It has been suggested that glutathione peroxidase rather than catalase is the principal defense against peroxide toxicity in some cells[11, 12, 15]. The above studies have demonstrated significant levels of catalase[12]. The enhanced peroxide detoxification capability of the MOPC 46 cells in the presence of glucose could be related to the generation of reduced cofactors for glutathione peroxidase during glucose metabolism. Thus these results are consistent with the view that catalase may not provide the major mechanism for peroxide detoxification by the MOPC 46 tumor cell and other neoplastic cells[16], and that glutathione peroxidase offers a likely alternative mechanism[17].

Résumé. Des péroxides détoxifiants peuvent empêcher la réduction de la production des immunoglobulines par les cellules de la tumeur MOPC 46 en l'absence de glucose. Le contenu en glutathion de péroxydase de ces cellules est élevé tandis que s'abaisse le contenu en catalase.

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12 October 1973.

The Distribution of Inorganic Phosphate in Blood Conserved at 4°C in Acid-Citrate-Dextrose with Adenosine and Persantin®

During the conservation of blood in acid-citrate-dextrose (ACD) solution at 4 °C, there occurs a relatively fast decomposition of phosphate compounds, especially 2, 3-diphosphoglycerate (2, 3-DPG). This fact is reflected in the increasing concentration of inorganic phosphate (P_i) inside the erythrocytes and its gradual penetration outside the red cell[1–3]. As we have stated before, the addition of adenosine in combination with persantin to the conserving medium causes the slower rate of decomposition of 2, 3-DPG and slower increase of P_i inside the red cell[2]. Similarly, Gibson et al.[4], who incubated blood at 37 °C, stated the slower increase of P_i in the presence of adenosine and persantin. Gerlach et al.[5] have found that persantin considerably reduced the rate of phosphate influx and efflux in the erythrocytes incubated at 37 °C, the efflux being more strongly influenced than the influx. Persantin retards also the adenosine transport across the red cell membrane and inhibits adenosine deaminase in whole blood[6]. The rate of the P_i increase in blood con-

[1] G. R. Bartlett and H. N. Barnet, J. clin. Invest. *39*, 56 (1960).
[2] B. Zachara, Acta haemat. *48*, 164 (1972).
[3] H.-G. Böer, P. Stehle, H. Roigas and S. Rapaport, Folia haemat. *90*, 265 (1968).
[4] J. G. Gibson II and F. J. Lionetti, Transfusion *6*, 427 (1966).
[5] E. Gerlach, B. Deuticke and J. Duhm, Pflügers Arch. ges. Physiol. *280*, 243 (1964).
[6] R. D. Bunag, C. R. Douglas, S. Imai and R. M. Berne, Circulation Res. *15*, 83 (1964).

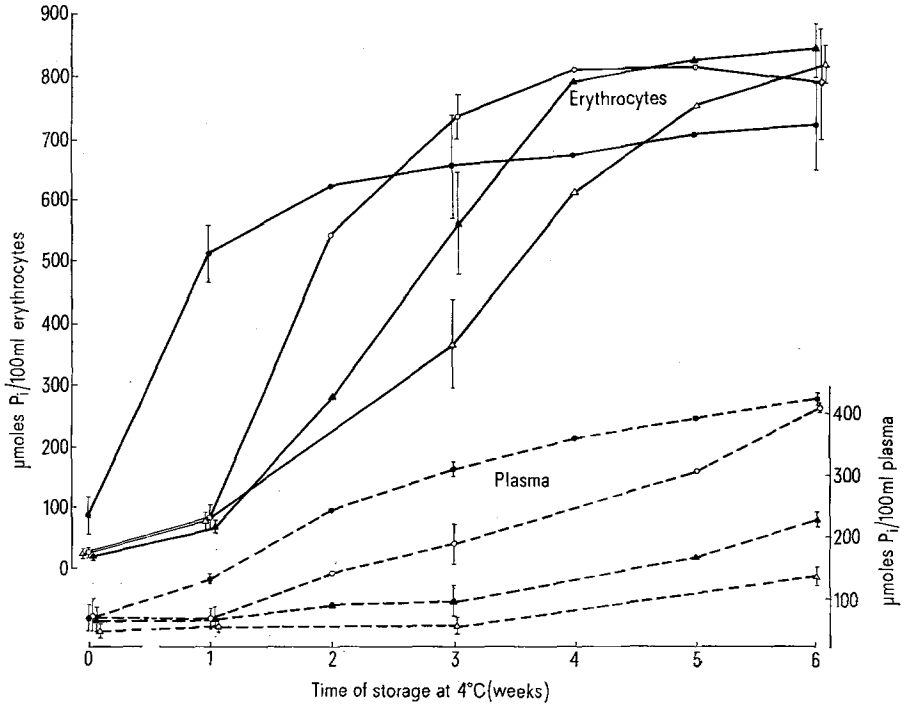

Fig. 1. The content of P_i in erythrocytes (—) and in plasma (- - -) in blood conserved at 4 °C in ACD (●), ACD-A (○), and in ACD-A-P ($\Delta = 1 \times 10^{-4}\,M$; $\square = 4 \times 10^{-4}\,M$). The content of P_i is expressed in µmoles per 100 ml of packed red cells (mean ± S.D. of 3 experiments) and per 100 ml of plasma (mean ± S.D. of 6 experiments).

served at 4 °C reflects the metabolism of the phosphate compounds of erythrocytes. Although there have been several reports on the influence of persantin on the phosphate transport across the red cell membrane, these investigations were carried out at 37 °C. The rate of phosphate transfer through the red cell membrane is very slow[7], and strongly depends on the incubation temperature[3,5].

The present investigation is on the changes of P_i content inside and outside the erythrocytes during blood storage at standard temperature of 4 °C in ACD, ACD with adenosine and both adenosine and persantin.

Material and methods. The blood was conserved in ACD solution (formula B), ACD with adenosine (2 mM; ACD-A solution) and in ACD-A with persantin (1×10^{-4} M and 4×10^{-4} M; ACD-A-P solutions[8] in glass bottles. Blood taken from the same donor was always examined in parallel in all conserving media. The first experiment was carried out about 2–4 h after the blood had been taken, the next ones mainly at 1, 3 and 6 weeks of conservation at 4 °C. After the red cells had been separated from plasma and washed with 0.9% NaCl solution, the phosphate compounds were extracted[2]. The extract was separated in ion exchange chromatography on Dowex 1 and the 2,3-DPG as well as P_i were determined in eluates[2,9,10]. Plasma was precipitated with trichloroacetic acid and the content of P_i was determined in the centrifuged supernatant[10]. The levels of 2,3-DPG and P_i were expressed in μmoles per 100 ml of packed red cells and per 100 ml of plasma.

Results and discussion. In the Figure 1 is presented the content of P_i in red cells and plasma conserved at 4 °C for 6 weeks in the media mentioned above. The initial content of P_i in erythrocytes conserved in ACD is 3- to 4-fold higher when compared with the 3 other media. It is connected with the rapid decomposition of 2,3-DPG of red cells taking place from the beginning of blood conservation in ACD solution[1,11]. Decomposition of 2,3-DPG follows in the further period of conservation, and P_i grows violently inside erythrocytes[1,2,11]. In the other media, especially in the presence of adenosine with persantin, the increase of P_i content is markedly slower. At the end of conservation, however, P_i content in red cells conserved in ACD-A and ACD-A-P solutions is higher than in ACD. The presence of persantin with adenosine in the conserving medium exerts a protective influence upon the phosphate compounds. In Figure 2, the contents of 2,3-DPG in the media examined is shown. While in the medium containing ACD, 2.3-DPG contents has been decreasing since the moment of blood taking, in the remaining media there was observed a slight increase of this compound in the first week of blood preservation. It is noticeable that in the 6th week the contents of 2,3-DPG in the medium containing adenosine and persantin is higher than in the 3rd week in ACD blood. The mechanism of persantin effect upon the metabolism of 2,3-DPG is not completely understood yet. Persantin is known to inhibit adenosine deaminase and uptake of nucleosides by erythrocytes[6]. Due to these facts, as it seems, adenosine undergoes a slower decomposition in plasma and gradually penetrates into the red cell, providing the substrate for 2,3-DPG synthesis. Further investigations concerning these problems are being carried out.

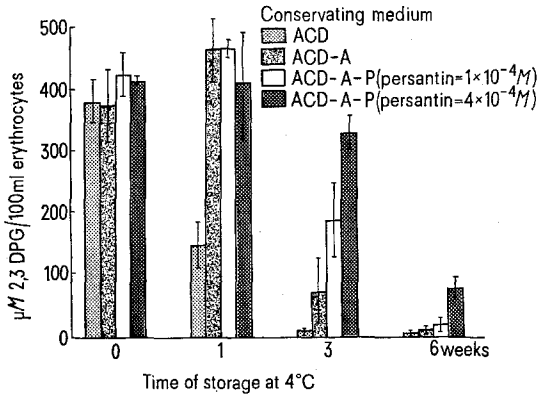

Fig. 2. The content of 2,3-DPG in erythrocytes of blood conserved at 4 °C in ACD, ACD with 2 mM adenosine and in ACD with adenosine and persantin (1×10^{-4} M and 4×10^{-4} M). The values are expressed in μmoles per 100 ml of packed red cells (mean ± S.D. of 3 experiments).

P_i liberated as a result of degradation of phosphate compounds, penetrates to the surrounding medium after having reached the saturation point[1]. BARLETT and BARNET[1] have found that the saturation point in erythrocytes conserved in ACD is reached only on about 15th day. As may be seen in the Figure 1, this observation is also confirmed by our work. The saturation point in ACD-A solution reaches its peak between 2nd–3rd week of conservation, while in ACD-A-P media between 4th–6th week, depending upon the concentration of persantin.

The content of P_i in plasma of blood conserved in ACD shows almost a linear increase from the initial period of conservation. In the presence of adenosine, no increase of P_i in plasma has been found in the first week. In the medium containing adenosine with persantin, the increase of P_i is hardly noticed during the first 3 weeks. Only in the further period of conservation, an increase of P_i level was found, but slower than in ACD and in ACD-A media. The lowest level was found in the medium containing the higher concentration of persantin.

The rate of P_i efflux depends upon the permeability of the red cell membrane. GERLACH et al.[5], who examined the phosphate transport across the membrane of erythrocytes, incubated at 37 °C, stated that adenosine and especially persantin diminish the phosphate transport across the red cell membrane, if added separately to the incubating medium. It can be suggested that, similarly to the incubation at 37 °C, the presence of both these compounds in the conserving medium at 4 °C shows a synergistic activity.

Other evidence of the lower permeability of the red cell membrane for P_i in our experiments is the ratio of P_i concentration inside the red cell to the concentration in plasma. The values are given in the Table. In ACD this ratio was about 3.9 in the 1st week, gradually diminishing afterwards. In ACD-A it reaches this value in the 2nd and 3rd week of conservation, while in the presence of adenosine and persantin in the 3rd week it was 5.9 and 6.4 for the lower and higher concentration of persantin, respectively. In the 6th week the differences are distinctly higher for the media containing persantin.

[7] B. VESTERGAARD-BOGIND and T. HESSELBO, Biochim. biophys. Acta *44*, 117 (1960).

[8] Persantin® (2,6-*bis* [diaethanoloamino]-4,8-dipiperidinopyrimido-5,4-pyrimidine; dipyridamole) was kindly supplied by C. H. Boehringer Sohn, Ingelheim, Germany.

[9] G. C. MILLS, D. O. BURGER, M. SCHNEIDER and W. C. LEVIN, J. Lab. clin. Med. *58*, 725 (1961).

[10] G. R. BARTLETT, J. biol. Chem. *234*, 466 (1959).

[11] H. YOSHIKAWA and N. NAKAO, Ser. Haemat. *10*, 48 (1966).

The ratio of P_i content in erythrocytes to the content in plasma

Conservating medium	Weeks of storage at 4 °C			
	0	1	3	6
ACD	1.2	3.9	2.1	1.7
ACD-A	0.3	1.2	3.8	1.9
ACD-A-P persantin (1×10^{-4} *M*)	0.3	1.0	5.9	3.7
ACD-A-P persantin (4×10^{-4} *M*)	0.5	1.4	6.4	6.1

In conclusion, it may be stated that during the conservation of blood at 4 °C in the medium ACD-adenosine-persantin: 1. The concentration of 2,3-DPG sinks more slowly and the content of P_i in red cells increases more slowly than in ACD and ACD-adenosine. 2. The red cell membrane in the presence of these 2 compounds reduces the P_i transport to the plasma.

Zusammenfassung. Persantin verzögert den Konzentrationsanstieg des anorganischen Phosphats in Erythrozyten und Plasma bei der Lagerung von Blutkonserven gegenüber der alleinigen Zugabe von ACD oder ACD-Adenosin.

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Department of Physiological Chemistry, AM, Pl. 9 may No. 1, P–90 647 Łódź (Poland), 20 February 1973.

[12] The author is very much indebted to Dr. Barbara Lewandowska for revision of the English text.

Immunohistochemical Demonstration of Corticotrophic Cells Concentrated in the Rostral Zone of the Pars Intermedia of the Mouse Hypophysis

Cells whose fine morphology makes them comparable to the corticotrophic cells of the pars distalis (PD), have been described in the pars intermedia (PI) of the rat and mouse hypophysis[1–4]. These cells are particularly abundant in the rostral zone of the PI. This is especially clear in the mouse, where the rostral zone of the intermediate lobe comprises essentially ACTH type cells which are obviously stimulated after adrenalectomy[1]. The mouse is therefore particularly suitable for an immunohistochemical study intended to reveal the corticotrophic nature of these cells in the PI and to distinguish them clearly from the MSH-producing cells.

Technique. 10 mouse hypophyses were fixed either with Elfmann's fluid or with Stieve's solution. The 'indirect' reaction was performed on 5 µm sections with anti-ACTH or anti-MSH antisera obtained from the rabbit using a synthetic ACTH (β 1–24 corticotropine: Synacthen, Ciba) or synthetic α or β MSH (Ciba), and with sheep antirabbit γ globulin coupled with fluoresceine isothiocyanate.

The specificity of the antibodies used was evaluated by immunofluorescence inhibition tests[5]. As for the antigenic affinity of the anti β 1–24 corticotropine antibodies, it should be noted that doses of synthetic α or β MSH 40 times higher than the minimum inhibiting dose of β 1–24 corticotropine do not inhibit the reaction.

The specificity of the reaction, on the other hand, is verified by successive application to the sections of: 1. rabbit anti ACTH or anti MSH antibodies, 2. unlabelled sheep anti-rabbit γ globulins and 3. fluoresceine isothiocyanate labelled sheep anti-rabbit γ globulins. No reaction is detected under these conditions.

Results. As we have already shown[1], the rostral zone of the mouse PI reveals a characteristic appearance by light and electron microscopy (Figure 1). The MSH-producing cells are immediately adjacent to cells which are small and clear, with extremely intricate prolongations and contain dense marginal secretory granules, between 160 and 230 nm in diameter. These cells are in direct relation – without intervening basement membrane – with the nervous tissue of the pituitary stalk. Some of these cells are detached from the epithelium and located either singly or in small clusters in the stalk and in the neural lobe. Cells of the same type, which sometimes contain finer granules, are encountered in the remainder of the PI, at the periphery of the lobe, i.e. underneath the lining epithelium of the hypophysial cleft and along the neural lobe.

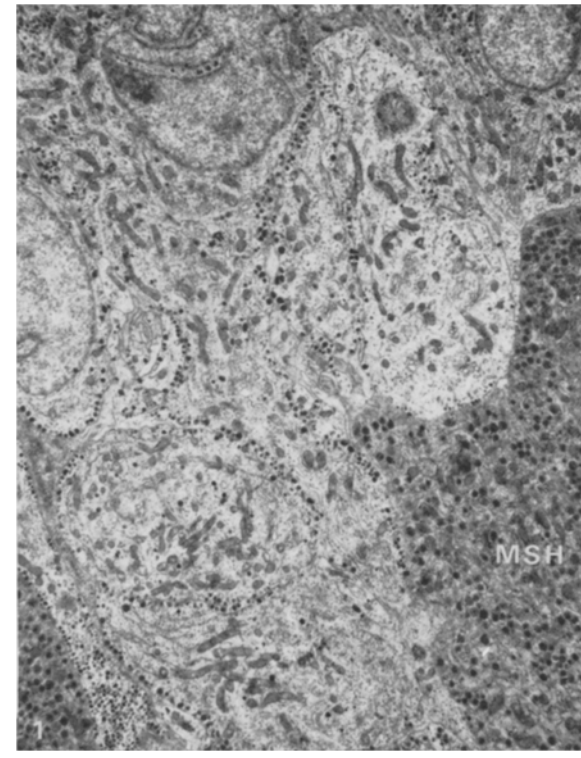

Fig. 1. Appearance, under the electron microscope, of a portion of the rostral zone of the pars intermedia showing the replacement of MSH cells by small corticotrophic-type cells containing dense marginal granules. ×4,500.

[1] M. E. Stoeckel, H.-D. Dellmann, A. Porte and C. Gertner, Z. Zellforsch. *122*, 310 (1971).
[2] G. C. Moriarty and N. S. Halmi, Z. Zellforsch. *132*, 1 (1972).
[3] D. V. Naik, Z. Zellforsch. *133*, 415 (1972).
[4] M. E. Stoeckel, H.-D. Dellmann, A. Porte, M. J. Klein and F. Stutinsky, Z. Zellforsch. *136*, 97 (1973).
[5] M. P. Dubois, Z. Zellforsch. *125*, 200 (1972).