polarizing pulse, the magnitude of the potential change thereby produced in I and L being 40 per cent of that in G and J. By applying the test pulse at various phases during the single or summated after-hyperpolarization, it was shown that the depression of the test hyperpolarization or depolarization approximately paralleled the amplitude of the after-hyperpolarization.

The prolonged after-hyperpolarizations following impulses in mammalian C fibres[7] and frog myelinated nerve[9] show also a temporal summation. The ionic process of these after-hyperpolarizations has been related to the active transport of sodium and potassium ions that would hyperpolarize the membrane by depletion of the extracellular potassium[7,8] or by driving an electrogenic sodium current[9]. In this respect, the after-hyperpolarization in motoneurones, which is generated by the increased potassium conductance, appears to be a unique phenomenon. The special significance of the motoneurone after-hyperpolarization has already been discussed[2,10].

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[1] Brock, L. G., Coombs, J. S., and Eccles, J. C., *J. Physiol.*, **122**, 429 (1953).
[2] Eccles, J. C., Eccles, R. M., and Lundberg, A., *J. Physiol.*, **142**, 275 (1958).
[3] Coombs, J. S., Eccles, J. C., and Fatt, P., *J. Physiol.*, **130**, 291 (1955).
[4] Frank, K., and Fuortes, M. G. F., *J. Physiol.*, **134**, 451 (1956).
[5] Coombs, J. S., Curtis, D. R., and Eccles, J. C., *J. Physiol.*, **145**, 505 (1959).
[6] Ito, M., *Jap. J. Physiol.*, **7**, 297 (1957).
[7] Ritchie, J. M., and Straub, R. W., *J. Physiol.*, **136**, 80 (1957).
[8] Greengard, P., and Straub, R. W., *J. Physiol.*, **144**, 442 (1958).
[9] Straub, R. W., *J. Physiol.*, **159**, 19P (1961).
[10] Kuno, M., *J. Physiol.*, **149**, 374 (1959).

Mechanism of Calcification : Inhibitory Role of Pyrophosphate

The mechanism of calcification has been considerably clarified recently[1]. Collagen fibrils were shown to possess *in vitro* the property of nucleating hydroxyapatite precipitation and of triggering mineralization[1-3]. But why does only part of the collagen present in the organism calcify ? Recently, we suggested that the activating sites of collagen and crystal growth could be blocked in the organism by a plasma inhibitor[4]. Indeed we showed plasma to contain one or several substances highly inhibitory to apatite precipitation. The technique used was to determine *in vitro*, at physiological conditions, the minimum ion product (Ca) × (P) necessary for hydroxyapatite precipitation. After addition of plasma, the rise of this minimum product revealed the presence of inhibitors. As the inhibitor was shown to be destroyed by alkaline phosphatase, and the polyphosphates to have a strong inhibitory activity at concentrations as low as 10^{-7} M, we suggested this inhibitor to be a polyphosphate. However, to our knowledge, no such compound has so far been demonstrated in plasma or in urine.

As the concentration of plasma of this hypothetical polyphosphate was bound to be very low (10^{-5} M), we first searched for it in urine, where it was implied to exist, according to our test, in a much higher concentration. After isolation, its nature was determined as inorganic pyrophosphate[5,6]. The mean daily excretion of pyrophosphate in healthy males was found to be 2·16 mg expressed as phosphorus ($\varepsilon = \pm 0{\cdot}20$); in adult young women it is below 1 mg, but it increases with age. By its highly inhibitory action, the urine pyrophosphate accounts for the supersaturation of urine in calcium and phosphorus. Our finding that its mean daily excretion falls to 1·06 mg ($\varepsilon = \pm 0{\cdot}17$) in urolithiasic men, suggests that its action is present both *in vivo* and *in vitro*.

The presence of pyrophosphate in urine suggests that the plasmatic inhibitor is of a similar composition. Indeed, in experiments recently completed, we could isolate from plasma a phosphorus compound highly inhibitory of hydroxyapatite precipitation which, after purification, migrates on paper chromatography in the same way as inorganic pyrophosphate, with three different solvents (Solvent I : isopropanol, 70 ; water, 30 ; 20 per cent ammonium hydroxide, 0·3 ; trichloroacetic acid, 4 g. Solvent II : methanol, 70 ; 2 N ammonium hydroxide, 30. Solvent III : *n*-propanol, 30 ; ethanol, 30 ; water, 39 ; 25 per cent ammonium hydroxide, 1). Its concentration, although very low (10^{-5} M), easily inhibits apatite precipitation. Its action at this level cannot be explained by a lower ionic calcium concentration due to the formation of a complex, but it is probably a 'poisoning' of crystal growth.

The following mechanism of calcification is therefore suggested : Hydroxyapatite precipitation can occur at a physiological concentration of calcium and phosphorus, due to the nucleating function of collagen. This property, together with crystal growth, is inhibited by plasma pyrophosphate, which protects the collagen that is not to be mineralized. For collagen to calcify, pyrophosphate must be destroyed *in loco* by the enzyme pyrophosphatase, which was shown to be present in mineralizing tissues[7-9].

We thank Prof. H. Pfaendler, interpreter, World Health Organization, for translating this manuscript.

This work was supported by the grant A-4909 of the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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[1] Neuman, W. F., and Neuman, M. W., *The Chemical Dynamics of Bone Mineral* (Univ. Chicago Press, 1958).
[2] Sobel, A. E., Laurence, P. A., and Burger, M., *Trans. N.Y. Acad. Sci.*, **22**, 233 (1960).
[3] Glimcher, M. J., *Rev. Mod. Phys.*, **31**, 359 (1959).
[4] Fleisch, H., and Neuman, W. F., *Amer. J. Physiol.*, **200**, 1296 (1961).
[5] Fleisch, H., and Bisaz, S., *Helv. Physiol. Acta*, **19**, C69 (1961).
[6] Fleisch, H., and Bisaz, S., *Amer. J. Physiol.* (in the press).
[7] Perkins, H. R., and Walker, P. J., *J. Bone and Joint Surg.*, **40**, B, 333 (1958).
[8] Cartier, P., and Picard, J., *Bull. Soc. chim. biol.*, **37**, 1169 (1955).
[9] Dulce H. J., *Z. Physiol. Chem.*, **319**, 272 ; **320**, 1 (1960).

HÆMATOLOGY

Accelerated Lysis of Blood Clots

In a recent article[1], we described a class of compounds which specifically inhibited cross-linking of the sub-units of the clot network. The possibility arose that clot lysis by the proteolytic enzyme system of blood (plasmin) might be greatly enhanced if clotting were allowed to take place in the presence of the foregoing inhibitors, of which glycineamide is a simple example. (Of eight glycyl dipeptides so