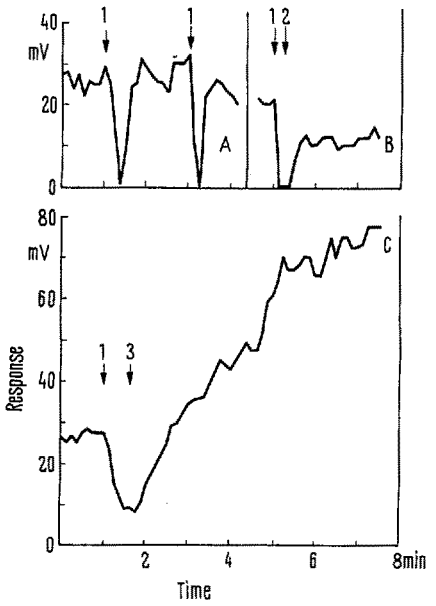
Depilated albino guinea-pigs (500–600 g body weight) are injected i.v. with about 1 ml of the labelled protein solution of approximately 200 μ curie/ml. Thirty min after injection, the animals are tied, back uppermost, to a firm frame. A disc of soft lead (8 cm diameter × 1.5 mm thickness) with a central hole (16 mm diameter) is sandwiched between 2 strips of adhesive tape cut to expose the central hole. The resulting 'belt' is laid on the back of each animal, with the hole exposing a selected area of skin which is then outlined with a fine felt marking pen.



Continuous recordings of changes in vascular permeability. Block (A) illustrates the return of the recording to its previous level following the removal of the test animal (1) and its replacement under the G-M counter. Block (B) records the effect of 0.1 ml physiological saline injected intracutaneously (2), and block C the effect of 0.2 μg histamine (base) in 0.1 ml saline (3). The fall in the recording at (1) in blocks B and C is due to partial removal of the guinea-pig from under the counter to inject saline and histamine respectively. The animal was then immediately re-positioned with the injected site directly under the counter.

A G-M counter with a thin mica end-window (Mullard type MX123) is clamped about 3 mm above the exposed skin and connected to an EKCO type N624 Ratemeter, which in turn is connected to a strip chart recorder; a full deflection of the ratemeter (0–100 pulses/sec) is equivalent to a displacement on the recorder of 110 mV.

When the animals were re-positioned under the G-M counter, the baseline of the record always returned in 45 sec or less to within $\pm$ 3 mV of its previous level (block A in Figure).

When physiological saline alone was injected, the tracing took 30–60 sec to stabilize at a new level, usually several mV below the original baseline (Figure, B). The injection of 0.2 μg histamine (base) induced a rapid rise in the level of the recording (Figure, C), typically 40–45 mV above the baseline. Such increases occurred in 4–5 min, the tracing then remaining at the new level for at least 30 min.

The older methods of assessing increased vascular permeability by the extravasation of serum albumin labelled with an aniline dye or I^{131} do not permit precise observations of the early development of the response. The present method overcomes this disadvantage by permitting the quantitative and continuous monitoring of changes in vascular permeability. By the use of labelled antigen or antibody, this technique may also prove useful for similar studies of immunological reactions in the skin of laboratory animals[6].

Zusammenfassung. Es wird die Anwendung radioaktiven Phosphanilsäure-Rinderserum-Albumins zur kontinuierlichen Messung von Änderungen der Gefässwandpermeabilität beschrieben.

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A Simple Method for the Collection of Leucocytes from Rat Blood

Leucocyte-rich plasma can be obtained from human peripheral blood in a number of different ways[1,2]. In our laboratory, however, attempts to obtain large yields of rat white blood cells by similar methods[3,4] proved inadequate due to poor sedimentation of the erythrocyte mass. The method described in this report has consistently aggregated Lewis rat red cells, thereby yielding plasma that contained large numbers of leucocytes.

Lewis rats were decapitated 5 min after i.v. injection of 10 U of heparin/g body weight: 8–10 ml of whole blood was obtained in this way from rats that weighed 250-300 g. Aggregation of the rat red cells was accomplished by the addition of serum or plasma from normal humans whose red cells were of group B. It was found that the optimum proportions were 2 parts of human plasma or serum to 1 part of heparinized rat blood. The samples, in 12 ml conical centrifuge tubes, were inverted 3 times and placed at a 45° angle for 1 h at room temperature

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before the supernatant fluids were harvested. The cell-rich supernatant, although somewhat reduced in its polymorphonuclear and monocyte components was found to contain numerous leucocytes and a high yield of lymphocytes (Table). In 8 separate determinations it was noted that plasma from normal group B humans consistently produced more red cell aggregation than plasma from persons of other blood groups. Saline, rat plasma, or phytohemagglutinin used in place of human plasma failed to show any sedimenting activity and rat whole blood virtually did not sediment at all in 1 h. Comparative results between the heterologous serum method and 3% dextran[5], 6% dextran[6] or 3% gelatin[7,8] are given in the Figure and in the Table. (Dextran *M*w-236,000, Lot T893, Pharmacia Fine Chemicals, Pistcataway, New Jersey. Gelatin-U.S.P., Knox Gelatine Inc., Johnstown, New York.)

By way of example, the 2 most successful methods are compared below in terms of time, lymphocyte yield and degree of red cell contamination. In the heterologous serum method, the mean lymphocyte yield was 94% (range 81–109%) in 1 h. The mean supernatant red cell count represented less than 1% of the original number of erythrocytes present (28,000 ± 5000/mm³). The 3% gelatin system provided a 75% lymphocyte yield (range 63–79%) with a mean red count of 20,000 (± 4000)/mm³. In 15 min a 75% mean yield of lymphocytes was obtained by the heterologous serum method as against a mean yield of 7% by the 3% gelatin method. The former method thus had the dual advantage of speed and increased yield.

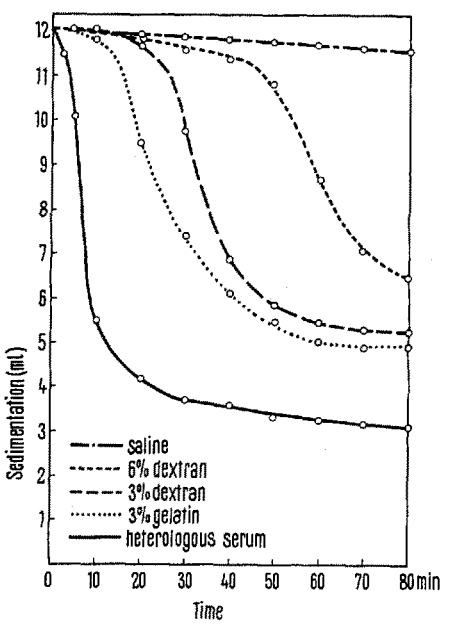
Heat inactivation at 56 °C for $^1/_2$ h, or prolonged storage at − 14 °C did not significantly alter the ability of the heterologous plasma to aggregate rat red blood cells. Of interest was the fact that anti-A_1 lectin (*Dolichos biflorus*, Hyland, lot No. 3192A2B) failed to aggregate rat red cells. Human anti-A plasma absorbed with A substance or A_1 or A_2 cells, while no longer capable of agglutinating human A cells, still aggregated rat red cells. Although no further attempt was made to characterize the agent or agents involved, these results indicated that human anti-A antibody was not the factor that aggregated the rat erythrocytes.

To test the viability of the leucocytes obtained by this method, 72-h cultures were set up on sterile samples using phytohemagglutinin (PHA) as a stimulus. The method used was that generally described to produce 'blast' transformation in lymphocytes, adapted from the technique of HUNGERFORD et al.[9]. In cultures of rat leucocytes prepared by the heterologous serum method and challenged with PHA, lymphocyte transformation occurred in about 15% of the cells at 72 h, while this phenomenon was not observed in leucocyte cultures prepared by the same method and not so treated. In all instances lymphocyte staining characteristics appeared normal and their survival or behavior in culture appeared to be unchanged either by the manipulation described or the exposure to human plasma. Definitive proof of cell viability and competence was obtained by the passive transfer of experimental allergic encephalomyelitis with Lewis rat blood white cells processed by the heterologous serum method[10].

Mean results using different sedimentation methods[a]

Method	Leucocyte yield (%)	Lymphocyte yield (%)	Supernatant RBC (per mm³)	Supernatant volume (ml)
3% Dextran	62	67	43,000	6.6
6% Dextran	25	30	30,000	3.2
3% Gelatin	65	75	20,000	6.9
H.S.[b]	79	94	28,000	8.8

[a] Mean values from 4 Lewis rats/method. [b] H.S., normal sera from humans with group B red cells. Volumes used: 8 ml, sedimenting agent: 4 ml blood. Sedimentation time: 1 h, room temperature.



Comparative sedimentation rates.

Zusammenfassung. Blutleukozyten von Ratten wurden mit menschlichem (normalem) Serum behandelt. Die Viabilität dieser Präparationen konnte dadurch demonstriert werden, dass die Zellen durch Phytohemagglutinin in «blast» Formen umgewandelt wurden.

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