CXIX. INTERMEDIARY CARBOHYDRATE METABOLISM IN AMPHIBIA

I. CARBOHYDRATE BREAKDOWN BEFORE METAMORPHOSIS

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CARBOHYDRATE metabolism seems to be of dual importance in embryonic life. In the earlier stages of development it is believed that the active substance which brings about neural induction (the evocator) forms a symplex with glycogen and protein and when this is broken down in the dorsal lip of the blastopore, the evocator is liberated [Waddington *et al.* 1935; Waddington *et al.* 1936; Needham, 1936]. In older stages the breakdown of carbohydrate probably provides much of the energy necessary for growth and differentiation.

An extensive study of carbohydrate metabolism in the chick embryo [Needham & Nowiński, 1937; Needham *et al.* 1937; Needham & Lehmann, 1937, 1, 2; Lehmann & Needham, 1937; 1938] showed that the way of carbohydrate breakdown in chick embryo probably differs from that in muscle. The formation of lactic acid in the embryo seems not to pass through a phosphorylating cycle.

The main characteristics of embryonic carbohydrate metabolism found by these authors may be summed up as follows: glycogen is not broken down to lactic acid, whereas glucose and mannose are readily used. The intermediate products of phosphorylation, such as hexose-monophosphate or -diphosphate are not used at all and the same applies to other carbohydrates such as fructose, galactose, sorbose, various pentoses, glucosamine etc. Embryo glucolysis somewhat resembles glucolysis in brain and in tumour tissue. It may be inhibited by *dl*-glyceraldehyde [Needham & Nowiński, 1937] as Mendel [1929] found for tumour tissue. Fluoride also inhibits glucolysis in embryo, but there is no accumulation of hexosediphosphate or phosphoglyceric and glycerophosphoric acids [Needham *et al.* 1937].

These observations on chick embryo seemed sufficiently interesting to warrant the question whether they are typical for embryonic tissue in general. In the present work the carbohydrate breakdown in tadpole tissues (*Rana temporaria*) before metamorphosis has been studied. A later paper will deal with the carbohydrate metabolism during metamorphosis. Studies on mammalian embryo are also planned.

Methods

The methods described previously [Needham & Nowiński, 1937; Needham *et al.* 1937] were used in the present work. Substrate preference and glyceraldehyde inhibition were studied manometrically but in smaller Warburg cups (6.0–7.0 ml.). The temp. of the water bath was 20°. The first reading was taken 20–30 min. after gassing with N₂/CO₂ mixture.

As the younger tadpoles after hatching still contain much yolk and the tissues are saturated with their natural substrate, no significant results could be expected from experiments on this stage. Work was begun, therefore, with stages in which no trace of yolk in the tadpole could be seen under the binocular microscope.

The absolute age of the animals was hard to determine as the eggs were sometimes found already fertilized in the laboratory tanks, but the youngest stages investigated were about 5 days after hatching. The size of the tadpole, as a criterion of age, is not very dependable, but it led to the interesting result that the chemical differentiation (of enzyme systems) of the tissues seems to be independent of growth in length.

If brei is used, the tadpoles being ground in a small mortar until morphological structure is destroyed, the Q_L^N obtained is so low (for autoglycolysis +1.65, for glucose as substrate +2.85) that the enzyme system is obviously inactivated by the process. Experiments on intact tadpoles were also not very successful as the Q_L^N for autoglycolysis and glucose as substrate were almost the same (autoglycolysis +12.7 and glucose +13.95). Probably the cutis is not permeable to these substances.

The embryos were therefore pulped with a scalpel, but not into too small pieces. The Q_L^N thus obtained were more satisfactory (e.g. in Exp. 5 for 10–12 mm. tadpoles: autoglycolysis +10.48; with glucose as substrate +19.2).

The appropriate carbohydrates were added before the experiments started. As the added substances were dissolved in NaHCO_3 -Ringer for the frog, no dilution occurred of Ringer solution present in the cup.

Autoglycolysis

Table I shows the autoglycolysis of tadpoles during development. In these experiments the total length of the tadpoles was taken as the criterion of age (the figures were mainly obtained from the same lot of tadpoles, measured as development progressed).

Table I

Size of tadpoles in mm.	Average Q_L^N
8–10	+19.35
10–12	+12.7
12–14	+12.1
16–18	+10.72
18–20	+11.9
20–22	+14.6

Fig. 1. Preference for glucose. A record of a single experiment.

The curve of autoglycolysis is quite similar to the curve of autoglycolysis in chick embryo [Needham & Nowiński, 1937]: it decreases rapidly with age (Fig. 1). The unusually high $Q_L^{N_s}$ of +14.6 for the 20–22 mm. tadpoles is probably due to "cannibalism": the tadpoles in my experiments were given no food, but in some cases it was impossible to prevent them from eating weak or dying individuals. Fed animals of course grow more quickly but though the size is greater (e.g. 20–22 mm.) the chemical differentiation is just the same as that of 10–12 mm. tadpoles, judged by substrate preference.

Substrate preference

As in the case of chick embryo, glucose but not glycogen is broken down by tadpole tissue. Fig. 1, a record of a single experiment, shows that the curve of glycogen breakdown (in ml. CO_2) runs closely with the curve of autoglycolysis (in all the cups there was approximately the same amount of tissue and the vessel constants differed only slightly), whereas the glucose breakdown gives much higher values, and at the end of the exp., when the glycogen breakdown reaches the value of $36\mu\text{l. CO}_2$, the CO_2 production with glucose as substrate rises to $67.5\mu\text{l.}$ The corresponding values for $Q_L^{N_s}$ in this experiment were: autoglycolysis +11.45, glycogen +11.9, glucose +22.5.

These facts were quite reproducible and the main results are collected in Table II. Though there is some scatter among the various experiments, nevertheless the preference for glucose during the course of development of the tadpole is quite obvious.

Table II

Length of tadpoles in mm.	$Q_L^{N_s}$ glycogen	$Q_L^{N_s}$ glucose
8–10	+19.0	+27.0
10–12	+10.48	+19.2
—	+13.5	+22.25
—	+8.5	+29.0
—	+15.2	+27.3
12–14	+16.1	—
14–16	+19.2	+30.3
—	+12.0	+21.0
—	+11.26	—
—	+7.64	+17.51
16–18	+12.6	—
20–22	+20.25	+25.0

Table III

Exp. no.		$Q_L^{N_s}$
8	Autoglycolysis	+13.5
	Hexosediphosphate	+12.0
	Glucose	+29.0
9	Autoglycolysis	+10.83
	Hexosediphosphate	+10.3
		+10.67
10	Autoglycolysis	+11.5
	Hexosediphosphate	+11.8
		+10.55
		+11.08

Table III shows that adding hexosediphosphate has no effect upon glycolysis, the $Q_L^{N_s}$ for hexosediphosphate corresponding closely to the $Q_L^{N_s}$ of autoglycolysis, whereas glucose (as in Exp. 8) gives a value 70 % higher.

From these results we may draw the conclusion that the mechanism of glucolysis in the tadpole is similar to the glucolysis of the chick embryo.

Glyceraldehyde inhibition

It was first observed by Mendel [1929] that *dl*-glyceraldehyde has the property of inhibiting glucolysis of tumour tissue completely. The same effect may also be produced on the glucolysis of brain [Ashford, 1933], but not on the glucolysis in muscle extract as mentioned by Holmes [1934] and confirmed by other investigators [Needham & Lehmann, 1937, 2; Baker, 1938; Cori *et al.* 1939]. It was therefore suggested that *dl*-glyceraldehyde is a specific inhibitor of glucolysis. As such it was used by Needham and co-workers [Needham & Nowiński, 1937; Needham & Lehmann, 1937, 2] for embryonic tissue and it was found that in chicken embryo glyceraldehyde inhibits the breakdown of glucose. This effect is due to *l*-glyceraldehyde only as was shown by Needham & Lehmann [1937, 2] and confirmed for tumour tissue by Mendel *et al.* [1938]. The specificity of this inhibition was contradicted by Boyland & Boyland [1938] and by Adler *et al.* [1937]. The latter authors claimed to show that glyceraldehyde inhibits not only glucolysis in tumour tissue but also the glycogen breakdown in muscle extract. This discrepancy between the results of these authors and those of Holmes, Needham & Lehmann, Baker and Cori *et al.* was removed by Lehmann & Needham [1938] who showed that freshly prepared glyceraldehyde (in dimeric form) inhibits both ways of carbohydrate breakdown, glucolysis and glycogenesis, whereas glyceraldehyde in its monomeric form (produced by standing for some time in solution) acts as a specific inhibitor of glucolysis.

It was of interest to investigate whether tadpole tissue behaves towards this specific inhibitor of glucolysis in the same way as tumour, brain or chick embryo. The results obtained in these experiments showed that it does. Of course stronger concentrations ($5 \times 10^{-2} M$) inhibit glucolysis: in Exp. 25 for instance Q_L^N for glucose as substrate was +19.8 but for glucose + glyceraldehyde +8.77 only. In another exp., where Q_L^N for autoglucolysis was +4.55 and for glucose +11.59, the Q_L^N for glucose + glyceraldehyde was +3.58.

On the other hand Lehmann & Needham [1938] emphasize the importance of a suitable concentration: too strong concentrations may inhibit glucolysis and glucogenesis [confirmed by Süllmann, 1939] as in the experiments of Boyland & Boyland and Adler *et al.* Lehmann & Needham obtained maximum inhibition with a glyceraldehyde concentration of $3-6.7 \times 10^{-2} M$. It was therefore necessary to test lower concentrations than stated above.

The experiments were carried out in Warburg manometers. Glucose and glyceraldehyde were added together and another manometer with glucose was used as a control. A further control consisted of tissue without substrate.

The rate of inhibition obtained from these experiments is shown in Fig. 2, being an average from various figures. It is obvious from it that with increasing concentrations the rate of inhibition rises. A glyceraldehyde concentration of

Fig. 2. Inhibition of glucolysis by glyceraldehyde.

$10^{-3} M$ inhibits 76 % of glucolysis; $2.5 \times 10^{-3} M$ inhibits about 90 %; whereas $5 \times 10^{-3} M$ inhibits glucolysis completely. The corresponding figures for Q_L^N are shown in Table IV.

Table IV

Concentration of glycerald.	Q_L^N glucose + glycerald.	Q_L^N glucose	Q_L^N no substrate
$10^{-3} M$	17.75	34.00	12.65
$2.5 \times 10^{-3} M$	10.21	21.15	8.77
$5 \times 10^{-3} M$	6.55	12.30	8.28
$10^{-2} M$	1.70	12.30	8.28
$5 \times 10^{-2} M$	8.79	18.00	8.73

Lehmann & Needham [1938] stated that maximum inhibition has to be produced by a concentration of $3 \times 10^{-3} M$ glyceraldehyde, but not stronger. My results are in accordance with this criterion as the maximum inhibition in my case lay between $5 \times 10^{-3} M$ and $2.5 \times 10^{-3} M$. We may, therefore, draw the conclusion that glucolysis in the tadpole resembles that of chick embryo, tumour and brain tissue, in that it may be inhibited by glyceraldehyde.

Triosephosphate accumulation

Needham & Lehmann [1937, 1] showed that in the case of the chick embryo triosephosphate accumulates during the experiment. The following typical experiment shows the method.

Exp. 20. One hundred tadpoles (length 12–15 mm.) were chopped and incubated anaerobically for 1 hr. in Thunberg tubes (some experiments were carried out in Warburg cups). The first tube (X 31) contained tissue without substrate, tube X 52 an appropriate amount of hexosediphosphate and tube X 60 glucose. The amount of tissue was exactly the same in each tube: 2 ml. of brei. After 1 hr. incubation (anaerobically) at 20° the contents of these tubes were ground ice-cold with P-free sand and 3 ml. 3 % trichloroacetic acid, and filtered through asbestos in a Gooch crucible. 1 ml. of the filtrate was removed for estimation of the inorganic P. 1 ml. of filtrate was made alkaline (2N NaOH) and after standing for 20 min. at room temp., the P estimation (Fiske-Subbarow) was carried out. The difference of the two estimations gave the amount of triosephosphate.

The results of my experiments are shown in Table V. The differences between the alkali-labile + inorganic P and the inorganic P are very small. They are within the range of colorimetric experimental error. It would therefore appear that hexosediphosphate is not attacked at all by tadpole tissue.

Table V

No. of cup	Substrate	Inorganic P (mg.)	Alkali-labile P + inorganic P (mg.)
X 31	None	0.075	0.055
14	None	0.079	0.080
11a	None	0.039	—
X 52	Hexosediphosphate	0.051	0.069
11	Hexosediphosphate	0.054	0.073
13	Hexosediphosphate	0.049	0.058
X 60	Glucose	0.054	0.062
13	Glucose	0.071	0.079
14	Glucose	0.062	0.045

SUMMARY

1. The anaerobic glycolysis of tissues of tadpoles between loss of yolk and metamorphosis was investigated.
2. As in the case of the chick embryo, tadpole autoglycolysis decreases in the course of development.
3. Tadpole tissue shows a preference for glucose; glycogen and hexosediphosphate are not broken down.
4. *dl*-Glyceraldehyde in a concentration $5 \times 10^{-3} M$ inhibits by about 90 % the glycolysis of tadpole tissue.
5. These facts suggest that glucose breakdown in the tadpole does not go through the phosphorylation cycle.
6. After addition of hexosediphosphate no triosephosphate accumulation in the tadpole was observed (in contrast to the chick embryo).

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