Alpha-Glycerophosphate Dehydrogenase and Glucose-6-Phosphate Dehydrogenase in Tissues of the Weddell Seal

Abstract. *High activity of alpha-glycerophosphate dehydrogenase and low activity of glucose-6-phosphate dehydrogenase in adipose tissue of Weddell seals suggest that neutral fat may be assembled there from exogenous sources of fatty acids. Low activity of glucose-6-phosphate dehydrogenase in other tissues tested precludes assignment of the function of fatty-acid synthesis to any specific tissue and emphasizes uniqueness of adipose mass in seals.*

The Weddell seal (*Leptonychotes weddelli*) occupies the coldest habitat known for mammals—the shore ice and seas surrounding Antarctica. Its polar habitat and amphibious habits are associated with metabolic and thermoregulatory characteristics which enable it to adapt to this harsh environment (*1*). Since the flux of free fatty acids and neutral (esterified) fat is central to problems of fuel and insulation, we attempted to determine whether specific alterations in metabolic pathways concerned with lipid metabolism were involved in such adaptations. Assays for two enzymes involved in the synthesis of neutral fat were made on the tissues of these animals: (i) alpha-glycerophosphate dehydrogenase which mediates the conversion of dihydroxyacetone phosphate, arising in glycolysis, to the active glycerol (α-glycerophosphate) which then combines with fatty acids to form neutral fat; and (ii) glucose-6-phosphate dehydrogenase which affords the first step in the shunt pathway for the direct oxidation of glucose and yields the reduced form of the coenzyme nicotinamide adenine dinucleotide phosphate (NADP), a requisite for the synthesis of fatty acids.

Seals were stalked on the pack ice and killed by rifle shot; portions of liver, adipose tissue (blubber), rectus, kidney, heart, and midbrain were excised as quickly as possible and placed in plastic bags for the trip to the laboratory. Fresh material was processed immediately at the Biological Laboratory at McMurdo Station (Ross ice shelf in the Antarctic). Material to be processed in New York was frozen at −20°C. Tissues were homogenized in distilled water; appropriate dilutions were made from stock homogenates (10 percent), which were then centrifuged at 850*g* for 10 minutes to remove coarse particles. Homogenates of adipose tissue were centrifuged a second time after aspiration of the congealed top layer of lipid. α-Glycerophosphate dehydrogenase and glucose-6-phosphate dehydrogenase were determined with tetrazolium salt (*2*). A more extensive program of enzyme determinations was not possible in the field. In selected cases, phenazine and cyanide were omitted from the standard assay of α-glycerophosphate dehydrogenase to ascertain whether flavoprotein activity, which is shunted by the phenazine component, constitutes a limiting factor in the transfer of electrons along the transport chain.

Alpha-glycerophosphate dehydrogenase activity was higher in all tissues than corresponding activities of glucose-6-phosphate dehydrogenase. Adipose tissue showed extremely high activity of the former with kidney, liver, muscle, and brain following in decreasing order (Table 1). Activity of α-glycerophosphate dehydrogenase in heart was negligible; its activity in brain was almost entirely a reflection of the particulate mitochondrial enzyme, which is not dependent upon the coenzyme nicotinamide adenine dinucleotide (NAD), while in all other tissues the activity largely reflects the soluble cytoplasmic enzyme which is NAD-dependent (Table 2). Activity of glucose-6-phosphate dehydrogenase was quite low in all tissues, the order of decreasing activity being kidney, liver, adipose tissue, and brain. Heart and muscle demonstrated only negligible activity (Table 1). Enzyme activities in fresh tissue were generally higher than in frozen tissue, but of the same order of magnitude. In skeletal muscle, there was a marked diminution of formazan deposition associated with omission of phenazine and cyanide; this indicated low flavoprotein activity, and occurred in the absence and presence of NAD.

An inverse correlation of body size and the activities of redox enzymes is generally encountered on an interspecific basis in various mammals (*3*). The low activity of glucose-6-phosphate dehydrogenase in all tissues of the seal and the comparatively low activity of α-glycerophosphate dehydrogenase in liver, muscle, and brain (Table 3) are consistent with this position. However, comparative assays of the enzymes involved in the major pathways of glycolysis and the Krebs cycle might be more appropriate indices of the existence of such an inverse size-metabolic activity correlation in the case of the pinnipeds.

The high activity of α-glycerophosphate dehydrogenase in seal adipose tissue—more than double that of mouse (Table 3)—suggests a high rate of synthesis of active glycerol, presumably for the synthesis of neutral fat. This is in accord with recent evidence of the major role of adipose tissue in the synthesis and storage of fat (*4*). However, the high activity of glucose-6-phosphate dehydrogenase in adipose tissue in other

Table 1. Enzyme activity in tissues of the Weddell seal expressed as micrograms of iodoformazan per microgram of tissue nitrogen (micro-Kjeldahl). Samples from six animals were used in all cases, except for fresh adipose and frozen heart (where only five were used).

Condition	α-Glycerophosphate dehydrogenase	Glucose-6-phosphate dehydrogenase
	Mean ± S.E.	Mean ± S.E.
	Liver	
Fresh	1.51 ± 0.34	0.56 ± .14
Frozen	1.74 ± 0.17	.55 ± .10
	Adipose	
Fresh	9.25 ± 2.14	.48 ± .36
Frozen	6.94 ± 1.7	.17 ± .09
	Muscle	
Fresh	0.88 ± 0.17	.06 ± .03
Frozen	.73 ± .11	.07 ± .07
	Kidney	
Fresh	4.77 ± .78	.60 ± .13
Frozen	3.36 ± .59	.38 ± .11
	Heart	
Fresh	0.33 ± .07	.13 ± .10
Frozen	.29 ± .06	.08 ± .02
	Brain	
Fresh	.79 ± .05*	.37 ± .08
Frozen	.83 ± .03	.21 ± .03
Fresh	.81 ± .10†	
Frozen	.07 ± .08	

* With NAD. † Without NAD.

Table 2. Influence of NAD on activity of α-glycerophosphate dehydrogenase in the Weddell seal. Results are expressed as micrograms of iodoformazan per microgram of tissue nitrogen.

Tissue	Fresh		Frozen	
	With NAD	Without NAD	With NAD	Without NAD
Liver	1.96	0.09	1.73	000
Adipose	14.92	.00	10.00	11
Muscle	1.12	.07	0.64	14
Kidney	4.28	.08	4.15	11
Heart	0.46	.06	0.50	18
Brain	.60	1.00	.78	76

Table 3. Activity of α-glycerophosphate dehydrogenase in tissues of seal, rat, and mouse, expressed as micrograms of iodoformazan per microgram of tissue nitrogen. Numbers in parentheses denote number of samples.

Tissue	Weddell seal	Rat	Mouse
Liver	1.47 ± .17 (6)	5.55 ± .52 (12)	4.07 ± .43 (16)
Muscle	0.73 ± .11 (6)	4.00 ± .08 (11)	4.42 ± .58 (13)
Kidney	3.36 ± .59 (6)	2.26 ± .22 (12)	5.02 ± .52 (13)
Brain	0.83 ± .03 (6)	1.20 ± .04 (12)	2.85 ± .33 (14)
Adipose	6.94 ± .17 (6)		3.33 ± .44 (14)
Heart	0.29 ± .06 (5)	0.79 ± .05 (11)	Negligible

mammals (*5*) is not found in the Weddell seal, which indicates either that alternative pathways for the generation of reduced NADP exist in seal adipose tissue or that this tissue is not a major site of fat synthesis, because fatty acid production cannot occur without adequate amounts of the reduced coenzyme. Synthesis of fatty acids may occur elsewhere, and these acids may then be carried by the blood to adipose depots for assembly. However, the low activity of glucose-6-phosphate dehydrogenase in all the tissues assayed is not in accord with this hypothesis. Possibly, fat synthesis is not a major function of the adipose mass, but rather the high α-glycerophosphate dehydrogenase is an adaptation to oxygen deprivation associated with arterial constriction during diving (*6*). This enzyme functions similarly to lactate dehydrogenase in providing for a reoxidation of the reduced NAD arising during glycolysis, with a concomitant production of reduced metabolite. This may also apply in the kidney, where activity of α-glycerophosphate dehydrogenase is twice that in rat (Table 3).

The absence of intra-abdominal fat stores and the relatively coarse reticular structure of the insulating subcutaneous adipose tissue in seals (*7*) support the concept that adipose physiology is quite unique in these aquatic mammals. The absence of a hexose shunt pathway, coupled with high α-glycerophosphate dehydrogenase activity, provides an enzymatic reflection of this metabolic uniqueness.

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References and Notes

1. C. Ray and M. S. R. Smith, *Zoologica*, in press.
2. G. H. Fried, N. Greenberg, W. Antopol, *Proc. Soc. Exp. Biol. Med.* **107**, 523 (1961).
3. G. H. Fried and S. R. Tipton, *ibid.* **82**, 531 (1953).
4. H. E. Wertheimer, *Handbook of Physiology: Adipose Tissue* (Williams and Wilkins, Baltimore, 1965), vol 5, p. 6.
5. G. Weber H. J. Hird, N. B. Stamm, D. S. Wagle, *ibid.*, p. 229; G. H. Fried and W. Antopol. *Amer. J. Physiol.* **211**, 1321 (1966).
6. K. M. Bron, H. V. Murdaugh, Jr., J. Eugene Millen, R. Lenthall, P. Raskin, E. D. Robin, *Science* **152**, 540 (1966).
7. J. Vague and R. Fenasse, *Handbook of Physiology: Adipose Tissue* (Williams and Wilkins, Baltimore, 1965), vol. 5, p. 30.
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Sulfur Mustard and X-Rays: Differences in Expression of Lethal Damage

Abstract. *Before or after treatment with sulfur mustard, simple changes in the incubation conditions of cultured Chinese hamster cells lead to changes as great as a factor of 6 in survival-curve slopes. With x-ray treatment, changes are similar but much smaller in magnitude. These results suggest that the modes of action of these agents are not entirely the same.*

Bifunctional alkylating agents, which are considered to be radiomimetic (*1*, *2*), and x-rays kill cells, yield survival curves of similar shapes, produce chromosome breaks, lead to similar patterns of hematological changes, and are thought to produce similar results with respect to other end points. Lethal-repair processes relative to both agents are considered similar. Recovery from lethal damage by drugs is reported in microbes (*3*, *4*), and differences in the survival responses of different lines of mammalian cells are thought to reflect corresponding differences in their repair capacities after treatment with either type of agent (*2*). Because of these similarities and the use of both types of agent (singly and possibly together) for treating cancer, we undertook comparative study of their toxic properties. Sulfur mustard (*5*) was chosen because its reactivity in aqueous media permits closer approximation to acute irradiation than do other readily available alkylating agents. For Chinese hamster cells growing attached to a surface, we now report the influence on survival of some simple procedures entailing changes in medium and temperature before and after treatment. Our results show that these procedures have a strong influence relative to sulfur mustard treatment and only a weak influence relative to x-ray treatment.

Typical curves for the survival of the Chinese hamster subline *V79-661*, after exposure to different doses of sulfur mustard, are shown in Fig. 1. Cells were grown overnight attached to glass or plastic, and, after treatments to be described, were assayed for colony-forming ability by use of techniques similar to those reported (*6*). The medium used for surface-attached growth is a modification of Eagle's (*7*, *8*) and contains 15 percent fetal calf serum. The doubling time for these cells is 8 to 9 hours (*9*). As a result, after the overnight incubation of initially single cells, the population appears to be in asynchronous log-phase growth (*9*), with colony-forming units comprising an average of about three cells. Since Chinese hamster cells survive damage by either x-rays (*10*) or sulfur mustard (*10a*) independently, multiplicities greater than one introduce no serious complications with regard to analysis of survival curves.

Sulfur mustard (di-2-chloroethylsulfide), dissolved in absolute ethyl alcohol and stored in a deep freeze, was diluted before each experiment in the same solvent and then maintained at ice temperature. Immediately before each exposure, the solution was further diluted in buffer at *p*H 7.4 (Dulbecco's saline supplemented with 1 percent medium), also at ice temperature. Drug concentrations were adjusted so that 1.0-ml portions could be added to the medium or buffer in petri dishes to obtain the final concentrations desired. Accordingly, the final concentrations of alcohol (less than 1 percent) were always too low to affect survival. Treatment solutions were removed after 8 minutes and replaced by fresh medium or buffer as