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Studies on the Problem of "Aerobic Glycolysis" Occuring in Mammalian Embryos

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In all mammalian cells energy derived from nutritional substrates is made available through the formation of ATP. This ATP may be formed by anaerobic reactions—either glycolysis or the two steps of "substrate phosphorylation"—or by aerobic metabolic steps initiated by different oxidoreductases which feed hydrogen or electrons into the electron transport chain. In most mammalian cells the majority of ATP molecules is formed by reactions linked to respiration and only very few cell types produce ATP by predominantly making use of the glycolytic reactions and thereby accumulating lactate.

The fundamental studies of Warburg (1923, 1926, 1955, 1956) have shown tumor cells to be the most significant exception from this rule. Most tumor cells—and especially those showing a comparatively high growth rate—even under aerobic conditions form lactate to a large extent and thus produce a substantial percentage of their ATP via the energy preserving reactions of the Embden-Meyerhof-pathway.

There have been contradicting reports on the ability of mammalian embryonic tissue to produce lactic acid under aerobic conditions and thus to gain energy by the glycolytic reactions. Negelein (1925) published results which indicated that rat embryos were capable of forming lactic acid under aerobic conditions; but he interpreted his findings as the results of a damage done to the rather soft embryonic tissue under the conditions of an in-vitro assay. He concluded that his studies should be interpreted as showing that embryonic tissue is *not* capable of an aerobic

* Some of the data included in this paper are part of a dissertation to be submitted to the Free University Berlin.

glycolysis to an appreciable extent. Based on these rather limited data and possibly on further data, too, Warburg in 1955 and 1956 still stated that embryonic tissue surely is not capable of an aerobic glycolysis.

We, therefore, have re-investigated this problem and in this paper we like to present results obtained in a great number of experimental series performed with rat embryos from several hundred pregnant animals the developmental stages of which had been accurately timed.

Wistar rats (S.W. 69) kept under a reverse day/night cycle were mated for 2 h (8.00—10.00 a.m.) and the 24-h period after detection of sperms in vaginal smears was called day 0. The embryos were removed as fast as possible in the cold room. Most investigations are based on the 11.00—13.00 o'clock period. For studies on glucose utilisation or lactate formation the embryos were incubated in 95% O_2 + 5% CO_2 in a serum medium at 37° C for periods of 3—20 min. The rat serum used was dialysed against Ringer solution. Glucose and lactate were measured with optical tests using the hexokinase and the lactate dehydrogenase reaction, respectively. DNA was measured with the Burton (1956) method. Amniotic fluid was won by puncture in ether or Evipan anesthesia.

All enzyme activities given in this paper are V_{max} values obtained from optical tests (Leitz-Unicam SP 800 recording spectrophotometer) using a 100,000×g supernatant. Data were derived from full kinetic measurements and calculations based on both graphical and mathematical methods from at least 5, in most cases more than 10, complete kinetics. It is worth mentioning that significant deviations in DNA content, wet and dry weight as well as in the activities of many enzymes may be observed (Köhler and Peters, 1970; Köhler, 1970) when different strains of Wistar rats are used.

Data are compiled in the Table. Especially two results appear worth discussing:

1. Embryos Removed as Fast as Possible from the Mother Animals always Showed a Relatively High Basal Content of Lactic Acid. This amount of lactic acid was rather independent of the time needed for the removal of the embryos from the mother animal, suggesting that these embryos also possess a relatively high concentration of lactic acid in vivo.

2. Lactic Acid was Produced under Aerobic Conditions in vitro by these Rat Embryos at a rather Constant Rate. At the same time glucose was taken up by the embryos from the medium. Following an incubation without glucose no formation of lactic acid could be observed excluding an appreciable degree of degradation of glycogen. Glucose uptake of 13-day old rat embryos has been measured before by De Meyer and De Plaen (1964) and by De Plaen (1970). Although we have confirmed these results we have performed most of our studies at earlier stages of development since in 13-day old or even older rat embryos such data are hard to interpret because the limiting thickness and an unimpaired permeability of oxygen and possibly also of substrates cannot be guaranteed, as Spielmann and Lücke (1971) have discussed in detail.

We have focussed our attention especially on the problem of the "intactness" of the embryonic cells the metabolism of which has been

Table. *Metabolism of glucose in rat embryos at the stage of organogenesis. M ± S.D. DNA and weight were measured with 50—200 embryos. All other data are average values from 6—10 estimations*

			day-old embryos		
			11	12	13
DNA content		$\mu g \times embryo^{-1}$	14.8 ± 2.7	80 ± 10.4	274 ± 35
DNA/dry weight		$\mu g/mg$	60	75	88
Wet weight		$mg \times embryo^{-1}$	2.6 ± 0.4	13.4 ± 2.8	37.0 ± 5.9
Glucose utilisation		p moles $\times \mu g$ DNA^{-1} $\times min^{-1}$	182 ± 24	39 ± 5	34 ± 5
Lactate formation		p moles $\times \mu g$ DNA^{-1} $\times min^{-1}$	291 ± 43	66 ± 10	61 ± 9
		μmoles $\times$ mg dry weight^{-1} $\times h^{-1}$	1.05	0.30	0.32
		$Q_M^{O_2}$	23.9	6.8	7.3
Lactate from glucose		%	80%	85%	89%
$^{14}CO_2$ from ^{14}C-1-glucose		p moles $\times \mu g$ DNA^{-1} $\times min^{-1}$	1.7 ± 0.3	1.2 ± 0.2	0.5 ± 0.1
$^{14}CO_2$ from ^{14}C-6-glucose			0.01	0.01	0.01
Oxygen consumption*		Q_{O_2}	11.5	11.5	11.5
Lactate content		mμ moles $\times \mu g$ DNA^{-1}	8.5 ± 1.5	3.2 ± 0.4	3.3 ± 0.4
Glucose concentration	maternal serum	mμ moles $\times \mu l^{-1}$		6.2 ± 0.5	
	amniotic fluid	mμ moles $\times \mu l^{-1}$		2.8 ± 0.4	
Lactate concentration	maternal serum	mμ moles $\times \mu l^{-1}$		3.2 ± 0.5	
	amniotic fluid	mμ moles $\times \mu l^{-1}$		9.1 ± 0.8	
Activity	maternal liver	p moles NAD $\times$ mg protein^{-1}		1650 ± 100	
α-GPD	embryo	$\times min^{-1}$		6 ± 0.4	6 ± 0.4

* Data from Spielmann and Lücke (1971).

studied. Since the studies of Druckrey (1936), Brock *et al.* (1938, 1939) and Herken (1939) it is well established that a high rate of glycolysis may be the result of a so-called "damaging metabolism". Such an assumption has been favoured by Negelein (1925) and Warburg (1955, 1956) for the lactate accumulation measured with rat embryos.

For the following reasons we feel that from our data such an explanation may be excluded in the case of rat embryos in the phase of organogenesis:

1. Numerous electron microscopical studies performed by Prof. Dr. H.-J. Merker (Anatomisches Institut der Freien Universität Berlin) on rat embryos incubated in a serum medium for varying time intervals (up to 30 min) and subsequently fixed in Karnovsky's solution revealed no changes. These results will be published in detail elsewhere (Merker *et al.*, 1971). Even such delicate structures as mitochondria or polysomes did not show any alterations by this incubation so that the incubated embryos could not at all be distinguished from embryos studied right after the removal from the mother animal without any incubation. It may be mentioned that this finding is quite in contrast to the appearance of liver tissue incubated in a saline medium for some time which shows a high degree of disintegration despite *no* appreciable lactate formation under aerobic conditions. Thus the damage done to any tissue cannot be the main reason for its capability to form lactate under aerobic conditions.

2. In contrast to the studies of Negelein (1925) performed with the manometric technique we have found a similar rate of lactate formation (72.9 ± 7.0) when 12- or 13-day old rat embryos were incubated still in the yolk-sac and surrounded by the physiological amniotic fluid.

3. The concentration of lactate found in rat embryos before incubation is very high. Since the rate of lactate formation has been found to be rather constant in vitro, it is unlikely that this lactate has accumulated during the isolation of the embryos.

4. High concentrations of lactic acid are present *in vivo* in the amniotic fluid (Table). This fluid was won from the animals by puncture in anesthesia.

These Data may be Taken as Evidence for Mammalian Embryonic Tissue being Capable of Forming Lactic Acid under Aerobic Conditions.

Boxer and Delvin (1961) have developed a concept for explaining the high rate of aerobic glycolysis in tumor tissue: They have found that this sort of metabolism may be the result of the inability of these tumor cells to re-oxidize the $NADH+H^+$ formed during glycolysis by "shuttle" mechanisms which guarantee the transport into the mitochondria and the oxidation of the hydrogen equivalents through the respiratory chain. From data shown in the Table it may be seen that mammalian embryonic

tissue at the stage of organogenesis has an extremely low activity of a hyaloplasmic NAD-dependent α-glycerophosphate dehydrogenase. On the other hand, mitochondrial α-glycerophosphate oxidase in this tissue has about $^1/_4$ of the activity of maternal liver.

Summarising, the Following Conclusions can be Drawn. 1. Mammalian embryonic tissue is capable of forming lactate under aerobic conditions if a sufficient supply of glucose is guaranteed.

2. This "aerobic glycolysis" is not the result of a "damaging metabolism".

3. The rate of aerobic glycolysis is in the same order of magnitude as that reported for many experimental tumors despite the fact that a high rate of respiration ($Q_{O_2} \sim 12$) can be measured under the same experimental conditions (Spielmann and Lücke, 1971).

4. The activity of α-glycerophosphate dehydrogenase is extremely low in embryonic tissue. An inability to transport hydrogen equivalents into mitochondria via the α-glycerophosphate "shuttle" (Zebe *et al.*, 1959) may be an explanation for the "aerobic glycolysis" observed.

5. The rate of aerobic lactate formation as well as the rate of glucose utilisation in the rat decreases sharply between day 11 and 12 of pregnancy concomitantly with the beginning of the placenta function.

6. Under aerobic conditions 80—90% of the glucose utilized is found as lactic acid showing that only a small portion of the glucose is metabolized via the reactions of the respiratory pathway. The high rate of respiration measured under the same experimental conditions must be maintained by other sources than glucose.

7. Since at present no data are available on the oxygen tension within rat embryos in utero, it may well be presumed that the capacity for respiration and for an "aerobic glycolysis" is not used by the embryo in vivo so that energy may be derived predominantly from "anaerobic glycolysis"—as it has been shown to occur within certain experimental tumors which grow under anaerobic conditions. An "anaerobic glycolysis"—exceeding that of many experimental tumors—has been shown to occur in rat embryos by Negelein (1925) and has completely been confirmed by our studies.

8. The high rate of glucose utilisation of 11-day old embryos—exceeding that of most maternal tissue—in the phase of most rapid growth and differentiation may not only be of significance for energy formation but it may also be of vital importance for synthetic processes. This has been revealed by studies which showed an incorporation of ^{14}C-glucose fragments into RNA, DNA, lipids and glycosaminoglycanes (Neubert *et al.*, 1970, Köhler *et al.*, 1970; Krowke *et al.*, 1971)—although the pentose phosphate pathway contributes to only 1—3% of the glucose utilized (Köhler and Peters, 1970; Köhler and Brand, 1970).

This special situation of the glucose metabolism in embryonic tissue which drastically deviates from the metabolic situation present in most tissue of the maternal organism may well be a target for the action of embryotoxic agents.

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