fish[16–18] and the presence of this biologically active amine, but not 5-hydroxytryptamine, has been demonstrated in the dermis of plaice[15]. Although SRS-A has been demonstrated in primate skin[19], its cellular origin remains obscure and there appear to be no reports of finding SRS-A in any tissues from poikilotherms.

Although the mechanisms involved in immediate hypersensitivity reactions have been the subject of intensive study[20–22], much has still to be learned about the chemical mediators involved and this is especially true of mediators found in mammals other than primates. The mediators, target cells and sensitivities of different tissues vary with species[11,23] and with poikilotherms the available knowledge on all of these aspects is extremely limited.

In our experiments the administration of antihistamines alone did not inhibit the plaice erythema reactions. This may indicate that histamine is not involved in the plaice skin reactions or that mediators other than histamine are also involved. However, inhibition of the fungal extract – and compound 48/80 – induced plaice erythema reactions by DSCG and diethylcarbamazine, compounds known to inhibit some type I allergic responses, supports our earlier conclusion[2] that we are dealing with a true immediate hypersensitivity reaction in a poikilotherm. Further experiments are in progress in an attempt to identify the individual pharmacologically-active compounds and the target cells which are involved in the flatfish cutaneous reactions.

Résumé. Comme le cromoglycate disodique et la diéthylcarbamazine (qui ne sont pas des antistaminiques) inhibent la réaction d'hypersensibilité immédiate chez *Pleuronectes platessa* à la suite d'une injection intradermique d'extraits fungiques, il semble que les médiateurs autres que l'histamine peuvent être impliqués.

B. A. BALDO and THELMA C. FLETCHER[24]

Clinical Immunology Unit, Princess Margaret Hospital, University of Western Australia, (Western Australia 6008), and N.E.R.C. Institute of Marine Biochemistry, St. Fittick' s Road, Aberdeen AB1 3RA (Scotland), 18 November 1974.

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Hexose-6-Phosphate Dehydrogenase from Human Tissues: an Electrophoretic Study in Health and Disease[1]

Hexose-6-phosphate dehydrogenase (H6PD; EC 1.1.1.47) is a microsomal enzyme which has been demonstrated in a number of tissues from different species[2–12]. It is identical with glucose dehydrogenase[5,13,14]. After biochemical and immunological experiments, and hybridization studies in trout, it was suggested that H6PD and glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) arose from a common ancestral type of G6PD, an interesting relationship of H6PD and G6PD on the evolutionary basis has been established[15–18]. In mammalian tissues considerable biochemical differences between H6PD and G6PD have been found[3–5,8,11,12]; a comparison of the two enzymes is given in the Table. Electrophoretic studies on human liver[3] and placenta[9] suggested that H6PD is a highly polymorphic enzyme. However, no distinct pattern of inheritance could be defined by the results reported. We have performed an electrophoretic study

Comparison of hexose-6-phosphate-dehydrogenase (H6PD) and of glucose-6-phosphate-dehydrogenase (G6PD)

Properties	H6PD	G6PD
Localization	Microsomal fraction	Soluble fraction
Genetic control	Autosomal	*X*-chromosomal
Substrates	G6P	G6P
	Gal-6P	(2-d-G6P)
	2-d-G6P	
	Glucose	
Coenzymes	NADP	NADP
	NAD	(NAD)
Molecular weight	223,000	102,000
Binding to CM-cellulose	+	+++
Electrophoretic mobility	slow	fast
Precipitation by anti-G6PD	—	+
Inactivation by anti-G6PD	—	+

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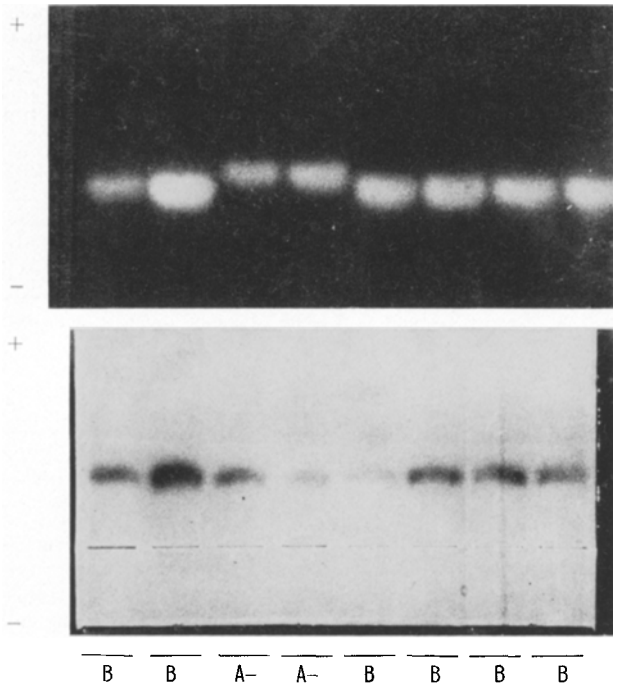

Fig. 1. Electrophoresis of glucose-6-phosphate dehydrogenase from human erythrocytes (upper part) and of hexose-6-phosphate-dehydrogenase from human leukocyte (lower part) from donors with glucose-6-phosphate dehydrogenase types 'B' (slot 1, 2, 5–8) and 'A-' (slot 3, 4). The slot order was identical in both experiments. Staining for glucose-6-phosphate dehydrogenase as 'fluorescence stain'[21], for hexose-6-phosphate dehydrogenase in the PMS-MTT linked reaction[9].

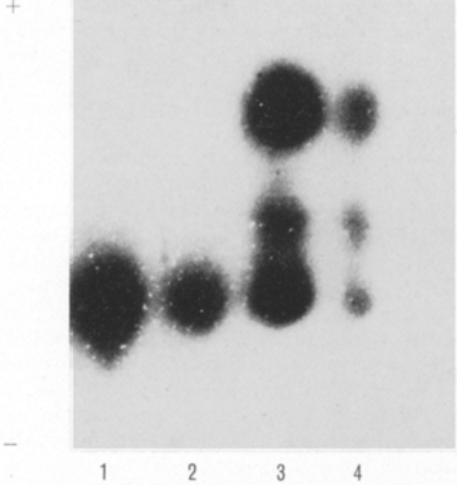

Fig. 2. Electrophoresis of hexose-6-phosphate dehydrogenase from normal human placenta (slot 1), normal human leukocytes (slot 2), and leukocytes from patients with chronic myelocytic leukemia (slot 3, 4). Staining for hexose-6-phosphate dehydrogenase activity in the PMS-MTT linked reaction[9].

on human tissues to elucidate the genetic situation and to discover the pattern of the enzyme under pathological conditions.

The biochemicals used in this study were purchased from the following manufactures: Nicotinamide-adenine dinucleotide phosphate (NADP), nicotinamide-adenine dinucleotide (NAD) and glucose-6-phosphate (G6P) from C.F. Boehringer, Mannheim, GFR; nitroblue tetrazolium, phenazine methosulfate, and D-glucose from Serva, Heidelberg, GFR; galactose-6-phosphate (gal-6P) and 2-desoxyglucose-6-phosphate (2-d-G6P) from Sigma, St. Louis, USA. *Tris* (hydroxymethyl) aminomethane (*Tris*) was obtained from E. Merck, Darmstadt, GFR, and Electrostarch from O. Hiller & Co., Madison, USA. Liver samples which were taken during laparoscopy, laparatomy or during autopsy, placenta specimen collected immediately after delivery, and leukocytes isolated from freshly drawn blood[19], were homogenized in a Potter-Elvehjem with subsequent sonication. After homogenization, the samples were centrifuged for 15 min at 40,000 $\times g$ at 0 °C and the supernatant solutions were used for electrophoretic analysis. Vertical electrophoresis[20] was performed in a 10% starch gel, prepared in 20 m*M* *Tris*-HCl pH 9.0; chamber buffer was 100 m*M* *Tris*-HCl pH 9.0. After 17 h at 200 V and 4 °C the gels were sliced and stained for H6PD and G6PD, respectively, following earlier described methods[5,9,21]. To demonstrate the genetic distinction between H6PD and G6PD we analyzed leukocyte H6PD and erythrocyte G6PD from donors with the G6PD type 'B' and with the 'A-'variant. The result is shown in Figure 1. Whereas H6PD exhibits an identical band in all samples, in the case of G6PD the well-konwn electrophoretic difference[22] between the genetic types can be observed.

We have studied the possible variability of H6PD by electrophoresis of 25 leukocyte samples, 15 liver specimen (histologically normal), and 25 placenta samples from 65 caucasian individuals, who were free from hematological disorders or liver diseases. The placentas were morphologically normal. In all 65 samples which were analyzed on the day of collection, we found a single band of H6PD activity in the typical position. In autopsy samples we also found H6PD in the following tissues: lung, kidney, spleen, heart, testicle, ovary, muscle, and thyroid gland. We saw no electrophoretic variation between the different tissues.

When 25 leukocyte samples from patients with hematological diseases (acute and chronic myelogenous or lymphocytic leukemias) were analyzed, we detected 2 samples from patients with chronic myelogenous leukemia which exhibited a 3 band pattern of H6PD, see Figure 2. Other patients with this disorder did not show the abnormality. Moreover, we were also able to produce the appearance of 3 bands by storing normal leukocytes over several weeks at 4 °C and −20 °C. Thus we believe that the additional bands are not a specific phenomenon. The same observation we made with samples from patients with chronic liver diseases (chronic hepatitis or cirrhosis of the liver). Faster moving bands could be observed in these diseases, but they also could be produced by aging normal liver samples in vitro.

To summarize, the genetic distinction between H6PD and G6PD can be demonstrated by electrophoretic means; we found no variability in samples from 65 caucasians; in chronic disorders additional bands of H6PD activity can appear, but this is not a specific biochemical feature of the diseases. It might be discussed whether the earlier described variability of H6PD[3,9] was due to chronic liver diseases in case of the autopsy specimen[3], or due to the length of storage of the placentas[9]. The nature of the fast

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moving bands, which appear in chronic diseases as more frequent and much faster than in normal samples, remains unclear sofar. Their presence might be explained by the formation of oligomers or by a disbalance in the oxido-reductive status of the enzyme molecule.

A similar study as reported herein has been published recently[23].

Zusammenfassung. Es wird über elektrophoretische Untersuchungen zum mikrosomalen Enzym Hexose-6-Phosphat-Dehydrogenase (H6PD) aus menschlichen Leukocyten, Plazentagewebe und Leber bei Gesunden und Kranken berichtet. Ferner wird durch Analyse des Enzyms H6PD bei zwei verschiedenen Glukose-6-Phosphate-Dehydrogenase (G6PD) Phänotypen gezeigt, dass die beiden Enzyme H6PD und G6PD beim Menschen nicht gemeinsamer genetischer Kontrolle unterliegen.

K. G. BLUME, G. M. SCHMIDT, H. H. HEISSMEIER and G. W. LÖHR

Medizinische Universitätsklinik, Hugstetter Strasse 55, D-78 Freiburg im Breisgau (German Federal Republic, BRD), 5 December 1974).

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Lymphocyte Transformation Studies of Sea Mammal Blood

A previous communication from this laboratory[1] described a technique for cytogenetic analysis of the dolphin (*Tursiops truncatus*). The following is a report of studies on the in vitro mitogen-induced blastogenic response of lymphocytes from Delphinidae. To our knowledge, these are the first in vitro cellular immune studies reported in sea mammals.

Materials and methods. A male and a female member of each of the species, *T. truncatus* (dolphin), *Orcinus orca* (killer whale), and 1 male *Globicephala melaena* (pilot whale) were studied. These aquatic mammals are captive specimens of an oceanarium collection (Sea-Arama of Texas, Galveston, Texas). Peripheral blood from each animal was drawn by venipuncture into a sterile heparinized (20 units/ml of blood) syringe. Lymphocyte cultures were established by a method similar to that used for the study of human peripheral blood lymphocytes[2]. Triplicate culture tubes containing 0.1 ml of heparinized whole peripheral blood in 3 ml of medium were innoculated with either 0.1 ml phosphate-buffered saline (PBS, control tubes), 0.1 ml of 1:2 dilution of phytohemagglutinin-M (PHA, Difco Laboratories, Detroit, Michigan), or 0.1 ml of pokeweed mitogen (PWM, Grand Island Biological Company, Grand Island, New York). The culture medium consisted of Earle's minimal essential medium (MEM) supplemented with 20% heat-inactivated fetal calf serum, 50 µg erythromycin, 200 µg streptomycin, and 1.5 µg sodium bicarbonate/ml. After 2 to 13 days of incubation at 37 °C and 5% CO_2, 0.5 µCi of tritiated thymidine were added to each culture tube. Approximately 24 h after labeling, the cells were washed twice with distilled water to lyse the erythrocytes, and the cell pellet was precipitated with 3 ml 5% cold trichloroacetic acid (TCA). The precipitate was then washed with methanol and digested with 0.2 ml of 0.1 *N* sodium hydroxide. 10 ml of scintillation cocktail consisting of 4 g of Butyl-PBD (Beckman Instruments, Fullerton, Calif.), 100 mg of POPOP (Packard Instrument Co., Downer's Grove, Ill.), and 100 ml Bio-Solv BBS-3 (Beckman)/1000 ml of scintillation grade toluene (Beckman) were added to each tube at least 2 h before counting in a Beckman LS-150 counter. The counts per minute (cpm) of triplicate tubes were averaged and the specific incorporation (SI) of tritiated thymidine was calculated by the formula SI = cpm/control cpm. In some experiments, additional culture tubes containing only blood and medium were set up for cell viability studies. On the day of harvest these cells were washed twice with PBS, and viability was determined by trypan blue exclusion. Additional studies were run to demonstrate that the specific incorporation results were actually due to thymidine incorporation in blastic and mitotic cells as had been found in other mammalian species tested by lymphocyte transformation. Autoradiographs of dolphin blood were done at 5 days on PWM stimulated cells at the same time as tubes were harvested for morphological counts of transformed cells and tubes for liquid scintillation counting. Culture of lymphocytes were labeled 24 h before harvesting.

Aliquots of the cell sediment from triplicate tubes were carefully smeared on hematology microscopic slides for staining (Wright's). Counts of transformed (lymphoblastic) and mitotic cells/1,000 cells were read in triplicate.

Radioactive labeled blood smears on glass microscope slides were immersed at 40 °C into a 50% solution of Kodak NTB-2 nuclear track emulsion and deionized distilled water. The slides were then room dried and stored in light-tight boxes for 40 days exposure to the radioactive label. They were then developed at 17 °C in a Dektol solution, fixed with Kodak acid fixer, and then washed in deionized water at 17 °C. After drying, the

Table I. Response of peripheral blood cultures from the killer whale (*O. orca*) to in vitro exposure to PHA

Day of assay	PBS (CPM)	PHA (CPM)	SI[a]	Cell viability (%)
3	77[b]	3179	40.7	83
	118[c]	4049	34.3	79
5	220	5011	22.8	72
	353	6864	19.4	76
7	138	4359	31.6	60
	412	4655	11.1	62
10	289	3306	11.4	36
	78	2086	26.7	40
14	330	395	1.2	30
	416	393	0.9	36

[a] SI, specific incorporation of tritiated thymidine = CPM PHA/CPM PBS (SI > 3.0 = positive). [b] Total leukocyte count = 5,600/mm³; differential = 32% mononuclear cells; 68% polymorphonuclear. [c] Total leukocyte count = 7,000/mm³; differential = 25% mononuclear cells; 75% polymorphonuclear.

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