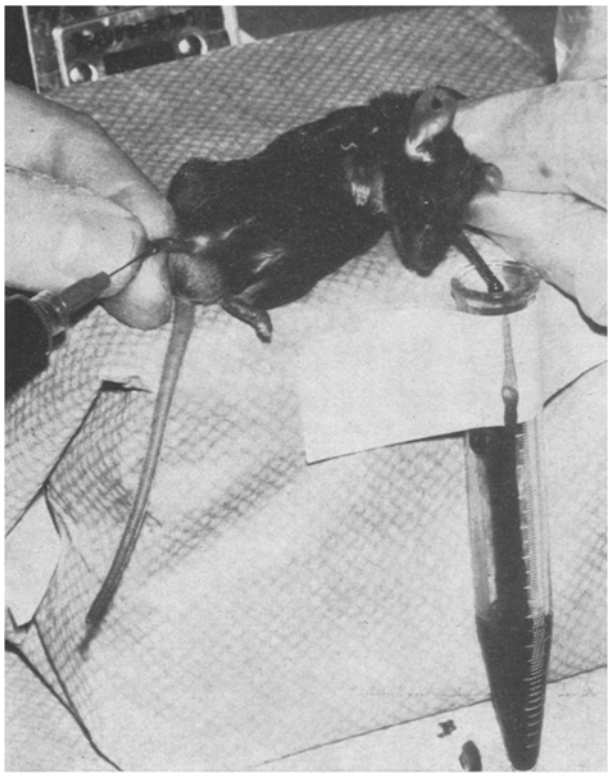

Illustration of blood exchange accomplished by injection via the corpus cavernosum of the penis and bleeding through the orbital sinus of the mouse.

Animals which survived the first 2 h after transfusion (> 90%) showed no observable ill effects during the following 3 months. When normal male C57Bl mice were transfused with 2 volumes of fresh normal heparinized blood, there was a 19% drop in the 1 h post-transfusion hematocrit (Table). This decrease was probably the result of leakage of greater than the 0.2 ml compensated for, and is difficult to avoid. When corrections were made for the change in hematocrit, approximately 14% of the initial ^{59}Fe-labelled red cell counts remained in the animal after transfusion.

A method has been described for transfusing mice by injection of fresh whole blood into the corpus cavernosum of the penis while blood is concurrently removed from the venus plexus of the orbital cavity. Both these routes are easily accessible and when reasonable precautions are taken, they facilitate rapid and relatively safe blood transfusion in small animals. The high survival rate when syngeneic blood is used makes it a useful procedure for longterm studies. Based on a 7% blood volume per weight mouse, only 2 volumes of blood were transfused in this study. However, it seems likely that larger amounts can be transfused, and a concomitant reduction in the endogenous blood obtained. If necessary, the procedure could be repeated at frequent intervals with no damage to the penis. The orbit is more prone to injury; however, as Riley[5] noted 1–2 bleedings of small volumes via this route per week seem to be harmless to the animal and daily bleedings are even possible. Furthermore, although chloral hydrate results in a decreased blood flow to the tail of the mouse, it is possible to transfuse blood via the tail vein. Thus, though more difficult, transfusion is possible in chloral hydrate anesthetized female mice.

A Simple Algorithm for the Solution of the '$n \times m$' Case of a Binding Equilibrium

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Summary. Accurate estimates of the equilibrium concentrations in the non-interactive reaction of several ligands with several classes of binding sites with univalent stoichiometry can be rapidly obtained by a simple method of successive approximations on a programmable desk calculator.

Binding reactions between macromolecules and other compounds (ligands) are common biochemical phenomena. Well-known examples are the formation of enzyme-substrate complexes, the specific and nonspecific binding of hormones to plasma or cell proteins, the antigen-antibody reaction, etc. In the '$n \times m$' case[2], m independent classes of noninteracting, univalent binding sites react with n univalent ligands simultaneously to reach an equilibrium defined by $n \times m$ equations given by

$$B_{ij} = K_{ij} (N_j - C_j) (S_i - D_i) \qquad (1)$$

where B_{ij} is the equilibrium concentration of the ith ligand bound to the jth class of binding sites ($i = 1,2,\ldots, n$; $j = 1,2,\ldots, m$), K_{ij} is the corresponding equilibrium association constant, $C_j = \sum_{i=1}^{n} B_{ij}$, $D_i = \sum_{j=1}^{m} B_{ij}$, and N_j and S_i are the total concentrations for the jth class of binding sites and the ith ligand, respectively. For a given positive integer m, it is possible to estimate the values of K_{ij} and N_j from experimental binding data and known S_i's by the method of least squares as applied to nonlinear regression[3]. Several computer programs are available for this kind of parameter fitting[4–8], including ours that incorporates a goodness-of-fit test to decide (for $n = 1$) which of several values assumed by m best describes the experimental data[9].

Equally important to researchers is the converse problem of calculating the equilibrium concentrations of bound and unbound ligands and of filled and empty bindings sites when the association constants and total concentrations of reactants are known or allowed to assume given values (simulation or modeling). Stucki[10] has described an algorithm for implicitly solving this problem when $n = 1$. Others have used various computer programs based on linearization (Taylor series) or differential equations when $n > 1$[11–13]. The present report describes a simpler method which requires only a programmable desk calculator.

Summation of the $n \times m$ equations given by (1) to obtain the m concentrations of jth ligand bound to all sites and the n concentrations of ith class of binding sites