Die Rolle, die die Purin-PRT in den Erythrozyten spielt, erscheint nicht eindeutig geklärt, ebenso die Frage nach dem Ursprung und der Form des Transportes der Purinbasen im Organismus, im Speziellen in die Erythrozyten. Durch das Fehlen der Purin-de-novo-Synthese fällt eine Funktion des preformed pathway Enzymsystems weg, nämlich die Regulation der de-novo-Synthese durch Rückkopplungsmechanismen. Ein Teil der Purine wird mit der Nahrung zugeführt oder stammt aus katabolischen Vorgängen. Da Nukleotide durch Zellmembranen meistens nicht permeieren können, bleibt nur die Möglichkeit des Transportes in der Form der Nukleoside oder Basen offen. Wie MAGER[11] an Rattenerythrozyten feststellte, gelangen eher die Purinbasen in die Erythrozyten als die Nukleoside. Ob diese Tatsache auch für andere Organismen, wie Hühnererythrozyten zutrifft, ist nicht bekannt.

In den letzten Jahren gewinnt die Methode der Zellfusionierung immer mehr an Bedeutung. Mit Hilfe einiger inaktivierter Viren (z.B. Sendai- oder SV-40-Viren) können Zellen verschiedenen Ursprungs miteinander fusioniert werden[12–14]. Dabei wurde gezeigt, dass Hühnererythrozyten, die mit Maus-, HeLa-Zellen oder Fibroblastenzellen fusioniert werden, wieder zur DNS- und RNS-Synthese befähigt werden. Ob dabei gleichzeitig eine Stimulation der Purin-PRTn- und eine Purin-de-novo-Synthese eintritt, soll in weiteren Versuchen untersucht werden.

Summary. Hen erythrocytes were investigated with respect to their Purinphosphoribosyl-pyrophosphate-transferase activities. These enzyme systems connected within the salvage pathway of purines have a low activity in contrast to human erythrocytes or leucocytes. This is possibly due to xanthineoxydase, which is present in hen erythrocytes.

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[11] J. MAGER, A. HERSHKO, R. ZEITLEN-BECK, T. SHOSHANI, A. RAZIN, Biochim. biophys. Acta *149*, 50 (1967).
[12] Z. DARŻYNKIEWICZ, Exptl Cell Res. *69*, 477 (1971).
[13] H. HARRIS und J. F. WATKINS, Nature *205*, 640 (1965).
[14] A. G. SCHWARTZ, P. R. COOK and H. HARRIS, Nature New Biol. *230*, 5 (1971).

Contamination of Commercially Available Intermediates of the Glycolytic Pathway

The commercial availability of substrates has placed the performance of studies of enzyme reactions in the grasp of many laboratories all over the world. Although commercial substrates are often available in a high state of purity, contamination with compounds of similar structure is often found. The extent of these impurities usually is very small, but can be of great practical importance under some circumstances. Therefore we have assayed glycolytic substrates supplied commercially by two different firms, applying enzymatic methods which permitted detection of contaminants even in quantities as small as 0.005%.

Substrates of Sigma Chemical Company, St. Louis, Mo., and Boehringer Mannheim Corp., New York, were investigated. Based on water content, purity, and corrected molecular weight, as far as these data were given by the companies, a 200 m*M* solution was prepared and carefully neutralized with HCl or KOH, if necessary.

Table II. 3-PGA contamination in different 2,3-DPG preparations provided by Boehringer Mannheim Corporation (B) and Sigma Chemical Company (S)

Lot	Actual 2,3-DPG concentration (molar %)	3-PGA contamination (molar %)
B 6419115	89.2	0.029
B 7200215	89.2	0.031
B 7332316	93.2	4.550
B 7470416	87.0	3.690
B 7412117	96.4	0.072
S 81C-3290	91.8	3.610
S 52C-5081-9	125.4	0.090

The lot numbers, the actual 2,3-DPG concentration in molar percent of the values by weight and the 3-PGA contamination in molar percent of the actual 2,3-DPG concentration are given.

For testing for contaminants, 0.400 ml of the 200 m*M* solution was added to the test system with a final volume of 1.0 ml. To measure the actual concentration of each reagent (for instance G6P[1] in the G6P preparation) 0.400 ml of a 0.1 m*M* solution was added to a system which also had a final volume of 1.0 ml.

The investigations were usually performed within one week after receiving the sample. During this time the substrates were kept dessicated at –20°C. In each assay a clear end-point was obtained. When a contaminant was detected, varying amounts of substrate were added to assure that linearity between amount added and quantity of contaminant was present.

G6P and F6P were measured in a system that contained 400 m*M* *Tris*-HCl buffer, pH 8.0, and 2 m*M* NADP. G6PD and GPI were added and the reduction of NADP was measured at 340 nm. Glucose was estimated in a similar system that contained additional ATP (10 m*M*) and $MgCl_2$ (100 m*M*). The reaction was started with HK.

For the assay of FDP, DHAP and GAP, the GAPDH system, containing 30 m*M* arsenate[2], *Tris*-HCl buffer (175 m*M*, pH 8.0), NAD (2 m*M*), β-mercaptoethanol

[1] The following abbreviations are used: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; DHAP, dihydroxy-acetone phosphate; 2,3-DPG, 2,3-diphosphoglyceric acid; FDP, fructose-1,6-diphosphate; F6P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GPI, glucose-6-phosphate isomerase; HK, hexokinase; LDH, lactic dehydrogenase; MPGM, monophosphoglycerate mutase; NAD, nicotinamide-adenine dinucleotide; NADH, nicotinamide-adenine dinucleotide, reduced form; NADP, nicotinamide-adenine dinucleotide phosphate; PCA, pentacyclohexylammonium; PEP, phosphoenolpyruvate; 2-PGA, 2-phosphoglyceric acid; 3-PGA, 3-phosphoglyceric acid; PGK, 3-phosphoglyceric phosphokinase; PK, pyruvate kinase; TCA, tricyclohexylammonium; TPI, triosephosphate isomerase; *Tris*, *tris* (hydroxymethyl) aminomethane; not measured.

[2] O. WARBURG and W. CHRISTIAN, Biochem. Z. *303*, 40 (1939).

Table I. Contamination in commercial intermediates of the glycolytic pathway

		Salt	Lot	Glucose	G6P	F6P	FDP	DHAP	GAP	1,3DPG	2,3DPG	3-PGA	2-PGA	PEP	Pyruvate	Contamination given by the manufacturer
G6P	B	diNA	07311445	n.m.	**85.0**	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.011	< 0.005	< 0.005	0.012	None given
	S	NA	121C-2541-9	n.m.	**97.4**	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	None given
F6P	B	diNa	7490319	n.m.	2.331	**73.3**	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.228	< 0.005	< 0.005	< 0.005	G6P < 2%
	S	diNa	91C-7071	n.m.	0.307	**82.4**	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.025	< 0.005	< 0.005	< 0.005	G6P < 1%
FDP	B	triNa	7301144	n.m.	0.797	0.165	**75.4**	< 0.005	< 0.005	< 0.005	0.017	0.464	0.074	0.021	0.017	None given
	S	tetraNa	32C-7300	n.m.	< 0.005	0.027	**77.4**	< 0.005	< 0.005	< 0.005	0.020	0.048	< 0.005	< 0.005	< 0.005	Substantially free of hexosemonophosphates
2,3-DPG	B	pca	7470416	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	**87.0**	3.690	0.318	0.157	< 0.005	None given
	S	pca	81C-3290	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	**91.8**	3.610	0.315	0.157	< 0.005	None given
3-PGA	B	triNa	7211212	< 0.005	< 0.005	< 0.005	0.011	< 0.005	< 0.005	< 0.005	0.144	**71.0**	0.135	< 0.005	< 0.005	None given
	S	Na	51C-5040	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	**84.2**	0.173	< 0.005	< 0.005	Essentially free of 2,3-DPG
3-PGA	B	tca	7230410	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.318	**90.4**	0.101	< 0.005	< 0.005	None given
	S	tca	41C-5090	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.340	**87.0**	0.242	< 0.005	< 0.005	Approximately 2% 2,3-DPG
2-PGA	B	triNa	7151112	n.m.	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	n.m.	0.034	**98.0**	< 0.005	< 0.005	None given
	S	Na	22C-0010	n.m.	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 00.05	n.m.	1.150	**100.4**	< 0.005	< 0.005	None given
PEP	B	tca	7192138	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	n.m.	< 0.005	n.m.	**79.6**	< 0.005	None given
	S	tca	41C-5070	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	n.m.	< 0.005	n.m.	**90.0**	< 0.005	None given
Pyruvate	B	Na	7042431	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	n.m.	< 0.005	n.m.	n.m.	**100.0**	None given
	S	Na	31C-1640	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	0.005	< 0.005	n.m.	n.m.	**100.0**	None given

The manufacturers (B = Boehringer Mannheim Corporation, S = Sigma Chemical Company), the salts and lot numbers, the actual concentrations of the investigated intermediates in molar percent of the values by weight (in bold face), and the contaminants in molar percent of the concentration of the substance assayed are listed. In the right column the contaminants given by the manufacturers in the 1971 catalogs are listed.

(7 mM), was employed. GADPH, TPI and aldolase were added subsequently.

The assay medium for 3-PGA contained *Tris*-HCl buffer (100 mM, pH 8.0), NADH (0.4 mM), $MgCl_2$ (10 mM), neutralized hydrazine sulfate (20 mM), ATP (1 mM), and β-mercaptoethanol (7 mM). After adding GAPDH the reaction was started with PGK.

For the determination of contaminating 2,3-DPG the method described by BEUTLER[3] was used. The 2,3-DPG concentration in the 2,3-DPG solution was estimated in a modified GAPDH system, based on the method of KEITT[4]. The assay medium contained K-phosphate buffer (pH 7.0, 5 mM), *Tris*-HCl buffer (pH 7.4, 10 mM), reduced glutathione (2 mM), neutralized hydrazine sulfate (10 mM), NADH (0.1 mM), ATP (2.0 mM), GADPH (3.5 U), and PGK (1.5 U). The reaction was started with 30 U MPGM λ and 0.5 mM phosphoglycollate.

The assay medium for PEP, 2-PGA and pyruvate contained 50 mM K-phosphate buffer, pH 7.0, 10 mM $MgCl_2$, 2 mM ADP and 0.4 mM NADH. LDH, PK and enolase were added sequentially.

The results of the assays are shown in Table I. The manufacturers (B = Boehringer, S = Sigma), the salts and lot numbers which were investigated, and the contaminating compounds are listed. The actual concentrations of the investigated intermediates in percent of the values by weight are listed in bold face of the Table I. The contaminating substances are expressed as molar percent of the concentration of the substance assayed. The contaminants given by the manufacturers in the 1971 catalogs are also listed.

The actual concentrations found ranged from 71 to 100%. No contaminants could be detected in the pyruvate and PEP preparations of both manufacturers. The highest impurity which could be found was 3.6% 3-PGA content in the 2,3-DPG preparations. Moreover, 2,3-DPG contained the greatest numbers of impurities. The 3-PGA contamination of different 2,3-DPG preparations are listed in Table II.

Since 3-PGA contamination in 2,3-DPG was not only the greatest degree of impurity we found in our investigations, but moreover, as will be pointed out, seems to be of some practical importance, we studied the influence of storage on the 3-PGA content in 2,3-DPG. During a storage period of 4 months the values fluctuated between 3.69% and 4.6% (Boehringer, lot 7470416). No differences in the 3-PGA content was found in frozen stored solutions and fresh prepared solutions of frozen, dessicated 2,3-DPG preparations. The 2,3-DPG concentrations also did not change significantly during storage. Apparently storage, at least for some months, does not increase the 3-PGA contamination of 2,3-DPG preparations.

On the other hand, as it is evident from the results in Table II the 3-PGA content in 2,3-DPG preparations seems to fluctuate remarkably in different lots. Therefore, if a 2,3-DPG preparation which is essentially free of 3-PGA contamination is needed, different lots should be tested for 3-PGA content. Another possibility is the purification of contaminated 2,3-DPG preparations[5].

Furthermore considerable contamination was present in the F6P, FDP, 3-PGA, and 2-PGA preparations.

Although the commercially available substrates of the glycolytic pathway are available in high quality, it is often unavoidable that the compounds contain impurities of similar structure or degradation products. This fact is due to the difficulties in isolating them from biological materials[6]. For certain purposes it is therefore necessary not only to measure the actual concentration of a commercial substrate, but also to test for contaminants. The enzymatic methods, applied in our investigations allowed the detection of even as small a quantity of contaminants as 0.005%. Using a full scale deflection of 0.4 OD units this concentration gave a change of 6% full scale on the recorder.

Although levels of contaminants found in our investigations were relatively low under some circumstances, recognition of the presence of impurities can be very important. The inhibition of PFK in human erythrocytes by physiological concentrations of 2,3-DPG, recently reported from this laboratory[7] was explained by STAAL and KOSTER[8] by a sequence of side reactions beginning with 2,3-DPG phosphatase and including the reserve 3-PGK and GAPDH reaction. However, this reaction was only observed when commercial 2,3-DPG preparations contaminated with 3-PGA were used[5]. Therefore it seems that Staal and Koster have been misled in their interpretations, at least partially, by using contaminated substrate. A few years ago erroneous reports of the presence of a 'galactose dehydrogenase' activity in mammalian tissues[9,10] and of 'low K_m' hexokinase[11] arose because of contamination of commercial reagents with ethanol[12,13].

In these investigations we have demonstrated that although the most glycolytic substrates are already commercially available in high quality, for some purposes it is important to test the preparations for impurities. The most suitable procedures are enzymatic methods which permit detection of concentrations of contaminants as low as 0.005%[14].

Zusammenfassung. Die Prüfung von kommerziell erhältlichen glykolytischen Substraten auf Verunreinigung mit anderen Verbindungen der Glykolyse ergibt eine bis zu 4.55% betragende Kontamination von 2,3-DPG mit 3-PGA. In den untersuchten 2,3-DPG Chargen findet sich ausserdem eine Kontamination mit 2-PGA und PEP. Weitere bemerkenswerte Verunreinigungen können in F6P, FDP, 3-PGA und 2-PGA nachgewiesen werden.

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[3] E. BEUTLER, *Red Cell Metabolism* (Grune & Stratton, New York 1971), p. 96.
[4] A. S. KEITT, J. Lab. clin. Med. *77*, 470 (1971).
[5] E. BEUTLER and E. GUINTO, in preparation.
[6] H. U. BERGMEYER, *Methods of Enzymatic Analysis* (Academic Press, New York and London, 1965), p. 1002.
[7] E. BEUTLER, Nature, New Biol. *232*, 20 (1971).
[8] G. E. T. STAAL and T. F. KOSTER, FEBS Lett. *23*, 29 (1972).
[9] P. CUATRECASAS and S. SEGAL, Science *154*, 533 (1966).
[10] P. CUATRECASAS and S. SEGAL, Science *153*, 549 (1966).
[11] J. BROWN, D. M. MILLER, M. T. HOLLOWAY and G. D. LEVE, Science *155*, 205 (1967).
[12] E. BEUTLER, Science *156*, 1516 (1967).
[13] J. C. KAPLAN and E. BEUTLER, Science *159*, 215 (1968).
[14] This work was supported in part by Grant No. HL 07449 from the National Institutes of Health, and the Max Kade Foundation.
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