only resting potentials are registered, FDP in the same concentration provokes a rhythmical electrobiological activity of the tissue investigated (Figure 1 b). The increase in the frequency of the action potentials induced by FDP is preceded by a short latent period. This effect of FDP lasts for about 2–5 min. In the course of our experiments, we have found that the increase in the number of the action potentials per unit of time, caused by FDP, depends on the initial electrobiological frequency 'f_i' (Figure 2). From the diagram it can be seen that FDP provokes the appearance of the action potentials in the tissue of the right auricle which previously showed only the resting potential. The frequency of these action potentials are the highest which we have encountered in the course of our experiments. The influence of FDP diminishes together with the increase of the spontaneous frequency of the action potentials.

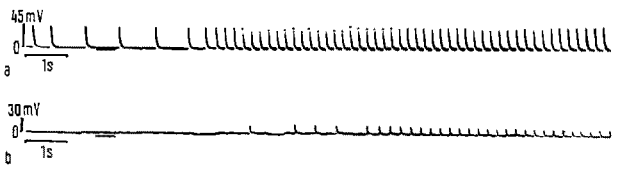

Fig. 1. a) Influence of FDP in concentration of 2.5 µg tyrosine on the frequency of the action potentials in the right auricle of the heart of rat, showing spontaneous activity. b) Appearance of rhythmical electrobiological activity influenced by FDP in the same concentration as in a).

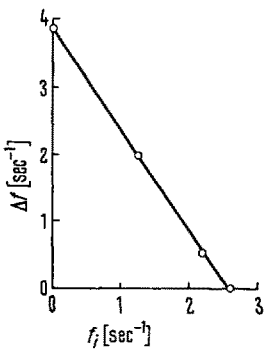

Fig. 2. The relation of the frequency of action potential increase under the influence of a constant dose (2.5 µg eq. tyr.) of FDP with initial frequency of the action potentials (f_i). The abscissa: difference (Δf) between the initial frequency (f_i) and the maximal frequency caused by FDP. The ordinate: initial frequency (f_i) i.e. the number of the action potentials in 1 sec.

Discussion. In our experiments we have observed the action potentials characteristic of the right auricular muscle[8] and the results presented indicate that FDP accelerates the appearance of the action potentials in the tissue which is electrobiologically active, and that FDP facilitates a spread of the activation wave in the right auricle, a manifestation of which is the appearance of electrobiological activity in tissue which had up till then exhibited only resting potentials.

The fact that the action of FDP depends on the initial electrobiological frequency and that under its influence action potentials appear in the tissue which possessed only the resting potential indicates that the essential action of FDP consists in its effect on the properties of the cell membrane and thus on the metabolic processes and excitability of the heart muscle cells. This view is supported by the results of previous papers particularly those showing that FDP influences the behaviour of electrobiological phenomena in the rat uterus cell[5–7].

The products formed during fibrinogen digestion by plasmin are always present in the plasma and their level constantly rises in pathological states in which there is an activation of the plasmin proteolysis[6]. The properties of FDP observed to date[1–4, 6, 9] and the results presented here indicate that FDP is an agent which, besides its role in the processes of haemostasis, acts not only on the local circulation, but also by influencing the electrobiological phenomena in the heart, may have, to some extent, an effect on the general circulation.

Résumé. Nous avons constaté que les produits de dégradation de fibrinogène (FDP) dûs à l'action de la plasmine exercent une influence sur les phénomènes électrobiologiques de l'oreillette droite isolée du cœur du rat.

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Metabolism of Different Histone Fractions in Ehrlich Ascites Cells after Environmental Change

Many investigators studied content, composition and metabolism of histones in various types of cells in an effort to understand the mechanism by which histones could specifically block the DNA template. While the analytical work has shown a remarkable similarity in types of histones isolated from different types of cells or organisms[1], the relationship between the rates of synthesis of individual histone fractions and between the metabolisms of histones and of DNA was found different from cell type to cell type[2]. To determine whether these relationships are characteristic for different types of cells, or are an expression of growth and metabolic conditions of the cells at the time of the experiment, the synthesis of individual histone fractions in Ehrlich ascites cells cultivated in vivo and in vitro was measured.

In in vitro experiments Ehrlich ascites cells were collected seven days after i.p. inoculation of about 4×10^6 cells into BDF1 strain of mice, washed free of erythrocytes with Dulbecco buffer, suspended in Earle's balanced salt solution to 2×10^6 cells per ml and swirled at 37 °C. Suspensions of cells were labeled at various intervals by addition of 1.0 µCi of amino acid ^{14}C (reconstituted protein hydrolysate, Schwarz Bioresearch Inc.; Schwarz Mixture) and 1.0 µCi of thymidine-methyl-^3H, Schwarz Bioresearch Inc., specific activity 7.0 Ci/mmole) per 2×10^8 cells 30 min prior to the time of harvest. In in vivo

experiments groups of 10 mice each were injected with labeled precursors on different days after i.p. inoculation of Ehrlich ascites cells into the mice. Each mouse in the group was injected i.p. with 1 μCi of thymidine-methyl-^{3}H in 0.2 ml of Dulbecco buffer and the cells were harvested 30 min after the injection.

After harvesting, the cells were immediately chilled and washed 3 times with Dulbecco buffer. Nuclei were isolated from cells by the procedure of CHAUVEAU et al.[3].

The arginine-rich histones were extracted from the nuclei with a mixture of absolute ethanol and 1.25 *N* HCl (4:1, v/v) and the lysine-rich histones were extracted from the ethanol-HCl residue with 0.2 *N* HCl[4]. The two groups of histones obtained were further fractionated by starch gel electrophoresis into the fractions F1 (very lysine-rich histones) F2a, F2b (lysine-rich histones) and F3 (arginine-rich histones). Bands corresponding to individual histones were cut from starch gels and each cut-out block was divided into 2 halves. One half was used to determine the amount of histones by the method of HABERMANN and SZOPA[5], the other half to measure the amount of incorporated ^{14}C in the histones by the method of KALBERER and RUTSCHMANN[6]. A part of the nuclei was used to prepare total histones by extraction with 0.2 *N* HCl[7]. After the histone extraction DNA was extracted from the nuclei with 0.5 *N* perchloric acid (15 min at 90 °C) and determined by the BURTON procedure[8]. The ^{14}C and ^{3}H were counted in Beckman's liquid scintillation spectrometer. The viability of cells was measured by staining with nigrosin.

If Ehrlich ascites cells after growing for 7 days in a mouse were transferred to a swirling culture in vitro, the synthesis of DNA declined and after 12 h the incorporation of thymidine-methyl-^{3}H into DNA was negligible (Table). During the same time period there was no increase in the percentage of dead cells. In contrast the synthesis of histones continued even after 12 h. But immediately after the transfer of cells from in vivo to in vitro, the relative ratios of amino acids incorporated into individual histone fractions changed. The relative activity of the F3 histones increased and simultaneously the relative activity of the F1 histones decreased. The change was more pronounced with increasing the time of swirling (Table).

Similar results were obtained if the relative incorporation of ^{14}C amino acids into histone fractions was measured in cells grown for 9 and 12 days in a mouse. 7 days after the inoculation cells are still in the logarithmic phase of growth, but after 9 days their growth is changing to a stationary phase. This change is reflected in declining incorporation of thymidine-methyl-^{3}H into DNA (Table), but not in the increasing number of dead cells. 12 days after inoculation, the percentage of dead cells in the population increased only to 13% from 11% of dead cells found in cells checked 7 days after inoculation. The decline in DNA synthesis was not coupled with a similar decline in the synthesis of histones. The ratios of incorporated amino acids into individual fractions changed in this experiment, too. The labeling of F1 histones was suppressed more than the labeling of the F2a and F2b histones and the labeling of the F3 histones even increased (Table).

If environmental conditions become unfavorable for cell growth, as in mice 12 days after inoculation with Ehrlich ascites cells, or in vitro after transfer of these cells to a balanced salt solution, the cells react to such a change by cessation of cell division, and by a change in the types of newly made RNA[9]. Histones are connected with the regulation of RNA synthesis; therefore, it is not surprising that environmentally caused changes in

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Specific activities of DNA and 4 histone fractions isolated from Ehrlich ascites cells cultivated in vivo and in vitro

	DNA	Total histone	Histone fractions							
			F1		F2a		F2b		F3	
	spec. act. DPM/mg	spec. act. DPM/mg	spec. act. DPM/mg	ratio to F2b	spec. act. DPM/mg	ratio to F2b	spec. act. DPM/mg	ratio to F2b	spec. act. DPM/mg	ratio to F2b
Cells were labeled in vivo										
5 days	12,700	620	852	1.91	618	1.39	445	1.00	680	1.53
7 days	12,080	632	949	2.03	474	1.02	465	1.00	759	1.63
9 days	2,030	506	605	1.41	368	0.86	429	1.00	750	1.75
12 days after inoculation of the cells into mice	455	412	302	0.77	330	0.85	390	1.00	865	2.21
Cells were labeled in vitro										
30 min	35,400	2,672	2,650	1.13	2,520	1.07	2,345	1.00	4,373	1.87
3 h	10,870	2,423	1,760	0.89	2,021	1.02	1,973	1.00	5,700	2.88
6 h	4,960	2,200	1,672	0.98	1,836	1.07	1,705	1.00	5,410	3.17
12 h	950	1,400	832	0.82	933	0.92	1,013	1.00	2,985	2.95

after transfer from mice to a balanced salt solution

gene transcription are expressed in a metabolism of histones which is independent from cell duplication and in a change of ratios of labeling of different histone fractions.

The function of different histones in the cell is not known. The independence of their metabolic patterns suggests that their functions are different. The tissues and species specificity of very lysine-rich histones (F1) as well as an increase in their content in nuclei during development[10,11] indicate that the F1 histones are involved in restricting the availability of the DNA template in the process of differentiation. Also the finding that removal of F1 histones from the chromatin will increase the extent to which RNA synthesized on this chromatin will hybridize with DNA[12] is in agreement with this suggestion. In contrast, removal of arginine-rich histones will only increase the rate of RNA synthesis but not its hybridizability[12]. Since the arginine-rich histones specifically interact with RNA polymerase[13], it is possible that this histone fraction regulates the response of the genome to environmental changes. Results reported in this communication support such a hypothesis.

The results presented have shown that the metabolism of histones, especially the arginine-rich histone fraction, can be detached from cell replication and is a part of the adaptive mechanism by which cells react to changed metabolic conditions[14].

Zusammenfassung. In Ehrlich-Asziteszellen, die ungünstigen Umweltbedingungen ausgesetzt sind, hört die Zellteilung auf. Doch auch ohne DNS-Synthese geht der Histon-Stoffwechsel noch eine Zeitlang weiter, wobei die Synthese der argininreichen Histone dreimal so gross ist wie die der anderen Typen. Diese von der Zellteilung unabhängige Histon-Synthese ist ein Teil des Mechanismus, mit welchem die Zellen versuchen, sich an die neuen ungünstigen Nahrungsbedingungen anzupassen.

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Establishment of Two Mosquito Cell Lines from Larval Tissues of *Aedes w-albus*

During the recent years several mosquito cell lines have been established from larval and adult tissues of *Aedes aegypti*[1–4], *A. albopictus*[2], *A. vexans*[5], *A. vittatus*[6], *Culiseta inornata*[5] and *Anopheles stephensi*[7]. This communication reports the establishment of two more cell lines from larval tissues of *Aedes w-albus*.

The culture medium employed for *A. w-albus* cell cultures was the same as used by SINGH[2] for *A. aegypti* and *A. albopictus* cell cultures. The techniques were also essentially the same as employed for the cultures of *A. albopictus* and *A. aegypti*, with minor modifications. Briefly, the mosquito eggs, weighing about 500 mg obtained from a laboratory-maintained colony, were surface sterilized with 70% ethanol for 2–3 min, followed by treatment with WHITE's[2] solution for 10 min. The surface sterilized eggs were further washed in sterile glass distilled water and allowed to hatch in Rinaldini's salt solution (RSS) under reduced atmospheric pressure. Within 4 h most of the eggs hatched and freshly hatched larvae were then minced in 0.1% trypsin solution in RSS and incubated at 37 °C for 10 min. The dispersed cells and tissue pieces were washed twice in RSS, suspended in 4 ml of the growth medium, and seeded into a 3 oz. prescription bottle. The culture was incubated stationary at 30 °C.

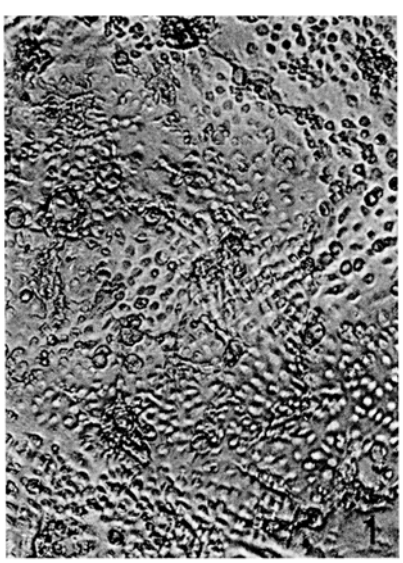

Fig. 1. *Aedes w-albus* cell line (ATC-136). Living culture, ×150.

On the 3rd or 4th day of seeding, a number of active foci of cell multiplication were observed; large patches of cells were observed by the 7th or the 8th day. A few small, bubble-like vesicles were also observed growing out from floating larval tissues, but no further growth of these vesicles was noted.

The first sub-culture of the attached cells was made on the 15th day after seeding, when almost a complete

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