plastic cage of the design commonly used in the animal colony (19×25×15 cm). The type of spring mounting used will depend on the size and weight of the animal and the cage; for 20–40 g mice, we have found that 4 cm cubes of soft sponge rubber are effective. The fidelity of the apparatus is improved by using fairly rigid spring mounts.

Methods. The apparatus was tested by placing a laboratory mouse into the activity cage with food and water in the morning and allowing acclimatization throughout the day. At approximately 16.00 h the impedance bridge was calibrated to an arbitrary level using a built-in calibration source and the pen recorder (6 mm/min) was started. Recording was continued until the following morning. In this situation, the activity cage was in the mouse room and connected to the electronic equipment in a separate room at a distance of about 30 m. Therefore, measurements were made in the animal's normal environment during the dark phase of the light-dark cycle, that is, when mice are normally most active. Tracings obtained from 10 animals were measured to determine total level of activity and the portion of the recording period spent sleeping. Total activity measurement was accomplished by integrating the area under the 'curve' obtained (figure 2) either manually or by electronic integration of the original signal.

Results and discussion. Mice used to test the device showed the greatest level of activity during the first 2 h of the dark phase and became progressively less active during the night. Simultaneous observation of the animal and the tracing revealed that only periods of sleep produced a perfectly flat recording; even minimal preening activity was obvious. Measurement of sleeping periods demonstrated that the mice spent an average (±SEM) of 68.2±1.6% of the night in waking activity. Total level of activity, in arbitrary units, was 2032±216 (n=10).

It was also found that eating the pelleted food which was provided produced a characteristic tracing pattern which could be measured to provide an estimate of the time spent eating, a parameter which is useful in studies of obesity in rodents[5]. Further, it is possible to construct several transducers which provide essentially identical tracings. Therefore, animals from more than 1 treatment group can be evaluated during the same time period. Calibration of more than 1 transducer is accomplished by first adjusting 1 device as described above and then placing both transducers on a single cage and adjusting the amplitude of the 2nd device until both tracings are identical.

1 K.J. Carpenter and J. Mayer, Am. J. Physiol. *193*, 499 (1958).
2 V.H. Denenberg, J. Gartner and M. Myers, Physiol. Behav. *15*, 505 (1975).
3 H. Murakami and K. Kinoshita, Experientia *34*, 671 (1978).
4 T.T.T. Yen and J.M. Acton, Proc. Soc. exp. Biol. Med. *140*, 647 (1972).
5 P.J. Wangsness, B.A. Dilettuso and R.J. Martin, J. Nutr. *108*, 256 (1978).

An improved method for the isolation of eosinophilic leukocytes from normal human blood

P. Portmann, P. Köppel and A. Jörg[1]

Physiologisch-chemisches Institut, Universität Freiburg, CH-1700 Freiburg (Switzerland), 2 May 1979

Summary. A relatively short and simple method for the isolation of the eosinophils from normal human blood is reported. With a recovery of about 45–55%, cell preparations, showing a degree of purity of 90–98%, are obtained. The isolated cells are morphologically intact and viable, as assessed by the trypan blue exclusion test and by active phagocytosis.

In connection with a better understanding of certain allergic reactions the study of the biochemical and the functional properties of isolated normal human eosinophils is of interest. Until recently all simple isolation methods used blood from patients with high eosinophilia (40–80%), the relatively diminished number of neutrophilic granulocytes facilitating the isolation of the eosinophils. However, recent investigations[2–4] have demonstrated that eosinophilia cells are, with respect of their biochemical and morphological properties (modified IgG receptors and enzyme content; vacuole formation and depleted granules), very different from those isolated from the blood of healthy subjects. The isolation of normal eosinophils is difficult because of the small density differences between human neutrophils and eosinophils. The method previously reported[5] for the isolation of the eosinophils from horse blood can not be used for human blood without modifications. Such a modified isolation method is described in this paper.

Material and methods. Isolation of human eosinophils: Cell sediments (about 3 l) of fresh citrated human blood, which, for various reasons, could not be otherwise used than for plasma production, were obtained from the local blood bank. These sediments, not older than 12 h, were again centrifuged (2500×g, 30 min, 4 °C; refrigerated 4 l-centrifuge). The plasma layer was aspirated, centrifuged until cell-free (Sorvall RC2B, 13,000×g, 20 min, 4 °C) and stored at 4 °C for further use (fraction 1). After aspiration of the buffy coat together with the upper fifth of the erythrocyte layer (fraction 2), the next 3 fifths of the red cell layer were removed and discarded. The remaining leukocyte-free red cell sediment, about 400 ml (fraction 3), was saved for further use. Fraction 2 was carefully mixed with half its own volume of plasma (ca. 200 ml) and centrifuged in a Sorvall RC2B centrifuge (rotor HS4, 4800×g, 30 min, 4 °C). The compact leucocyte layer was carefully and completely removed by aspiration. The erythrocyte layer (ca. 400 ml), containing the eosinophils, was mixed with 1.5 vol. of a solution containing 20% of the fraction 1 and 80% of a polyvinylpyrrolidone solution (60 g of polyvinylpyrrolidone K-60 (Fluka) and 10 g of NaCl in 1 l of H_2O). After spontaneous sedimentation of the red cells (30 min at room temperature) in a 1-l glass cylinder, the supernatant containing the eosinophils and some neutrophils was aspirated. The erythrocyte layer was resuspended in 1.5 vol. of fresh plasma-polyvinylpyrrolidone solution and resedimented. Thus, a 2nd portion of eosinophilrich supernatant could be aspirated. The combined supernatants (ca. 1.2 l) were centrifuged (250×g, 15 min). The cell sediment, resuspended in 40 ml of physiological saline, was divided into 4 equal portions, which were each separately superposed over cushions of red cells (24 ml of fraction 3 mixed with 6 ml of fraction 1) and centrifuged (Sorvall RC2B, rotor HB4,

5800× g, 30 min, 4 °C). During the centrifugation, most of the eosinophils penetrate into the erythrocyte layer. The other leucocytes formed a small buffy coat which was very carefully and completely aspirated and discarded. The wall region above the erythrocyte layer was also carefully cleaned with a small cotton compress. The eosinophil-rich red cell layers of the 4 centrifuge tubes were combined (80–90 ml), suspended in 150 ml of the plasma-polyvinyl-pyrrolidone solution and sedimented in a 500-ml glass cylinder. The erythrocyte layer was resuspended and sedimented once more. After each sedimentation, the supernatants were aspirated, combined and centrifuged as above. The cell sediment containing the eosinophils and some remaining red globules was resuspended in 50 ml of fraction 1. The erythrocytes were haemolyzed by the addition of 400 ml of distilled water followed, 50 sec later, by 100 ml of 5% NaCl in order to re-establish the isotonicity and the eosinophils were centrifuged (250× g, 15 min, 4 °C). To completely eliminate the red cell ghosts and the haemoglobin, the cell sediment was suspended in 5 ml of 1% NaCl and superposed over 5 ml of a 30% albumin solution (w/w) in physiological saline contained in a 15-ml centrifuge tube. The yellowish sediment, obtained after centrifugation (500× g, 10 min) was suspended in a 1% NaCl solution, examined for viability with the trypan blue exclusion test and for purity by light microscopy of stained cell smears (May-Grünwald-Giemsa).

The phagocytosis was estimated by the chemiluminescence technique[6] using a Picker liquid scintillation counter, operating with a window for H^3-measurement.

With the method of Markert[7] the O_2-consumption was determinated by measuring the haemoglobin formation from the added oxyhaemoglobin at 435,8 nm.

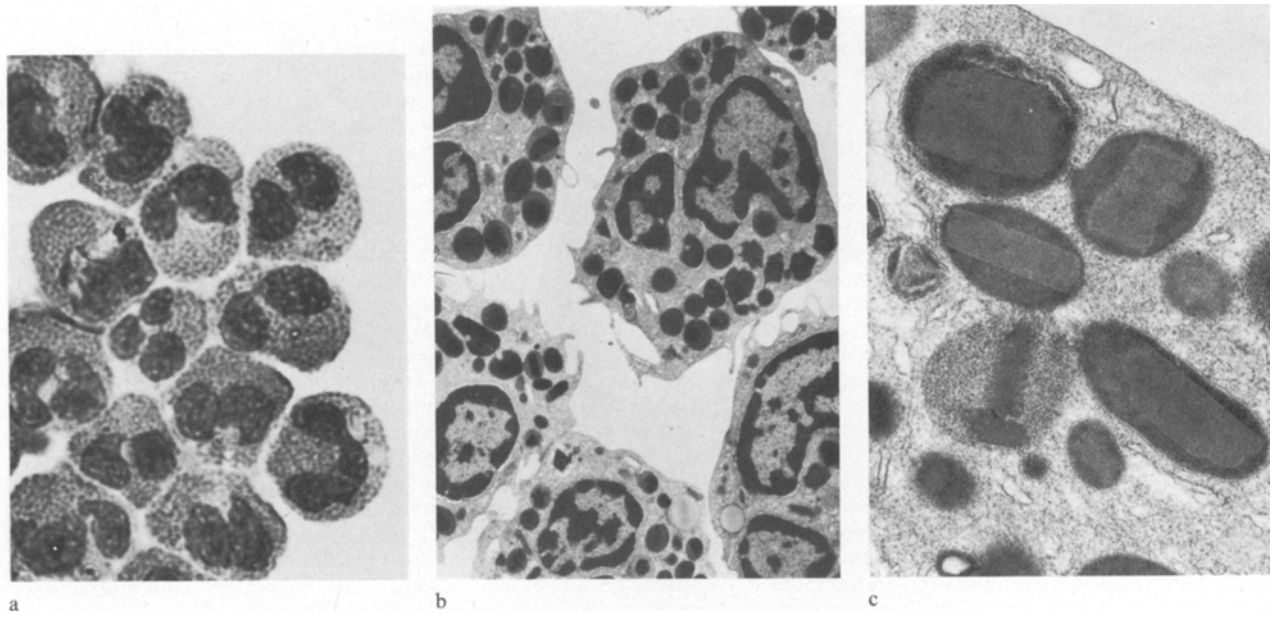
Superoxide anion production was estimated by determination of superoxide dismutase inhibitable ferricytochrome c reduction[8].

Results. Application of the method described to 3 l of a cell concentrate (approximately 2.5 l of cells and 0.5–0.6 l of plasma) obtained from about 12 blood conserves, permitted the isolation of circa 150 mg of eosinophils in 8–9 h. The purity of these preparations, assessed by differential cell counting, was estimated to be 90–98%. Admitting a total leucocyte count of 4500/mm^3 with an eosinophilia of 3% for the blood of these normal donors and estimating the mean cell volume of human eosinophils to be 400 μm^3, this amount represents a recovery of 45–55%. Figures 1a and 1b show the good morphological cell conservation, the purity of the preparations and the characteristic crystalloid internum of the human eosinophil granules. With the trypan blue exclusion test only 1–2% of the freshly isolated eosinophils were stained. The viability of the cells was also tested by their ability to phagocytose Zymosan granules. The phagocytosis was evaluated by microscopy and by measuring the concomitant increase of the chemiluminescence, the oxygen consumption and the production of superoxide anion.

During the phagocytosis of preopsonized Zymosan[9] the chemiluminescence light emission was increased more than 8-fold, the oxygen consumption about 3-fold (resting cells 2.2 nmoles O_{at}/min/10^6 cells, stimulated cells 7.1 nmoles O_{at}/min/10^6 cells) and the superoxide anion generation was about 2-fold greater in stimulated cells than resting cells. Under the same conditions, freshly isolated human neutrophils showed the following increases of these values: chemiluminescence 10-fold, oxygen consumption 1.5-fold (resting cells 0.2 nmoles O_{at}/min/10^6 cells, stimulated cells 0.34 nmoles O_{at}/min/10^6 cells), superoxide anion generation was about 1.5-fold greater in stimulated than in resting cells.

Discussion. The reported method permits the isolation, in good yield, of pure and intact eosinophils from normal human blood. This isolation does not need special density gradient centrifugations, in contrast to Grover's method[10], but it uses a cushion of erythrocytes as a density barrier. The smaller density differences between human neutrophils, eosinophils and erythrocytes, compared to those of horse cells, necessitates higher centrifugation forces and repetitive sedimentations to ensure a sufficient and selective penetration of the eosinophils into the erythrocyte layer. These modifications increase the isolation time to 8–9 h, which still remains reasonably short.

The purity of the preparations depends highly on the complete removal of the buffy coat, especially after the 2nd centrifugation at 5800× g. The use of the smallest possible centrifuge tubes facilitates this removal. With some ex-



Human eosinophils: *a* light micrograph (× 1540); *b* electronmicrograph (× 3550); *c* detail view (× 17,000).

perience it is easy to reach a purity degree of at least 90%, in most cases 98%. Until now, a comparable purity and recovery was only obtained from blood of patients having an eosinophilia of 40–85%. In these cases the method of Day[11] gives good results, but it can not be used for the isolation of the eosinophils from normal human blood.

Other special and more complicated methods published recently (Spray[12], Shar[13] and Parrillo[14]) use the differences in IgG binding capacity, the different iron phagocytosis or differences in the adherence on a nylon wool surface of the neutrophilic and eosinophilic granulocytes, for isolation of the eosinophils from normal blood.

1 The authors are indebted to the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' for financial support.
2 C.J.F. Spry, Schweiz. med. Wschr. *108*, 1572 (1978).
3 C.J.F. Spry and P.C. Tai, Clin. exp. Immunol. *24*, 423 (1976).
4 J.T. Connell, J. Allergy *41*, 1 (1968).
5 A. Jörg, P. Portmann, G. Fellay, J.L. Dreyer and J. Meyer, Experientia *34*, 1654 (1978).
6 H. Rosen and S.J. Klebanoff, J. clin. Invest. *58*, 50 (1976).
7 M. Markert and J. Frei, Experientia *34*, 914 (1978).
8 B.M. Babior, R.S. Kipnes and J.T. Curnutle, J. clin. Invest. *52*, 741 (1973).
9 L. Ignarro, J. exp. Med. *139*, 1935 (1974).
10 W.H. Grover, H.H. Winkler and D.E. Normansell, J. Immunol. *121*, 718 (1978).
11 R.P. Day, Immunology *18*, 955 (1970).
12 P.C. Tai and C.J. F. Spry, Clin. exp. Immunol. *28*, 256 (1977).
13 R. Sher and A. Grover, Immunology *31*, 337 (1976).
14 J.E. Parrillo and A.S. Fauci, Blood *51*, 457 (1978).

A simple and versatile apparatus for the continuous superfusion of nervous tissue preparations

R.C. Cantrill and M.J.W. Brennan[1]

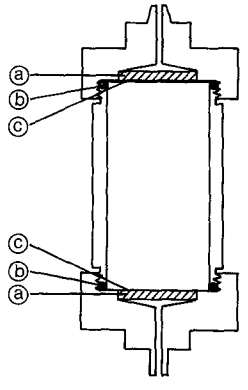
Department of Medical Biochemistry, University of the Witwatersrand Medical School, Johannesburg 2001 (South Africa), 2 April 1979

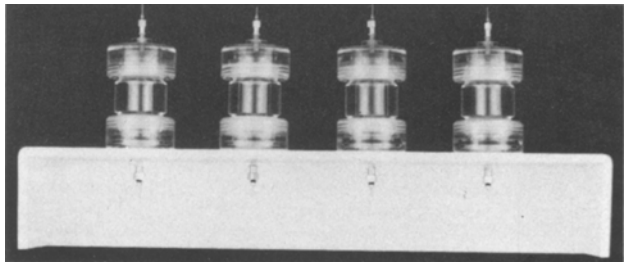
Summary. A bimodal apparatus for the continuous superfusion of both subcellular particles and whole tissue slices is described.

The measurement of the rate of release of neurotransmitter substances from slices or subcellular fractions of nervous tissue has been confounded by the presence of very active transport systems. These uptake processes, which exist for many transmitters in nerve endings, synapse-associated glial cells and post-synaptic perikarya, are responsible for the removal of the released substance from the surrounding medium before an accurate measurement of release can be made. Recently, this problem has been overcome by the development of a superfusion apparatus shown to minimise reuptake of released transmitter by continuous removal of the medium and released transmitter substance[2,3]. In this communication, we describe a simple apparatus which can be used for the continuous superfusion, at constant volume, of subcellular particles or whole brain slices.

The chamber depicted in the figure consists of a central barrel of machined perspex which is screwed into 2 identical perspex endpieces. Each endpiece is fitted with a Millipore 25 mm filter support frit and a rubber 'O' ring (Millipore) to seal the connection. The outlet of the endpiece is machined to give minimum dead volume. The apparatus can be used in the conventional manner to superfuse a bed of subcellular particles resting on the lower Millipore filter. In this mode, an appropriate volume of medium can be introduced into the chamber and the volume thereafter kept constant since the apparatus is airtight. It is also possible to superfuse subcellular organelles or whole tissue slices against gravity by pumping the medium into the chamber from the bottom of the apparatus and drawing it out of the top. Such upward displacement of the medium keeps the tissue preparation in suspension and prevents blockage of the lower filter, a problem often encountered in superfusion of tissue slices by conventional techniques. Since it is necessary to fill the chamber completely with medium when operating in this mode, the volume of the chamber can be varied by altering the length of the barrel.

An apparatus consisting of 4 chambers of the type described above arranged in parallel has been used extensively in the investigation of the efflux of the amino acid neurotransmitters gamma-aminobutyric acid and L-gluta-





Superfusion apparatus. *a* Diagram of the construction of the perspex apparatus in cross section. All dimensions are based on the 25 mm Millipore filter shown at (c). Rubber 'O' rings are shown (b) together with the Millipore support frits (a). *b* The complete apparatus mounted in a bank of 4 chambers as used in experiments to determine neurotransmitter release.