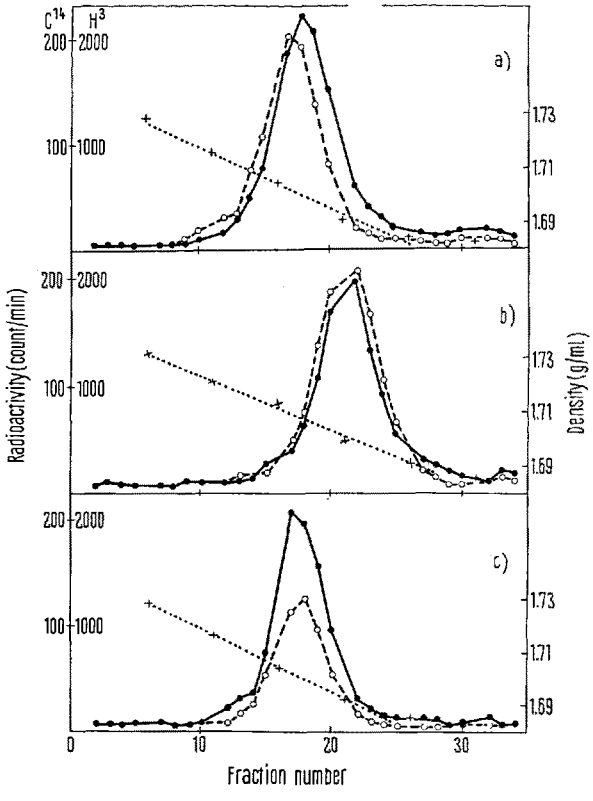
Early Replicating DNA of *Physarum* is Denser than Late Replicating DNA

The myxomycete *Physarum polycephalum* can be grown as plasmodia, in which most nuclei go through naturally synchronous mitoses[1]. The main nuclear DNA is replicated during a 3 h period immediately following mitosis; under the conditions used here the whole intermitotic time is about 9 h. It has been shown previously that DNA molecules that replicate in a subfraction of one S-period again replicate in the corresponding subfraction of the following S-period[2]. This shows that DNA molecules are replicated in a specific time sequence, at least under standard growth conditions. The following experiment shows that DNA replicating at different times of the S-period has a somewhat different density.

Surface cultures of *Physarum* were grown by routine methods and the times of mitosis determined by phase contrast microscopy. Three cultures were prelabeled from inoculation of surface cultures until 3 h after mitosis 2 with H^3-thymidine (0.5 μC/ml) and then transferred to an unlabeled medium. After mitosis 3, cultures were pulse labeled at different times of the S-period with C^{14}-thymidine (2 μC/ml). Immediately following the labeling, nuclei were isolated, DNA extracted and cesium chloride gradients prepared by methods described previously[2]. It can be seen from the Figure that H^3 and C^{14} do not band at the same position at all times. DNA replicated early in the S-period is denser than bulk DNA (Figure a) and DNA replicated late in the S-period is somewhat less dense than bulk DNA (Figure c). Although nuclear satellite DNA (density 1.714 g/ml) does not replicate together with the bulk nuclear DNA (density 1.702 g/ml), it cannot have any influence on the outcome of these experiments[3]. The nuclear satellite DNA represents only 2% of the total DNA and it replicates from the middle of the S-period to the end of the G-2-period[4].



Cesium chloride gradients of pulse labeled DNA. Cultures were prelabeled with H^3-thymidine, then allowed to continue growth in unlabeled medium and pulse-labeled for 10 min with C^{14}-thymidine at the following times during the S-period: a) from 10 min after mitosis 3 to 20 min after mitosis 3; b) from 50 min after mitosis 3 to 60 min after mitosis 3; c) from 90 min after mitosis 3 to 100 min after mitosis 3. ●—●, H^3-radioactivity; ○---○, C^{14}-radioactivity; × ... ×, density. Bottom of gradients at the left.

For mammalian cells in culture very similar results have been obtained: DNA replicated early in the S-phase has a higher G + C content than DNA replicated late in the S-phase[5–7]. It follows that in very different eukaryotic organisms DNA replication proceeds in a well defined time sequence at least under standard growth conditions. Whether this sequence is also maintained during differentiation remains to be established. It is not clear what functional significance this fixed time sequence of replication has, nor by what mechanism it is controlled: conceivably activating proteins may induce the replication of one segment after another[8].

Résumé. Le DNA du myxomycete *Physarum polycephalum* a été marqué pendant des temps de 10 min à différentes étapes de la phase S. L'analyse sur gradients de densité préparatifs de chlorure de césium a montré qu'une partie de ce DNA, reproduit tôt dans la phase S est plus dense que le reste du DNA, celui-ci étant lui-même plus dense que le DNA reproduit tardivement.

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1 H.P. RUSCH, in *Advances in Cell Biology* (Ed. D.M. PRESCOTT, L. GOLDSTEIN and E. MCCONKEY; Appleton-Century-Crofts, New York 1970), vol. 1, p. 297.
2 R. BRAUN and H. WILI, Biochim. biophys. Acta *174*, 246 (1969).
3 A. ZELLWEGER, U. RYSER and R. BRAUN, J. molec. Biol., in press.
4 R. BRAUN and T.E. EVANS, Biochim. biophys. Acta *182*, 511 (1969).
5 A.M. TOBIA, C.L. SCHILDKRAUT and J.J. MAIO, J. molec. Biol. *54*, 499 (1970).
6 W.G. FLAMM, J.N. BERNHEIM and P.E. BRUBACKER, Expl. Cell Res. *64*, 97 (1971).
7 C.J. BOSTOCK and D.M. PRESCOTT, Expl. Cell Res. *64*, 267 (1971).
8 J.E. CUMMINS, in *The Cell Cycle, Gene-Enzyme Interactions* (Ed. G.M. PADILLA, G.L. WHITSON and I.L. CAMERON, (Academic Press, New York 1969) p. 141.
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The Effect of Pressure Changes on Esterification of Cholesterol and Hydrolysis of Cholesterol Esters in Rat Aorta and Serum

Enzyme activity in the tissues may be influenced by various factors. It is known that there is adaptation of enzymes on alterations in the diet[1], after the administration of hormones[2], drugs[3], etc. It was found previously that also the activity of enzymes esterifying and hydrolyzing cholesterol esters in vascular wall and blood serum is affected by diet changes[4]. When studying other factors which could control the activity of these enzymes in the

Esterification of [4-^{14}C] cholesterol and hydrolysis of [7-^{3}H] cholesterol palmitate in rat aorate and blood serum under elevated pressure

Group (pressure)	No. of animals	Esterification/cholesterolesters						Hydrolysis
		saturated	1 =	2 =	3,4 =	X =	$\lesssim$	free cholesterol
Aortae								
Controls	11	0.69	0.82	0.32	0.18	0.10	2.13	14.27
(atmospheric)		± 0.075	± 0.14	± 0.068	± 0.042	± 0.002	± 0.31	± 1.30
(200 mm Hg)	10	0.58	0.60	0.22	0.11	0.07	1.42[b]	9.58[b]
		± 0.074	± 0.010	± 0.063	± 0.030	± 0.025	± 0.50	± 1.18
Blood serum								
Controls	10	0.55	0.74	1.41	4.00	0.71	7.84	2.56
(atmospheric)		± 0.12	± 0.21	± 0.75	± 1.24	± 0.30	± 1.54	± 0.97
(200 mm Hg)	10	0.71	0.82	2.44	4.45	0.94	9.37[a]	0.98[a]
		± 0.09	± 0.16	± 0.36	± 0.48	± 0.29	± 0.99	± 0.09

Values represent % of the total activity in the tube esterified (or hydrolyzed) per 2 h (mean ± S.D.).
1 =, Mono-unsaturated cholesterol esters; 2 =, Di-unsaturated cholesterol esters; 3,4 =, Tri- and tetra-unsaturated cholesterol esters; X =, Poly-unsaturated cholesterol esters; $\lesssim$ Total cholesterol esters. [a] $P < 0.05$. [b] $P < 0.01$, statistically significant difference vs. controls.

arterial wall, the effect of pressure changes has been examined in the present experiments. Enzymes in the rat arterial blood and/or wall are working under the pressure of about 100–150 mm Hg, while the measurements of enzymatic activity have been done previously under ordinary laboratory conditions, i.e. at atmospheric pressure.

Normal female Wistar rats on standard diet were used. The blood was obtained by decapitation. Then the aortae were removed, the tunica adventia was discarded and the weighed tissue (40 mg) was cut into small pieces with scissors and incubated. The incubation medium was phosphate-acetate buffer to which was added Mg^{2+}, ATP and coenzyme A[5] together with a tracer dose of [4-^{14}C] cholesterol or [7-^{3}H] cholesterol palmitate (Radiochemical Centre, Amersham) in 0.1 ml acetone to 2 ml incubation mixture. The control samples of aorta and/or serum were incubated at atmospheric pressure. The experimental samples were incubated in a hermetically closed chamber under strictly identical conditions, but during the whole incubation period (2 h) the pressure was elevated to 200 mm Hg. Immediately after the incubation the reaction was stopped by lipid extraction according to FOLCH et al.[6]. The radiochemical procedures were described previously[7].

From the Table it follows that under high pressure conditions in rat aortic tissue the esterification of the cholesterol is inhibited and there is also significantly less [7-^{3}H] cholesterol palmitate hydrolyzed when compared with the aortae incubated under lower (atmospheric) pressure.

It is also evident that in the case of blood serum there was also decreased hydrolysis of cholesterol palmitate, but significantly elevated esterification of cholesterol.

It is of some interest that, in the case of rat aortic tissue, the esterification in saturated and mono-unsaturated cholesterol esters predominates, while in serum the tri- and tetraunsaturated esters prevail. Whether the different pattern of cholesterol esters is involved in the different response of cholesterol esterification in the serum and aortic tissue to the pressure elevation awaits further studies.

Assuming that cholesterol-esterifying and cholesterol-esters-hydrolyzing enzymes are working in blood and aortic wall in vivo under similar pressure conditions (i.e. 100–200 mm Hg) as has been simulated in our experiments, then present results may serve to explain the marked difference between the faster hydrolysis than esterification rate in our previous work[4]. Furthermore the addition of coenzyme A in incubation medium in the present experiments apparently also facilitated esterification of cholesterol[8].

The reasons why a rise in pressure alters the studied processes are not clear at present. The changed accessibility of substrates to the enzymes under pressure conditions cannot be excluded. It is also possible that steric configuration of enzymes molecules (with respect to active sites) is modified under the influence of elevated pressure. These and other possibilities are currently under study in this laboratory.

It can thus be summed up that under the presence of 200 mm Hg the hydrolysis of cholesterol palmitate in rat aortic wall and blood serum is inhibited while the esterification of cholesterol in the blood serum is increased.

Zusammenfassung. Experimente in vitro zeigen, dass unter Überdruck von 200 mm Hg die Hydrolyse von Cholesterolpalmitat in den Aorten und im Blutserum von Ratten gehemmt wird: Die Esterifikation von Cholesterin, in Aorten ebenfalls erniedrigt, zeigt im Serum einen deutlichen Anstieg.

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[1] S. NUMA, M. MATSUHASHI and F. LYNEN, Biochem. Z. *334*, 203 (1961).
[2] R. T. SCHIMKE, E. W. SWEENEY and C. M. BERLIN, Biochem. Biophys. Res. Commun. *15*, 214 (1964).
[3] I. M. ARIAS, D. DOYLE and R. T. SCHIMKE, J. biol. Chem. *244*, 3303 (1969).
[4] V. FELT and P. BENEŠ, Enzym. biol. clin. *11*, 511 (1970).
[5] G. SHYAMALA, W. J. LOSSOW and I. L. CHAIKOFF, Proc. Soc. exp. Biol. Med. *118*, 138 (1965).
[6] J. FOLCH and M. LESS, J. biol. Chem. *22*, 497 (1957).
[7] V. FELT and P. BENEŠ, Biochim. biophys. Acta *176*, 435 (1969).
[8] S. MUKHERJEE, G. KUNITAKE and R. B. ALFIN-SLATER, J. biol. Chem. *230*, 91 (1958).
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