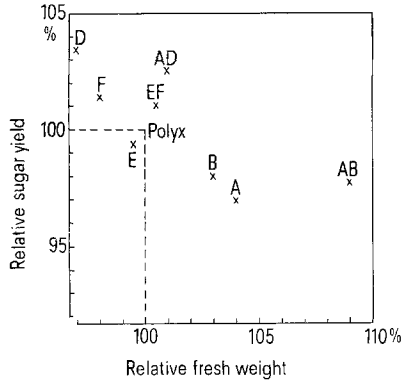
Peroxidase as Biochemical Measure of Fresh Weight and Sugar Yield in Sugar-Beet

The correlation between mean fresh weight in full-grown sugar-beet tubers and sugar yield is well known by practicians in the sugar-beet industry: the bigger are the tubers, the lower the sugar yield (per kg), in general. Selection and hybridization studies tend naturally to increase both fresh weight and sugar yield at the same time.

Preliminary experiments[1] on tubers indicated a positive correlation between sugar richness and peroxidase. The present experiments were undertaken to question the predictive value of peroxidase activity in seedlings of new hybrids for establishing the degree of superiority of yield in full-grown tubers.

Peroxidase and protein were measured by methods already described[1,2], comparatively in extracts from 7-day-old seedlings (culture in sand, 25 °C, dark) and from corresponding field full-grown tubers.



Distribution of parents and sugar-beet hybrids on the basis of their relative fresh weight and sugar yield compared to those of the Polyx variety taken as reference.

Peroxidase activity (measured by gaïacol) expressed in equivalents µg HRP (horseradish peroxidase Fluka) per unit protein, in tubers and seedlings roots of the sugar-beet populations A, B, D, E, F, AB, AD and EF (see Figure)

	A	B	D	E	F	AB	AD	EF
Tubers	0.83	0.78				0.59		
Seedlings	2.02[a]	2.96				1.80		
	1.81[a]	1.92				1.60		
	2.26[a]		4.21				3.16	
	2.19[a]		3.69				2.54	
				3.16	3.05			2.85
				4.21	4.50			2.95

[a] Mean values from different seed samples, studied at different times of a year.

Characteristics of parents and hybrids used here are indicated in the Figure. Comparisons of peroxidase activity in seedlings and tubers are given in the Table.

Let us consider the hybrids AB and AD obtained from the parents A, B and D. As can be seen in the Figure, the hybrid AB is bigger than the parents A and B, the size of the hybrid AD is between those of A and D. This situation is well reflected in peroxidase activities: peroxidase activity in AB is lower than that in A and B; it is intermediate between A and D in AD. Results obtained with E, F and EF are in agreement with the preceding ones. Important is the fact, as already mentioned[1], that this peroxidase index for tuber growth can be measured early in young seedlings, as well as in full-grown tubers (example A, B, AB). So this correlation between peroxidase and capacity of growth is automatically associated with sugar yield in sugar-beet and can serve as biochemical measure in selection studies.

The relationship between peroxidase and capacity of growth is well-known since the first experiments of VAN OVERBEEK[3] in maize dwarfism. It can be interpreted in terms of auxin available for growth through its control by the auxin-oxidase activity of peroxidase[4]. The link between sugar and peroxidase is not yet understood. But knowing that sugar is involved in ethylene biosynthesis[5] and that this gazeous hormone controls peroxidase[5], it would not be surprising to find a correlation between sugar yield peroxidase and ethylene biosynthesis in sugar-beet too. Experiments are being undertaken to clarify further the sugar yield capacity in this material[6].

Résumé. L'activité peroxydasique est toujours nettement plus élevée dans les tubercules de populations de Betterave caractérisés par un poids frais moyen peu élevé et par une forte teneur en sucre. Cette corrélation est décelable dans les plantules de quelques jours: elle peut donc servir de critère biochimique de sélection.

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The Influence of Histone Fraction F[1] and ATP on the Amplitude of Contractions of the Isolated Right Ventricle of Rats

In our previous work[1] we established that addition of different fractions of histones to the perfusion fluid produces changes in the amplitude of contractions of the isolated rat's left auricles. The most effective among them was fraction F_1 (lysine very rich histones), which most powerfully inhibited the amplitude of contractions. As ATP is indispensable for muscle contraction as the direct

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donor of the energy, and histones inhibit its snythesis[2-4], we considered it would be interesting to investigate the interaction among histones and ATP on the amplitude of contractions of the isolated rat's right ventricle.

Materials and method. The experiments were carried out on adult rats of both sexes weighing 200–300 g each. The heart was removed immediately after killing the animal, the right ventricle was dissected and put on the boot electrode. The electrode was then placed in an oxygenated bath with Tyrode's solution. The volume of the bath was 30 ml and its temperature kept at 36–37 °C. Stimulation was done with square wave impulses from a stimulator. The frequency of stimulation was 12/min, the duration of impulses was 1.5 msec and the intensity between 4–10 V. The amplitude of contractions was recorded by a light isotonic lever on smoked paper of the kymograph.

The preparations were divided into 4 groups. In the 1st group only the fraction F_1 of histones (40 mg/ml) was added to the bath; in the 2nd, fraction F_1 (40 mg/ml) and when the ventricle's contractions stopped, an equivalent amount of ATP (40 mg/ml) was added; in the 3rd, ATP (40 mg/ml) and after 15 min fraction F_1 (40 mg/ml), and in the 4th, simultaneously histone F_1 (40 mg/ml) and ATP (40 mg/ml). In all groups the ventricles were left in the bath for 30 min for stabilization before the substances were added.

Histone fraction F_1 was extracted from calf thymus according to the JOHNS[5] procedure (method 1). Commercial ATP (Boehringer, Mannheim) was used.

Results. The results are presented in the Figure. In the 1st group of experiments (13 specimens) the addition of histone F_1 inhibits the contraction of the right ventricle in about 10.5 min (the average value). In the 2nd group (10 specimens) contractions stopped at nearly the same time after addition of fraction F_1 as in the 1st group. 15 min after the contractions were stopped, ATP was added to the bath and 7 min later (average value) contractions restarted but the amplitude was smaller than before addition of histone F_1. These contractions persisted till the end of the experiment (120 min). In the 3rd group (7 specimens), 15 min after addition of ATP, which produces only a slight increase of the amplitude of contractions, histone F_1 was added to the bath. The addition of histone F_1 produces only a decrease of the amplitude of contractions, which persisted till the end of the experiment (120 min). In the 4th group (6 specimens), simultaneous addition of histone F_1 and ATP abolishes the contractions after 9 min (average value), and, till the end of experiment (120 min), they remained inhibited.

Discussion. Starting from the fact that histones inhibit the synthesis of many important tissue metabolites, among them ATP[2,3], as well as the fact that ATP is indispensable for the muscle contraction, we investigated the interactions between histones and ATP. The results of our experiments showed that histone fraction F_1 has significant inhibitory influence on the amplitude of contractions of the isolated rat's right ventricle, while the addition of ATP after cessation of contractions produced by histone F_1 restarted their contractions. The contractions were restored because, up to the time when ATP

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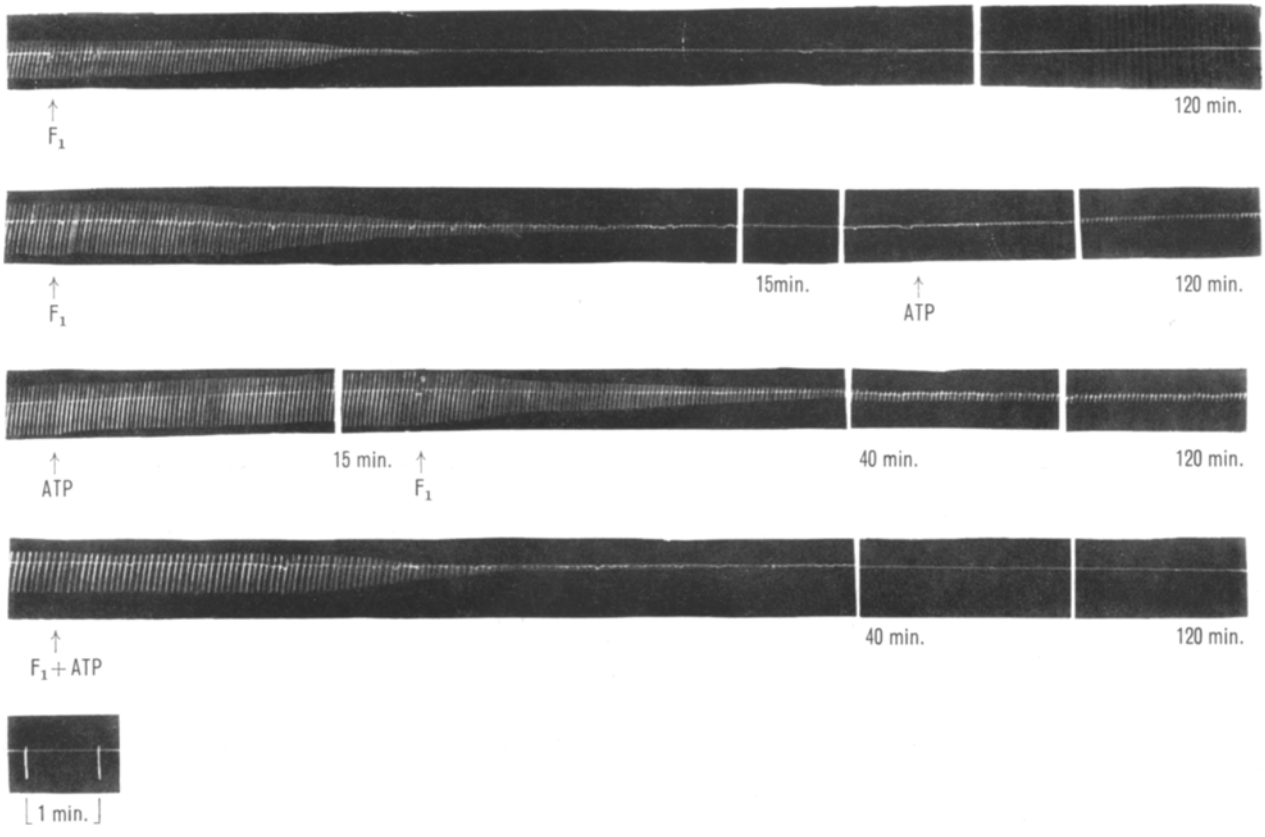

Effect of histone F_1 and ATP on the amplitude of contractions of the isolated right ventricle of rats. For details see the text.

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.

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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

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