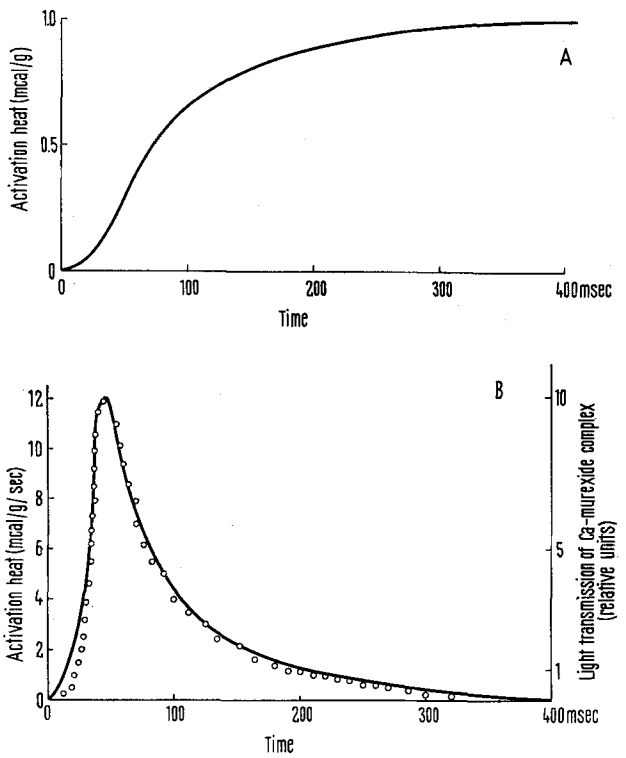
heat liberation during the initial 40–70 msec. However, over the 400 msec period 0.072 ± 0.008 µmole ATP/g muscle was hydrolyzed, a value which agrees with similar findings of DAVIES et al.[4].



Time course of activation heat (A) and the relationship between the first time derivative thereof and the release and reabsorption of calcium (B). Measurements of light transmission and heat production were obtained in parallel experiments. The solid line in Figure B is the time derivative of activation heat; the open circles represent the Ca^{2+} concentration at the various time intervals averaged from 10 successive twitches. The Ca-murexide concentration is given in relative units, all points are fractions of the light absorption value at 200 msec.

The difference in the time course of heat production and Ca^{2+} release over the initial 25 msec can readily be explained in terms of conformational changes accompanying the propagation of membrane depolarization[5]. As these changes are very similar to those observed upon nerve excitation[5], the value of 0.009 mcal/g obtained as heat per impulse in crab nerves[6] may be used for comparison. The heat value of about 0.015 mcal/g suggested from the difference in Figure B may reflect the greater membrane surface of muscle[7]. The finding that about 0.24 mcal heat/g liberated during the initial 50 msec are unaccompanied by any ATP splitting, suggests that all the ATP is split during the time in which Ca^{2+} is pumped back into the sarcoplasmic reticulum. SRETER[8] reports that for any molecule of ATP split, 8–10 Ca^{2+} ions are taken up by the sarcoplasmic vesicles. Therefore, on the basis of the observed ATP hydrolysis, the calcium level should be about $6 \times 10^{-4} M$ at its maximum in activated frog sartorius muscle at 0 °C.

As a working hypothesis it is suggested that the fraction of heat produced initially in parallel to the build-up of the free Ca^{2+} concentration reflects some conformational change as a result of Ca-binding to the myofilaments.

Zusammenfassung. Für den Froschmuskel wird ein enger Zusammenhang zwischen der zeitlichen Änderung der Aktivierungswärme und der Änderung der Kalziumkonzentration nachgewiesen.

R. A. CHAPLAIN and E. PFISTER

Department of Biocybernetics,
Technische Hochschule Otto von Guericke,
301 Magdeburg (DDR), 23 December 1969.

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[6] B. C. ABBOTT, A. V. HILL and J. V. HOWARTH, Proc. R. Soc. B *148*, 149 (1958).
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The Influence of Dimethyl Sulphoxide on Metabolic Activity of Ehrlich Ascites Carcinoma Cells and Microorganisms

When studying the mechanism of biological activity of isothiocyanates and their synthetic producers, we have so far constantly been up against the problem of appropriately dosing the above-mentioned substances in a wider range of concentrations related to cell suspensions in the culture medium. The possibility of choosing the solvent in which the required isothiocyanate concentrations can be obtained will naturally be limited by the fact that the solvent employed must not influence the metabolic processes of the organism under investigation. For the purposes mentioned we have for several years utilized methanol, ethanol, diethylene glycol, monoethylether of diethylene glycol and other solvents miscible with water. It was not until recent years that we started employing as a solvent dimethyl sulphoxide (DMSO), mainly for its high dissolving capacity in respect to a wide spectrum of inhibitors rather less soluble in the other organic solvents miscible with water. Its physico-chemical properties, biological effects, metabolism and utilization have already been described in a number of papers[1,2]. There has also been published a number of communications concerning the influence of DMSO on microorganisms[3–5]. Rather less, however, is known about the influence of low concentrations of DMSO on the metabolic processes of cells.

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Our experiments with Ehrlich ascites carcinoma cells in Krebs-Ringer phosphate medium with addition of ascitic serum and glucose[6] have shown that DMSO in its final concentration of 1% in the course of 2 h incubation did not influence either the production of lactate (Figure 1), the intensity of respiration (Figure 2) nor the incorporation of adenine-^{14}C and 1-valine-^{14}C, and further precursors, in nucleic acids and proteins (Table I). After 48 h action, DMSO in 1% concentration did not influence the growth of HeLa cells in tissue culture[7].

Likewise, DMSO in a concentration up to 2% did not influence the growth of the fungi *Aspergillus niger*, *Rhyzopus oryzae* and *Penicillium cyclopium* on Czapek-Dox medium. Only at about a concentration of 5% was a rather marked inhibition noted in *R. oryzae*, while the growth of the fungi *A. niger* and *P. cyclopium* had remained uninhibited. On the other hand, ethanol and diethylformamide had, under the same conditions, totally suppressed the growth of all 3 fungi.

Table I. Influence of DMSO on incorporation of adenine-^{14}C, uracil-^{14}C, thymidine-^{14}C and L-valine-^{14}C into TCA insoluble fraction of Ehrlich ascites carcinoma cells

^{14}C-precursors	Incubation time 1 h		2 h	
	Dimethyl sulphoxide concentration (%)			
	0	1	0	1
	(count/min)			
Adenine-^{14}C	600	610	1100	1070
Uracil-^{14}C	3920	3970	6650	6600
Thymidine-^{14}C	2300	2180	3720	3780
L-Valine-^{14}C	2390	2450	4560	4690

Concentration of glucose 3 mM/l. Otherwise as in Figure 1.

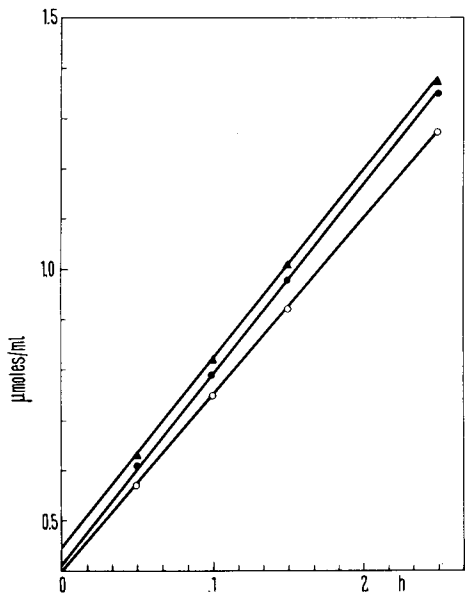

Fig. 1. Influence of DMSO on production of lactate (determined by Warburg optical test) of Ehrlich ascites carcinoma cells incubated in Krebs-Ringer phosphate buffer (pH 7.4) containing 2.5% of ascitic sera and 1% of glucose. Temperature 37 °C. Final concentration of DMSO in %: ○—○—○--○=0; ●—●—●—●=1; ▲—▲—▲—▲=2.

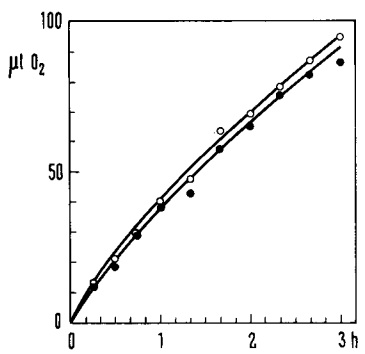

Fig. 2. Influence of DMSO on respiration (determined manometrically) of Ehrlich ascites carcinoma cells. Otherwise as Figure 1.

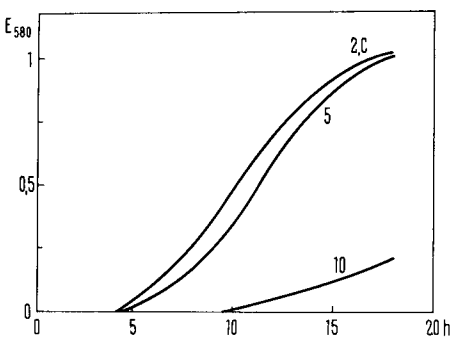

Fig. 3. Growth of *Candida albicans* on synthetic glucose-ammonium sulphate medium[10] at 28 °C under aeration. C, control; 2, 5 and 10, final concentration of % DMSO.

Table II. Incorporation of adenine-^{14}C and leucine-^{14}C into TCA insoluble fraction of *Candida albicans* cells in synthetic medium under aeration in absence and presence of 1% DMSO

^{14}C-precursors	Incubation time 1 h		2 h	
	Dimethyl sulphoxide concentration (%)			
	0	1	0	1
	(count/min)			
Adenine-^{14}C	1370	1300	3450	3420
Leucine-^{14}C	530	620	1080	1130

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DMSO has also proved its merits as a solvent of metabolic inhibitors in experiments with yeasts. On a synthetic glucose medium, both in static cultivation and in cultivation on a shaker, it did not influence to any appreciable extent the course of growth curves of the yeasts *Saccharomyces cerevisiae* and *Candida albicans* in concentrations up to 5%. Only at about a concentration of 10%, prolongation of the lag phase and decrease in the rate of growth was observed. However, not even 10% concentration of DMSO proved sufficient to stop the proliferation of cells of *C. albicans* (Figure 3). In 1% concentration it did not influence the intensity of respiration nor change the respiration quotient. In the same concentration it did not influence the incorporation of adenine-^{14}C and leucine-^{14}C either (Table II). DMSO has proved likewise useful in studying the influence of isothiocyanates and antibiotics on the protoplasts of *S. cerevisiae* where in 1% concentration it did not influence respiration (Figure 4), incorporation of adenine-^{14}C and leucine-^{14}C in its protoplasts in a hypertonic synthetic medium[8].

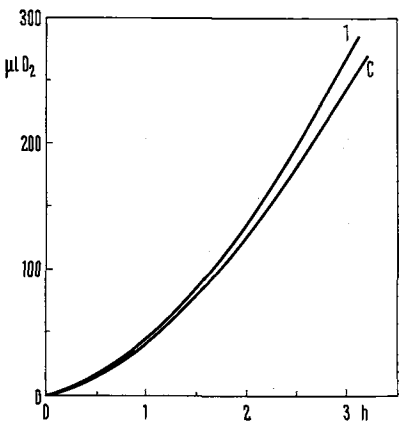

Fig. 4. Oxygen consumption by *S. cerevisiae* sferoplasts in synthetic medium[10] containing 0.6 *M* KCl[8]. C, control; 1, % DMSO. Measured manometrically at 28 °C.

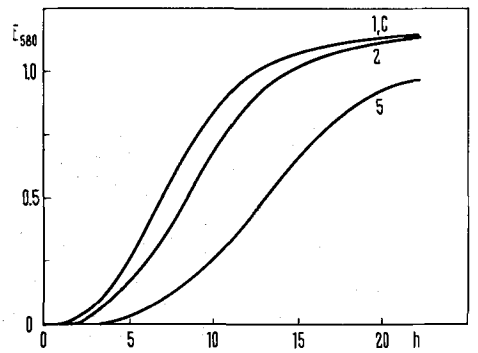

Fig. 5. Growth *E. coli* B on synthetic medium at 37 °C under aeration. C, control; 1, 2 and 5 represent final concentration of % DMSO.

DMSO has also proved effective as a solvent when studying the influence of inhibitors on metabolic processes in bacteria. As it appears from Figure 5, in 1% concentration it did not influence proliferation of the bacteria *E. coli* B. In a concentration of 5% its inhibitory influence had already become manifest. When investigating biologically effective substances against *M. tuberculosis* H_{37}Rv, solutions of these substances in DMSO could likewise be utilized to advantage[9].

As we were able to show, certain protozoa are particularly sensitive to the presence of organic solvents in the culture medium used. For instance, dimethylformamide and ethanol in 5% concentration will irreversibly stop mobility of the protozoa *Euglena gracilis*. DMSO in a peptone medium in 5% concentration will only cause a reversible loss of mobility of *E. gracilis* cells. After several hours their mobility will become entirely or partially restored. In a concentration under 2% DMSO has no inhibitory influence on the mobility of *E. gracilis* cells.

Zusammenfassung. Es wird gezeigt, dass 1–2%iges Dimethylsulfoxyd sich als unschädliches Lösungsmittel für antibakterielle Substanzen besonders gut eignet.

L. DROBNICA, J. AUGUSTÍN and M. MIKO

Department of Microbiology and Biochemistry, Faculty of Chemistry, Slovak Polytechnical University, Bratislava (Czechoslovakia), 6 November 1969.

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The Relative Antiquity of Fenestrated Blood Capillaries and Lymphatics, and their Significance for the Uptake of Large Molecules: an Electron Microscopical Investigation in an Elasmobranch

Lymphatics remove large molecules from the tissues. Fenestrated blood capillaries may also remove large molecules[1–4]. The probable mechanisms involved have been discussed elsewhere[2,3].

One of the very few studies of the relative effectiveness of the blood vessels and the lymphatics in the removal of large molecules concluded that the blood capillaries are the major mechanism for the removal of extravascular protein from skin[5]. Recently, SZABO[6] has found that in the cortex and in the medulla of the kidney, where fenestrae are very numerous[1], the blood removes about 100 times more protein than the lymph does[6]. In the skin, the blood and lymph systems are about equally effective. Here there are few fenestrae[1,7] and hence many of the molecules removed by the blood may enter the capillaries via the small endothelial vesicles[1,8–10].

In privative fish, the absence of lymphatics has made it difficult to understand how the tissues are cleared of large molecules[11]. In spite of earlier confusion, it is now considered that true lymphatics are absent in most species of elasmobranchs[12,13], including the Port Jackson shark, *Heterodontus portusjacksoni* (Meyer), which we studied. (We also injected colloidal carbon into the intestinal wall, and found no lymphatics.) Pieces of intestine, kidney,