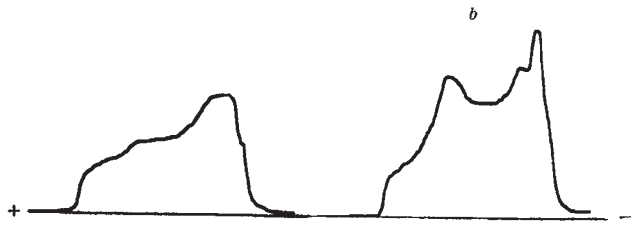

Fig. 1. Change in electrophoretic pattern on using heterologous thrombin (bovine) and fibrinogen (human). (*a*) Lysed fibrin dissolved in urea; (*b*) lysed fibrin mixed with urea

results. These findings prompted the following investigations:

(*a*) Thrombin–fibrinogen interaction without fibrin clot formation: this was achieved by maintaining the reaction at *p*H 4·85 throughout the experiments, thus allowing thrombin to act on fibrinogen but preventing the final formation of fibrin mesh[4]. The same concentrations of reagents mentioned here were used; urea being added after 48 h in order to compensate for the slowing of the thrombin–fibrinogen interaction occurring at *p*H 4·85. No electrophoretic changes could be elucidated on using heterologous reagents. It would therefore appear that the formation of fibrin is essential for the development of the new band.

Table 1. The Effect of Thrombin Species on the Electrophoretic Pattern of Lysed Fibrin

Species		
Thrombin*	Fibrinogen*	New band
Bovine	Human	Present
Human	Bovine	Present
Human	Human	Absent
Bovine	Bovine	Absent

* Freshly prepared solutions used after being clarified by centrifugation at 3,000 r.p.m. for 10 min.

(*b*) Other methods of lysis: for these, clotting of fibrinogen was induced by a lower concentration of thrombin, 10 units/ml. The lysis of this fibrin by the addition of streptokinase (activating plasminogen in fibrinogen solution) or by human plasmin 'Ortho-Actase' gave electrophoretic results similar to those described for the lysis of fibrin clot by thrombin. These findings indicated that the new electrophoretic band, appearing in case of heterologous reagents, was not related to the lytic agent used but rather to the molecular configuration of formed fibrin.

The exact nature of this electrophoretic band remains to be investigated. However, this variance in the electrophoretic pattern of lysed fibrin, depending on the source of thrombin, should be borne in mind when applying experiments on, or using reagents of, different species.

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[1] Nour-Eldin, F., *Nature*, **196**, 782 (1962).
[2] Lorand, L., and Middlebrook, W. R., *Science*, **118**, 515 (1953).
[3] Guest, M. M., and Ware, A. G., *Science*, **112**, 21 (1950). Lorand, L., *Physiol. Rev.*, **34**, 742 (1954).
[4] Laki, K., and Mommaerts, W. F. H., *Nature*, **156**, 664 (1945).

PHYSIOLOGY

Simple Separation of Rabbit Marrow Myeloid Cells from Erythroid Cells

Mel has recently reviewed the sedimentation properties of nucleated and non-nucleated cells in normal rat bone marrow[1]. The complex nature of the factors that are involved in the separation of marrow cells by sedimentation technique are emphasized in his comprehensive paper. Tsuji reported a method for separation of guinea-pig bone marrow cells by using layers of gum acacia solutions of varying densities[2]. Myeloid cells were obtained in practically pure state. Gradient techniques involving several kinds of solutions, such as sucrose and bovine plasma albumin, have been used by others[3].

Procedures for separating bone marrow cells of the rabbit have been investigated in this laboratory for a number of months. Approximately 2 ml. of cells are obtained from the femurs, tibiae and humeri of one animal and suspended in isotonic NaCl solution. They are then filtered through a nylon filter (No. 9331, The MacBick Co., Cambridge, Mass.). After centrifugation and removal of buffy material the cells are placed in 10 ml. of 6 per cent sucrose solution and gently layered on top of seven other layered sucrose solutions, in concentrations from 15 per cent to 75 per cent, with an increment of 10 per cent in successive layers from above downwards. Each of these solutions is 10 ml. in volume and the test-tube is 6 in. in length and 1¼ in. in diameter. The cells in solution are spun at 350*g* for 6 min. The non-erythroid elements are distinctly banded in the lower 3–4 density zones. They can be reconstituted in isotonic NaCl solution and are practically free of erythroid forms. The latter, which are in the top layers, can be obtained by similar reconstitution.

Our most successful method, however, and one which does not involve subjecting the cells to distortion by hypertonic solutions is the simple procedure of adding 6 ml. of isotonic saline to 1·5–2·0 ml. of marrow cells in a 10 ml. centrifuge tube, mixing, and allowing the tube to stand undisturbed at 6° C for 1 h. Under the influence of 1*g* there is marked sedimentation of predominantly myeloid cells. The erythroid cells are almost entirely restricted to the top solution. The time is fairly critical since after 1 h the erythroid cells begin to settle on top of the myeloid cells. Similar successive resuspensions of the bottom section will result in practical isolation of non-erythroid and erythroid elements. This can be accomplished in about four such treatments.

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[1] Mel, H. C., *Nature*, **200**, 423 (1963).
[2] Tsuji, Kenzo, *Osaka City Med. J.*, **8**, 91 (1962).
[3] Goodman, J. W., *Exp. Cell Research*, **21**, 88 (1960).

Presynaptic Inhibition induced by Muscle Stretch

The existence of a powerful inhibitory mechanism acting at the level of the primary afferent fibre has been well demonstrated[1]. The method by which this presynaptic inhibitory pathway acts is generally accepted to be through a prolonged depolarization of the central terminals of the primary afferent fibres with, as a consequence, depression of the amount of excitatory transmitter substance liberated by their synaptic terminals. This depolarization of the central terminals of a particular group of primary afferent fibres is effected in a rather specific manner by afferent impulses from some types of receptors and not from others. For example, the group I afferent impulses entering the spinal cord through the sensory fibres of the nerve to a flexor muscle (but not an extensor) will produce a prolonged depolarization of the central terminals of the primary afferent fibres from annulo-spiral endings of muscles of that limb, both flexors and extensors.

So far, presynaptic inhibition has been tested by electrically stimulating a given afferent nerve and noting the effects on the central terminals of other primary afferent fibres and on the excitatory synaptic action by

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