spielt also die Gewöhnung eine Rolle. Die den Müttern applizierten Oestrogene beschleunigten den Eintritt des ersten Oestrus. Die Wirkung trat in den meisten Fällen nach der zweiten Salbenapplikation ein, der erste Oestrus meist gleichzeitig mit der Vagina-Eröffnung, bei einem Jungengewicht von nur 10–13 g, mit turgeszenter, geröteter Vulva und massiven Schollenabstrichen. Das den Jungen injizierte Oestrogen wirkte schon in sehr geringen Dosen beschleunigend. Die Dosis von 5 µg bewirkte bei allen Weibchen nach einem Tage den Abbau des Vaginalverschlusses, dem am nächsten Tage ein massiver Schollenabstrich folgte, der sich als bis zu 60 Tagen dauernder Daueroestrus erwies. Es erfolgten zahlreiche Deckakte (bei 12 Weibchen 67 in 60 Tagen), die aber nur zu 8 Graviditäten führten. Die Jungen gediehen nicht, und viele starben.

c) Die erste Begattung ist ein weniger massgebendes Kriterium als die beiden ersten, weil sie nicht nur von den Weibchen, sondern auch von den Männchen bestimmt wird. Zudem kann sie, auch bei täglicher Kontrolle, übersehen werden, weil die Kopulationen meist in der Nacht erfolgen und der Vaginalpfropf oft schon nach wenigen Stunden herausfällt oder auch bei der Inspektion noch nicht erhärtet ist. VANDENBERGH[1] stellte den Pfropf bei 13 von 50 Weibchen fest, die später warfen (26%), wir haben ihn bei 217 von 280 Weibchen gefunden (77,5%).

d) Der erste Wurf. Noch komplexer als Kriterium der Geschlechtsreife ist das Datum des ersten Wurfes. Konzeption und Gravidität erfordern die Koordination aller Faktoren der Genitalfunktion. Die Beschleunigung einzelner Komponenten bedeutet nicht den Eintritt der Geschlechtsreife, die erst mit der Fortpflanzungsfähigkeit verwirklicht ist. Auch beim frühen und intensiven Oestrus der Versuchsgruppe 9 traten trotz zahlreicher Begattungen die Graviditäten erst im dafür normalen Alter ein. Die fehlende Koordination beeinträchtigt wie beim juvenilen auch beim alten Weibchen die Fertilität, das trotz fortdauernder Zyklizität nur noch wenige Graviditäten aufweist (BLOCH und FLURY[7]).

VANDENBERGH, WHITSETT und LOMBARDI[8] (Kriterium: Uterusgewicht juveniler Weibchen) bezweifeln auf Grund teilweiser chemischer Isolierung des Pheromons des männlichen Urins, die nicht auf einen flüchtigen Stoff schliessen lässt, dessen olfaktorische Wirkung, obwohl sie von Männchen, die durch ein doppeltes Drahtgitter von den Weibchen getrennt sind, und auch durch den auf die Nase der Weibchen aufgetragenen Urin der Männchen ausgeübt wird.

Die olfaktorische Wirkung des männlichen Urins auf die Nidation und die Provozierung der Zyklen (Bruce-effect, Whitten-effect) galt bisher auf Grund zahlreicher Untersuchungen als erwiesen, es wäre aber möglich, dass die Wirkung auf die einzelnen Kriterien der Geschlechtsreife von verschiedenen Komponenten des Pheromons ausgeübt wird und dass neben der olfaktorischen Wirkung auch taktile, perkutane oder andere Übertragungsweisen vorkommen, vielleicht auch durch Belecken des eigenen (oder in unseren Versuchen des mütterlichen) Fells oral eingenommene Substanzen wirksam sind. Bei den mit Oestrogen injizierten Jungen wurden nicht behandelte Wurfgeschwister, die unter den behandelten aufwuchsen, nicht beeinflusst, was für diese Behandlungsart nicht auf eine olfaktorische Wirkungsweise schliessen lässt. Die Möglichkeit verschiedener Wirkungsweisen sollte durch weitere Untersuchungen geklärt werden.

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PRO EXPERIMENTIS

Enrichment in Spontaneous and Complement Dependent Rosette Forming Cell Populations Using Ficoll-Hypaque Gradient Centrifugation[1]

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Summary. Peripheral blood human T (TL) and B (BL) lymphocytes were separated by continuous Ficoll-Hypaque (FH) gradients. BL were found at the 1050 density interfase (70.5% EAC rosettes) with no TL (0% EAC rosettes); while at the 1068 density interfase there was an enrichment of TL (68% E rosettes) with very few BL (8.5% EAC rosettes).

Several methods have been used to separate subpopulations of lymphocytes. Continuous and discontinuous gradients with bovine serum albumin, acacia gum, and Ficoll-Hypaque (FH) have been employed to isolate human lymphocytes[6,7] and other mammalian[8–15]. We now report a quick and simple method based on FH gradients, able to separate peripheral blood human T and B lymphocytes and apparent alteration on the cell membranes.

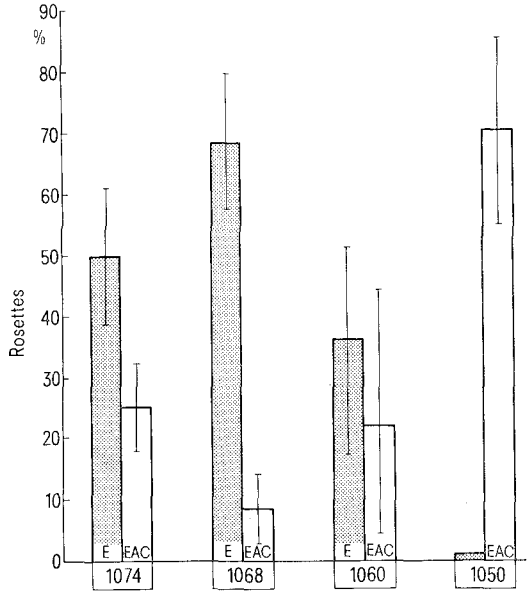
Materials and methods. Total lymphocyte separation. 15 ml of defibrinated blood from normal donors were diluted with saline 1:2 and layered on a FH gradient with a 1074 density, according to THORSKY and BRATLIER[16]. The gradient was centrifuged at 200 *g* for 1 h at room temperature and the lymphocyte population was collected from the interphase.

Selection of appropriate gradients. In order to find the correct density for the separation of lymphoid subpopulations, continuous linear gradients were prepared with densities ranging between 1074 and 1048; 2.5×10^7 total lymphocytes (from a 1074 density gradients) were layered over the continuous gradient and centrifuged as above. The type of lymphocytes that were layered at different densities were identified by their surface markers. The relevant densities were determined through refractometry.

Identification of T and B cells. T cells were identified by the spontaneous rosette technique[17,18] and B cells by the complement dependent rosette test[19]. Cell viability was tested by trypan blue exclusion.

Discontinuous gradients. Based on the results obtained with the continuous gradients, densities were controlled

by refractometry, densitometry and picnometry. 3 kinds of gradients for each experience were layered with defibrinated diluted blood: a) 3 ml of 1074, with 4 ml of blood, for the total population; b) 2 ml of 1068 and 2 ml 1060, with 2 ml of blood for the T enriched population and c) 3 ml of 1050, with 3 ml of blood for the B enriched population. All gradients were centrifuged as above and lymphocytes were collected from the interphases.



Separation of T and B lymphocyte populations.

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Table I. Distribution of E and EAC rosettes in lymphocyte populations isolated through discontinuous FH gradients

Experiment	1050		1060		1068		1074	
	RFL 'E'	RFL 'EAC'	RFL 'E'	RFL 'EAC'	RFL 'E'	RFL 'EAC'	RFL 'E'	RFL 'EAC'
1	0	65.00	60.00	10.50	69.00		49.20	25.20
2	0		45.00		77.00	2.30	55.00	
3	0	45.00	41.66	7.89	65.00	11.90	59.00	30.14
4	0	57.14	29.69	23.52	59.00	0	50.60	26.10
5	0	57.69	33.30	28.78	52.60	0	47.00	28.57
6	0	71.00	12.70	31.50	66.00	19.00	51.40	25.20
7	0	82.00	68.00	10.00	70.00	16.17	35.00	19.20
8	0	72.22	38.80	44.77	73.00	12.00	57.53	32.60
9	0	87.00	21.00	71.00	50.00	6.12	27.75	28.80
10	0	70.00	28.40	9.32	70.00	4.02	50.00	22.00
11	0	98.00	13.15	6.38	95.83		68.00	7.19

Table II. Distribution of E and EAC rosettes in lymphocytes from normal subjects separated through discontinuous Ficoll-Hypaque gradients

Density	Rosettes E (%)			Rosettes EAC (%)			Enrichment (+) Losses (−)	
	Nr.	$\bar{x}$	S	Nr.	$\bar{x}$	S	Rosettes E (%)	Rosettes EAC (%)
1050	11	0	± 0	10	70.52	± 15.2	− 100	+ 187.83
1060	11	35.60	± 18.00	10	24.36	± 20.00	− 28	− 0.57
1068	11	68.00	± 12.50	10	8.55	± 5.63	+ 36	− 65.10
1074	11	50.00	± 11.00	10	24.50	± 7.20		

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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