was added, a part of the histone was split under the influence of proteolytic enzymes in the myocardium and the addition of ATP to the bath allowed ATP dephosphorylation outside the cells with liberation of inorganic phosphate and adenosine. Adenosine passes into the cell where ATP resynthetizes. At the same time the liberated inorganic phosphate passes slowly into the cell and ATP is formed intracellularly[6]. Therefore, the contractions which were stopped under the influence of histone F_1, restarted after addition of ATP to the bath. The amount of ATP resynthetized in this way is less, on the one hand because of increased activity of ATP-ase stimulated by histones[7], and on the other hand with smaller presence of ATP. If, on the contrary, ATP is added before histone F_1, contractions only decrease, but persist till the end of experimental period (120 min). This can be explained as a result of more ATP which produces a slight increase in the amplitude of contractions, and later addition of histone F_1 only decreases the amplitude without complete inhibition. The mentioned surplus of ATP in the cell cannot be completely decomposed with ATP-ase stimulated by histones. But if histone F_1 and ATP were added simultaneously there is a direct interaction between them, so that the effect of ATP is blocked, and, once inhibited, contractions cannot be restored.

Résumé. L'action de la fraction F_1 des histones et de l'ATP sur l'amplitude des contractions a été examinée sur le ventricule droit des rats blancs. On a constaté que l'ATP appliqué après l'arrêt des contractions dû à l'histone F_1 provoquait la reprise de celles-ci. La présence de l'ATP avant l'application des histones F_1 abaisse seulement l'amplitude, mais les contractions persistent jusqu'à la fin de l'expérience. L'application simultanée de l'ATP et des histones F_1 provoque une inhibition irréversible des contractions ventriculaires.

D. PETKOVIĆ, M. PAVLOVIĆ and M. CVETKOVIĆ

Department of Physiology and Department of Biochemistry, Medical Faculty, Visegradska 26/2 Beograd (Yugoslavia), 13 July 1972.

6 PH. C. HOFFMANN and G. T. OKITA, Proc. Soc. exp. Biol. Med. *119*, 273 (1965).
7 A. SCHWARTZ, Circulation *30*, 155 (1964).

Alkaline Phosphatase Activity in Relation to DNA Synthesis in Synchronized HeLa S3 Cells

The biological role of alkaline phosphatase in the cell is not yet understood and its substrate(s) in vivo are not yet defined. In vitro experiments with mammalian cell extracts show that the enzyme is a nonspecific phosphomonoesterase, able to cleave a wide variety of phosphorylated substrates[1–3]. The facts that it can dephosphorylate nucleotides (nucleotidase activity)[2] and transfer phosphate groups from nucleotides to nucleosides (transphosphorylase activity)[2,4] prompted GEORGATSOS[2] and MELNYKOVYCH et al.[3] to suggest that alkaline phosphatase might be involved in nucleic acid biosynthesis by regulating the levels of free nucleotides in the cell.

In addition to the transphosphorylase activity associated with alkaline phosphatase, purified preparations of the enzyme from various sources, including mammalian cells, possess inorganic pyrophosphatase activity. It has been suggested[5–10] that all these activities are associated with a single enzyme protein.

Since inorganic pyrophosphate is a by-product of several biosynthetic reactions, including DNA and RNA synthesis, it has been suggested that it might be the natural substrate of alkaline phosphatase acting as a pyrophosphatase[5–9]. This would provide a mechanism by which the polymerization of nucleic acids would be irreversible.

If alkaline phosphatase is indeed involved in the regulation of the level of free nucleotides in the cell (as a transphosphorylase), or if it is involved in the control of the polymerization of nucleic acids (as a pyrophosphatase), then its activity in the cell should have some predictable relationship to DNA synthesis.

Materials and methods. HeLa S3 cells (P 1287) were grown as monolayers in a medium consisting of equal parts of Eagle's modified medium and medium M199 with 10% calf serum, 5 ml/l of 3% glutamine, 200 U/ml penicillin G, and 20 U/ml dihydrostreptomycin. Cells were synchronized by the double thymidine blocking treatment as previously described[3,11]. At given times after release from the second thymidine block, 11.6 Ci/m*M* ^{3}H(methyl) thymidine (^{3}H TdR) was added to cell cultures to a final concentration of 0.8 μCi/ml. After 60 min at 37 °C the radioactive medium was removed and cells were washed 3 times with Earle's balanced salt solution (BSS) containing 2 m*M* unlabeled thymidine. Cells were then collected by trypsine-versene, washed with BSS, counted, and sonicated (MSE sonicator, 9 microns between peaks) in 3 ml distilled water for 30 sec (2×15). The sonicate was centrifuged at $10{,}000 \times g$ for 60 min at 4 °C. Next 0.2 ml sonicate was pipetted into 2 ml of cold 6% solution of trichloroacetic acid (TCA) and the acid insoluble fraction was collected by suction on milipore filters which had been prewashed with unlabeled 2 m*M* thymidine to prevent nonspecific binding of the isotope to the filter. Filters were then dried and counted in 10 ml of scintillation fluid (5 g PPO, 200 mg POPOP, 1 l toluene) in a Packard Tri-Carb Spectrometer.

Alkaline phosphatase was assayed in 2.5 ml reaction mixture of 0.1 *M Tris*-HCl, pH 9, 2 m*M* disodium *p*-nitrophenyl phosphate (Fluka), and 50 μl cell sonicate. After incubation for 30 min at 37 °C, 0.5 ml of 2 *N* NH_4OH

1 F. HERZ and H. M. NITOWSKY, Arch. Biochem. Biophys. *96*, 506 (1962).
2 J. G. GEORGATSOS, Arch. Biochem. Biophys. *121*, 619 (1967).
3 G. MELNYKOVYCH, C. F. BISHOP and M. A. SWAYZE, J. Cell Physiol. *70*, 231 (1967).
4 R. HARKNESS, Arch. Biochem. Biophys. *126*, 513 (1968).
5 R. P. COX and M. J. GRIFFIN, Lancet *2*, 1018 (1965).
6 R. P. COX, P. GILBERT JR. and M. J. GRIFFIN, Biochem. J. *105*, 155 (1967).
7 F. MELANI and M. FARNARARO, Biochim. biophys. Acta *178*, 93 (1969).
8 M. J. GRIFFIN, Arch. Biochem. Biophys. *132*, 299 (1969).
9 P. L. MILES and P. R. V. NAYUDU, Enzymologia *41*, 27 (1971).
10 M. M. CHAN, R. B. RUCKER, F. ZEMAN and R. S. RIGGINS, Proc. Soc. exp. Biol. Med. *141*, 822 (1972).
11 G. GALAVAZI, H. SCHENK and D. BOOTSMA, Expl Cell Res. *41*, 428 (1966).

was added and the yellow color of the *p*-nitrophenol released from the substrate by the enzyme was read at 400 nm. A unit of enzyme was defined as the amount required to hydrolize 1 µmole substrate per min. ε 400 of *p*-nitrophenol is 12,000[12].

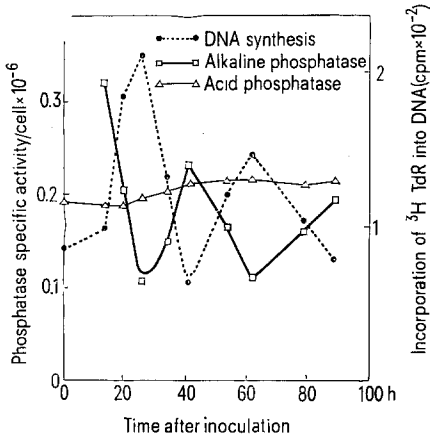
Acid phosphatase was assayed as previously described[13] except that the buffer in the reaction mixture was 0.1 *M* sodium acetate, pH 5.

Results and discussion. Alkaline phosphatase activity was highest when DNA synthesis was lowest and vice versa (Figure). Acid phosphatase activity, however, remained almost constant throughout several cell cycles as previously described[14–16].

The inverse relationship between DNA synthesis and alkaline phosphatase might indicate a way in which the enzyme is involved in DNA metabolism. If the natural substrate in the cell for alkaline phosphatase is inorganic pyrophosphate and this pyrophosphatase activity is needed to render nucleic acid biosynthesis irreversible by cleaving the pyrophosphates, which are by-products of nucleic acid synthesis[5–10], then its activity in the cell should be highest during the S-phase. Our results do not support this hypothesis. It is possible that alkaline phosphatase and pyrophosphatase, which are thought to be associated with a single enzyme[5–10], are actually separate entities. This idea has recently gained support from a work by HERZ and KAPLAN[16] which shows that in contrast to its striking effect on alkaline phosphatase activity in several cell lines, human serum does not modulate the activity of inorganic pyrophosphatase to a significant extent.

If alkaline phosphatase is involved as transphosphorylase in controlling the level of nucleotides in the cell as suggested[2,3], then its activity in the cell should be highest after completion of the S phase. At this stage the cell should start building up its free nucleotide pool, which is exhausted during DNA synthesis, in preparation for the next cycle of DNA syntheis. Our results support this hypothesis but do not exclude the possibility that alkaline phosphatase also has other biological duties in the cell.

Our results disagree with REGAN's study[17] which failed to find such relationship between alkaline phosphatsae and DNA synthesis in HeLA cells that were synchronized with 5-aminouracil.



Acid phosphatase and alkaline phosphatase activities in relation to DNA synthesis in synchronized HeLa S3 cells. 3 synchronized cultures were pulse labelled at various time intervals with ^{3}H TdR; cells were harvested, sonicated, and assayed in triplicate for acid and alkaline phosphatase and for acid-insoluble ^{3}H TdR incorporation into DNA. The detailed procedure is described in Materials and Methods. △, acid phosphatase; □, alkaline phosphatase; ●, acid-insoluble cpm ^{3}H TdR incorporated into DNA.

Zusammenfassung. In synchronisierten HeLa S3-Zellen wurde die Aktivität der alkalischen Phosphatase in Bezug auf den Zellteilungszyklus untersucht. Die geringste Aktivität wurde während der S-Phase und die höchste beim Minimum der DNS-Syntheseaktivität festgestellt, und es wird angenommen, dass die alkalische Phosphatase als Transphosphorylase zur Regulation der Polgrösse von Nucleotiden dient.

H. SLOR[19] and H. BUSTAN

Department of Human Genetics, Tel Aviv University Medical School, Tel Aviv (Israel), 16 April 1973

[12] W. E. RAZZEL and H. G. KHORANA, J. biol. Chem. *236*, 1144 (1961).
[13] H. SLOR and T. LEV, Biochem. J. *123*, 993 (1971).
[14] H. M. NITOWSKY and F. HERZ, Biochem. Biophys. Res. Commun. *11*, 261 (1963).
[15] R. P. COX and C. M. MACLEOD, Cold Spring Harbor Symp. quant. Biol. *29*, 233 (1964).
[16] F. HERZ and E. KAPLAN, Expl Cell Res. *74*, 307 (1972).
[17] J. D. REGAN, Experientia *22*, 708 (1966).
[18] Acknowledgment. This research was supported in part by a grant from the Israel Cancer Association.
[19] Present address: Laboratory of Radiology, University of California, San Francisco (California 94122, USA).

Substratum Specificity of Purified Peroxidase Isoenzymes of Horse Radish Root

Peroxidase is an enzyme present in almost all higher plants[1], in human saliva[2], in the medular part of the suprarenal gland[3], in liver[4], kidneys[5], leukocytes[6]. The peroxidase isolated from horse radish root was much studied, being almost completely characterized[7–9]. This enzyme being highly specific for hydrogen peroxide, in whose presence it catalyses the oxidation of certain substances such as: phenols, aromatic amines, triptofan, bilirubin, pyrogalol, benzidine, etc.

The purpose of the present work consists in separating horse radish peroxidase isoenzymes by the chromatography on column method, and in analyzing the specificity of different peroxidase fractions for peroxidic groupings contained in the H_2O_2 ROOH, ROOR type compounds (in which R is the cumyl radical).

[1] E. F. HARTREE, in *Modern Methods of Plant Analysis* (Eds., K. PAECH and M.V. TRACEY; Springer-Verlag, Berlin 1955), vol. 4; p. 231.
[2] W. MOSIMAN and J. B. SUMNER, Arch. Biochem. *33*, 487 (1951).
[3] I. HUSZAK, Biochem. Z. *312*, 330 (1942).
[4] W. G. HEIM, D. APPLEMAN and H. T. PYFROM, Am. J. Physiol. *186*, 19 (1956).
[5] G. BANCROFT and K. A. C. ELLIOTT, Biochem. J. *28* 1911 (1934).
[6] K. AGNER, Acta chem. scand. *12*, 89 (1958).
[7] L. M. SHANNON, E. KAY and J. Y. LEW, J. biol. Chem. *241*, 2166 (1966).
[8] E. KAY, L. M. SHANNON and J. Y. LEW, J. biol. Chem. *242*, 2470 (1967).
[9] B. C. SAUNDERS, A. G. HOLMES-SIEDLE and B. P. STARK, *Peroxidase*, Butterworths London, (1964).