

On the Concentration of Eosinophile Leucocytes

The eosinophile leucocytes are still very little known as far as their function is concerned. The great increase in their count in parasitic infestation and in allergic conditions has attracted great interest. The concentration and separation of these cells from other cellular elements is, however, a necessary condition for most studies on their cytochemistry and physiology. With this aim in view, some methods have been elaborated¹. These imply rather rough treatments of the cells which must be considered to suffer considerable damage. It was therefore felt important to investigate the possibility of concentrating eosinophile cells with the aid of a counter-streaming centrifuge, constructed by the senior author². This centrifuge, which will soon be described³, is constructed with the aim of separating differently sized particles of the same density. Liquid holding the particles to be separated is forced through a conical centrifuge tube against the centrifugal force. Here the larger particles down to a certain limit are accumulated whereas the smaller ones are carried off to a second tube where they are collected.

Because of the eosinophils being extremely large and the sedimentation rate of the erythrocytes high in the horse, blood of this species was used. As is well known, leucocytes have a tendency to agglutinate. This was counteracted by the use of versene which also served as an anti-coagulant.

Blood was drawn from the jugular vein, mixed with isotonic phosphate buffer (pH 7.4) containing versene as to give a final concentration of 0.1% versene. The erythrocytes settled down on standing, and the supernatant, containing the leucocytes, was drawn off, sedimented at 170 g for 3 min and washed several times with Ca⁺⁺-free Tyrode solution, containing 0.05% versene. At this stage, we speak of the material as the "original suspension". The separation in the counter-streaming centrifuge was carried on for 60 min with 300-450 R.P.M. and a streaming velocity of 0.28 ml/sec. The flowing medium in each experiment is given in the Table. The Tyrode solution was Ca⁺⁺-free and contained versene as above. The greatest diameter of the conical tube was 1.60 cm, and the distance between this and the theoretical point of the cone 18.0 cm, the tube being mounted so that this point was 24.0 cm from the centre of rotation⁴. In some experiments the cells were given two runs in the counter-streaming centrifuge.

After this treatment, the state of the material was examined under the microscope at 38°C. The cells appeared morphologically intact and showed normal amoeboid motility. The eosinophile count was determined with the aid of a Fuchs-Rosenthal chamber after staining according to RANDOLPH⁵. In the original suspension, this count varied between 2 and 8% of the total leucocytes.

Eosinophile counts (per cent eosinophils of total leucocytes) in the original suspension and in the conical tube after running in the counter-streaming centrifuge. In

Experiment No. 3 and 4 the same material was given two runs.

Experiment No.	Medium	Original suspension	Fraction in conical tube
1	Isotonic Tyrode	6	21
2	Isotonic Tyrode + saccharose (specific density 1.114)	8	34
3	First run Hypotonic Tyrode (corresponding to 0.85% NaCl)	8	27
	Second run Hypertonic Tyrode (corresponding to 0.95% NaCl)	27	50
4	First run Hypotonic Tyrode (corresponding to 0.85% NaCl)	2	25
	Second run Hypertonic Tyrode (corresponding to 0.95% NaCl)	25	50

As seen from the Table, the separating-out of eosinophils is better the lower the count of the original suspension, the concentrating effect being 3-4 times in the lower, and 2 times in the higher concentration range. By using the counter-streaming centrifuge, samples with eosinophile counts of 20-50% were obtained. On account of individual variations among the horses, it appeared difficult to standardize the procedure.

We are indebted to the Swedish Medical Research Council and to Ely Lilly and Company for financial support.

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Zusammenfassung

Eine Methode zur Anreicherung lebender eosinophiler Leukocyten des Pferdes mit Hilfe einer Gegenstrom-zentrifuge wird ausgearbeitet. In den angereicherten Suspensionen wurde eine Konzentration von 20 bis 50% erreicht.

The Effect of Protamine on the Action of ACTH

The problem of ACTH-heparin antagonism has been extensively dealt with in recent literature, since a number of authors attributed a physiological role to heparin in the inactivation of ACTH¹. We have been unable to confirm this view since it was found that not even pharmacological doses of heparin had any influence on the adrenal ascorbic acid depleting effect of ACTH².

In view of the antagonism existing between heparin and protamine in the mechanism of blood clotting, we have examined protamine for possible effects thought to be specific for ACTH, starting from the assumption that although exogenous heparin cannot enhance the action of physiological heparin, it may be possible that, by neutralising heparin circulating under normal conditions, the effects of ACTH are increased.

¹ R. VERCAUTEREN and G. PEETERS, Arch. Int. Pharm. 39, 10 (1952). - M. BEHRENS und H. R. MARTI, Exper. 10, 315 (1954). - K. M. LINDAHL, 8, 569 (1954).

² P. E. LINDAHL, Nature 161, 648 (1948).

³ I.V.A.'s Tidskrift för Tekniskt-vetenskaplig Forskning (Stockholm).

⁴ P. E. LINDAHL, Nature 161, 648 (1948).

⁵ T. G. RANDOLPH, Proc. Soc. Exper. Biol. et Med. 52, 20 (1943).

¹ Z. Z. GODLOWSKY, Brit. Med. J. 1951, Bd. 4711, 854. - L. WEISS-BECKER and A. SCHRÖTER, Klin. Wschr. 31, 288 (1953). - F. KOLLER and W. FRITSCHY, Helv. med. Acta 14, 263 (1953).

² Gy. FEKETE, Acta med. Acad. Sci. Hung. (in press).