CLIV. INTERMEDIARY CARBOHYDRATE METABOLISM IN EMBRYONIC LIFE

I. GENERAL ASPECTS OF ANAEROBIC GLUCOLYSIS

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INTRODUCTION

THE intermediary processes of the carbohydrate metabolism of the young embryo attract the interest of the biochemist for two reasons, first because of the peculiar succession of sources of energy which seems to occur during embryonic development, and secondly because of the relationships recently discovered between carbohydrate metabolism and organiser phenomena.

A considerable amount of evidence was brought forward six years ago [Needham, 1931, 2, 986 ff.] in support of the view that the utilization of carbohydrate, protein and fat occurs in a definite sequence during embryonic development. This evidence was derived originally from studies on the hen's egg, but other classes of animals, such as the echinoderms and the Amphibia, proved to be quite in agreement with it. No purpose would be served by recapitulating these facts here, but it is not too much to say that every relevant investigation since that time has further strengthened the generalization. For example, high respiratory quotients, indicating carbohydrate metabolism, are associated with the earlier phases of development, while values of approximately 0.7, representing the combustion of fat, predominate in the later. Indeed, the consensus of agreement for widely differing organisms is surprising. Fukahori [1933] has confirmed the falling R.Q. of the intact hen's egg, and the manometric experiments of J. Needham [1932, 1] agreed with those of Dickens & Šimer [1931] in showing an R.Q. of unity for the chick embryo in isolation up to the age at which its size no longer permits of manometric work. In the case of the minnow, *Fundulus*, Amberson & Armstrong [1933] found an R.Q. falling from above 0.9 on the first day to somewhat above 0.7 for the greater part of the developmental

period, and for the latter part of the development of the trout, *Salmo*, Schlenk [1933] obtained figures between 0.65 and 0.72. Then Baldwin [1935] observed that the R.Q. of the eggs of the pond snail, *Limnaea*, is maintained at unity throughout their development, side by side with a synthesis of fat sufficient to mask any fall of the quotient. The eggs of the crab, *Carcinus*, studied by J. Needham [1933, 1], gave again a markedly falling R.Q., and the work of Nolf [1932] on the worm, *Trichuris*, also supports the rule of a succession of energy sources. Finally, and perhaps most remarkable of all, the succession has been demonstrated in an insect, the grasshopper, *Melanoplus*, by Boell [1935]. In the first few days of development the R.Q. falls from as high as 1.05 to 0.74, after which it keeps at 0.7.

Some attempt has been made to ascertain the causes of this association. It is out of the question to suppose that the carbohydrate supplies of the chick embryo are exhausted at the time when it begins an appreciable protein catabolism. By injecting glucose into the egg on the fourth or eighth day of development [Needham, 1926], it proved impossible to postpone the normal rise in uric acid production. Conversely, it was later shown [Needham, 1932, 2] that the aerobic action of fluoride on the chick embryo in the early stages is to abolish glucose utilization and to substitute for it protein catabolism ending in ammonia, the R.Q. remaining unchanged. The normal and almost exclusive use of carbohydrate, therefore, cannot be due to the lack of the requisite machinery for protein deamination and combustion. "Some as yet obscure necessity", it was said, "governs the succession of energy sources, something other than the required machinery, something other than the availability of the raw materials." But the possibility yet remained that of all the possible substrates, glucose was the easiest to deal with (deamination and desaturation being out of court), and hence arises the question whether the embryo, like the classical cases of muscle and yeast, phosphorylates its carbohydrate, or whether some even simpler mechanism takes the place in it of the complicated cycles now known to exist in phosphorylating tissues [Parnas, 1936; Parnas & Ostern, 1936; Needham, D. M., 1937]. This and the succeeding papers in this series will largely be devoted to the problem of phosphorylation in the embryo.

The second reason for the importance of the study of embryonic carbohydrate metabolism is its association with organizer phenomena. The pioneer work of Spemann and his collaborators [reviewed by Huxley & de Beer, 1934], which demonstrated the function of "organization centres" in embryonic development, particularly the primary organizer in the dorsal lip of the amphibian blastopore, paved the way for an analysis of the physico-chemical properties of the various embryologically distinguishable regions of the gastrula. The progress in the investigation of the nature of organizer substances may be found described in the reviews of Weiss [1935] and of Needham [1936]; here attention is mainly directed to the discovery of Fischer & Wehmeier [1933] that glycogen as ordinarily prepared possesses organizer properties, being capable of inducing a supernumerary neural tube, and to the subsequent finding of Waddington *et al.* [1935] that this is due to the adsorption on it of an ether-soluble substance or substances. Following on the suggestions of earlier workers, Heatley & Lindahl [1937] have since found that glycogen specifically decreases in amount in the dorsal lip of the blastopore, while its inducing properties have been further studied by Heatley *et al.* [1937]. All this leads to the supposition that the ether-soluble organizer substance is associated with glycogen and protein *in vivo* in the form of a complex. And for this reason rapid extensions in our knowledge of carbohydrate metabolism in early embryonic life are greatly to be desired,

since the very progress of normal morphogenesis itself is now seen to be intimately dependent on and linked with normal metabolic processes by way of the morphogenetic hormones called organizers.

METHODS

All the work described in this series of papers was carried out on chick embryos. Development took place in electrically regulated incubators under the general conditions prescribed by Murray [1925]. The eggs used were mainly White Leghorn and Rhode Island Red, from the pure-bred flocks of the University Farm, Messrs Chivers of Histon, Cambs, Messrs Jackson of Fowlmere, Cambs, and the Papworth Industries. A few experiments, however, were made with Rhode Island Red/White Wyandotte and Rhode Island Red/Light Sussex crosses.

Individual chemical methods applied for specific purposes will be mentioned in the appropriate places, but the manometric experiments, whether two-bulb Cremer cups or Dickens-Simer R.Q. cups [see Dixon, M., 1934] were used, took place in an electrically regulated water-bath maintained at 38°. When Warburg N₂/CO₂ gas mixture was employed, as for nearly all the anaerobic experiments, the removal of traces of oxygen was effected by passing the gas through a tube containing metallic copper, heated in an electric oven.

The choice of suitable Ringer-bicarbonate solution for use with embryonic avian tissue presented at first some uncertainty. The formula used by Benzinger & Krebs [1933] for their experiments on uric acid formation by avian tissue slices was tried but proved unsatisfactory, for a spontaneous evolution of gas takes place in the manometer, giving as much as 5 cm. excursion in an hour. Experiments in parallel on various modifications of the formula, together with the mammalian Ringer-bicarbonate of Krebs & Henseleit [1932] led to the conclusion that the higher K and Ca concentrations of the avian Ringer affected the bicarbonate dissociation constant so as to throw the solution out of equilibrium with the standard gas mixture. Since recent work on the composition of bird serum [Dyer & Roe, 1934; Morgan & Chichester, 1935] shows that the amount of potassium and calcium in avian serum is not so high as was previously thought, it was decided to adopt the usual mammalian Ringer solution.

In the present series of papers, nomenclature and symbols follow the usage of Dickens & Greville [1932] and of K. C. Dixon [1935].

AUTOLYCOLYSIS

When a chick embryo of 3 or 4 days' development is placed in Ringer-bicarbonate under manometric conditions, a gas output is observed which ceases or continues at an extremely low level after half an hour. Since this autolysis is very small in proportion to the glycolyses produced when certain substrates are present, we have not devoted much attention to investigating its nature. The most important question concerning it is whether or not it should be subtracted from the glycolysis values. K. C. Dixon [1935], comparing the respirations of rabbit brain in the presence and absence of lactate, noted that, although the autorespiration fell off greatly, the respiration remained relatively steady in each successive hour when lactate was present. He therefore concluded that the autorespiration was suppressed by lactate addition. Unfortunately the same calculation is not dependable in the present case, for whereas the initial autorespiration was 70% of the respiration with

substrate, our initial autoglycolyses were never more than 15–20 % of the glycolyses with substrate. It has therefore seemed safer to adhere to the practice of subtracting the autoglycolyses in all cases.

It is possible, of course, that the autoglycolysis may not be glycolysis at all, but acid production of some other kind. That its magnitude may be covered by the amounts of free and combined carbohydrate already present in the embryo, however, is easily shown. From the data given by Needham [1927] Table I has been drawn up:

Table I

Day of development	Carbohydrate per embryo (mg.)		μ l. if all were used		Dry wt. (mg.)	$Q_L^{N_2}$ if all used in $2\frac{1}{2}$ hr.
	Free	Total	Free	Total		
4	—	0.166	—	41	5	+ 3.3
5	0.017	0.330	3	82	15	+ 2.2
6	0.063	0.536	16	134	29	+ 1.85
7	0.100	0.838	25	218	—	—
8	0.210	1.310	52	325	—	—

Yet the average $Q_L^{N_2}$ value for autoglycolysis between the fourth and sixth days is only 1.17, and in general the gas output is only a small fraction of what it could be if all the carbohydrate, including that combined in proteins, were utilized.

On the other hand, it is observed that neither fluoride, bisulphite nor glyceraldehyde inhibits the autoglycolysis. This is shown in Table II.

Table II

A plus sign indicates $Q_L^{N_2}$, the remaining figures are μ l. from equal quantities of brei.

"Inhibitor"	Exp. no.	Day	Conditions	Autoglycolysis	Autoglycolysis in presence of "inhibitor"
Fluoride $M/50$	223	8	Brei	206	206
Bisulphite $M/20$	29	$3\frac{1}{4}$	Intact	+ 1.02	+ 6.65
	30	$3\frac{1}{2}$	Intact	+ 2.5	+ 5.5
<i>dl</i> -Glyceraldehyde 0.12 %	56	$2\frac{3}{4}$	Intact	+ 4.91	+ 5.41
	57	$2\frac{1}{2}$	Intact	+ 9.7	+ 11.5
	58	3	Intact	+ 6.83	+ 8.13
	248	8	Brei	62	130

Furthermore, as will be shown in a subsequent paper in the series, the relative amounts of pyruvic acid formed are quite different in the presence and absence of glycolysable substrate.

One of the most evident characteristics of the autoglycolysis is a tendency to fall with the age of the embryo. In this it resembles the glucolysis of glucose. The fall is probably but an index of enzymic activity in general as related to dry weight. It is illustrated in Fig. 1.

The position of *dl*-glyceraldehyde in Table II above is somewhat different from that of the other inhibitors, since it could itself be a substrate for fermentation. Hence we can only speak of an increase of autoglycolysis with much reserve; the greater acid production may come from the glyceraldehyde itself. In a later communication [Needham & Lehmann, 1937] the question of glyceraldehyde disappearance will be taken up.

Fig. 1. Decline of rates of autoglycolysis, glycolysis and mannolysis with age of the chick embryo (autoglycolysis not subtracted). ○ glucose; • mannose; × autoglycolysis.

GLYCOLYSIS OF VARIOUS SUBSTRATES

The extent of anaerobic glycolysis with a variety of substrates is shown in Table III. Among the monosaccharides it is at once clear that only mannose is used comparably with glucose. Although at first galactose appeared to be used, this was shown to be due to the presence of traces of glucose as impurity, since galactose purified according to the method of Stephenson & Yudkin [1936] by previous fermentation with *Saccharomyces cerevisiae* gave values no higher than the autoglycolysis. Glucosamine also remained quite unutilized.

The decline of glycolytic power with age was strikingly exhibited. As far as glucose is concerned this only confirms the earlier work of Kumanomidō [1928] and the concurrent work of Hanaoka [1936], but it was interesting to find that for mannose also exactly the same relation held good, suggesting that the same enzyme system is at work. The curves are shown in Fig. 1.

The mannolysis of the chick embryo is of significance in view of the isolation by Levene & Mori [1929] of a trisaccharide from egg white, containing glucosamine and mannose. Coagulated egg white contains 1.9% of the substance, ovalbumin 0.26% and ovomucoid 5.1%. It was also obtained from yolk. According to Levene & Rothen [1929] the prosthetic group of the molecule

Table III

Substrate	Exp. nos.	Day of develop- ment	Average $Q_L^{N_2}$
Glucose	1, 2, 21-25, 27, 28, 31-33, 35, 37-39, 40-42, 57, 94	3-4 4-5	16.75 12.5
Glucosemonophosphate	54	4-5	0.0
Mannose	5, 6, 40-42, 58, 60, 94	3-4 4-5 5-6	17.98 7.37 6.69
Mannosemonophosphate	43	4-5	0.0
Hexosediphosphate	3, 4, 23, 33	4-5	0.18
Hexosemonophosphate	3, 4, 21, 31, 32, 35	4-5	0.52
Glycogen	1, 2, 22, 23, 24, 25, 27, 28	3-4 4-5	0.28 0.17
Glucosamine	44-46	4-5	0.54
Fructose	5, 6	4-5	0.68
Galactose	6a	4-5	2.38
	8 (purified)	4-5	0.07
Sorbose	51, 52, 94	4-5	0.09
Arabinose	47-49	3-5	0.0
Xylose	17	4-5	0.0
Maltose	7, 8	4-5	0.28
Lactose	7, 8	4-5	0.01
Trehalosemonophosphate	50	4-5	0.04
Raffinose	17	4-5	0.0
Autoglycolysis	—	4-5	0.65

All carbohydrates used at a concentration of 0.25 %, i.e. 5 mg. per cup. Autoglycolysis always subtracted.

consists of four trisaccharides each containing one molecule of glucosamine and two of mannose. The presence of mannose in egg albumin had first been shown by Frankel & Jellinek [1927], and after the work of Levene and his collaborators the subject was further pursued by Sørensen [1934], who found amounts of mannose and galactose varying from 1.7 to 14.9 % in the various proteins of egg white. Onoe [1936] explained the findings of Levene on yolk by isolating a vitellomucoid, the carbohydrate moiety of which contained glucosamine and mannose in the same proportions as Levene's trisaccharide.

Closer examination was made of the egg protein trisaccharide and other related compounds at a later stage, as will be referred to in its place.

Continuing the description of Table III, it will be seen that fructose is untouched. So also is the parent pentose of glucose and mannose, arabinose and the monosaccharide sorbose, which, like galactose, belongs to a stereoisomerically more distant group of sugars. Correspondingly, xylose was, as expected, as inert as arabinose. The disaccharides maltose and lactose, and the trisaccharide raffinose, were all unattacked.

Somewhat contrary to expectation, however, was the absolute failure of the embryo to glycolyse glycogen or any of the phosphorylated hexoses. The hexosediphosphate in the table is 1:6-diphosphofructofuranose (Harden & Young); the hexosemonophosphate obtained commercially was probably a mixture of 6-phosphofructofuranose (Neuberg) and 6-phosphoglucopyranose (Robison) together with some 15 % of mannosemonophosphate and minor amounts of two other sugars. We also had at our disposal, however, pure specimens of glucosemonophosphate and mannosemonophosphate [Robison, 1932; Jephcott &

Robison, 1934]. Trehalosemonophosphate [Robison & Morgan, 1928] was also tested, with negative results.

This is the place to obviate certain possible criticisms. In the first place, embryos placed in manometers in Ringer solution with no bicarbonate give rise to practically no pressure change over a couple of hours (Exp. 148), thus showing that the formation of a fixed acid is taking place in experiments conducted normally. That the acid produced is lactic acid was shown by the following experiment (156):

Groups of manometers were set up as usual and after varying times removed from the bath and the lactic acid then estimated according to Friedemann *et al.* [1927]. The observed acid production and the estimated lactic acid differ by an amount roughly equal in each case to the glycolysis which had been proceeding during the preparation and equilibration of the cups in the bath (5.2 mg. calculated from the $\frac{1}{2}$ hr. reading).

Time after beginning readings	Av. Q_L^N	Total μ l.	Calculated mg.	Plus un-registered glycolysis mg.	Estimated mg.
$\frac{1}{2}$ hr.	+ 8.85	1396	5.61	10.8	10.8
1 hr.	+ 7.12	2373	9.54	14.74	14.57
2 hr. 20 min.	+ 4.84	2467	9.95	13.85	12.04

That the negative results obtained with many substrates could not be due to rapid consumption before the beginning of the readings, was shown by special experiments in which the substrates were tipped in from side-bulbs after gaseous equilibrium had been attained (Exps. 161, 168 and 170-175).

It has often been suggested that a failure to glycolyse phosphorylated hexoses and glycogen may be due rather to an impermeability of the cells to these substances than to any metabolic peculiarity. In a later paper in this series [Needham & Lehmann, 1937] we shall bring forward strong evidence to show that the phosphorylated hexoses do penetrate the embryonic cells. For polysaccharides the contention is, indeed, more plausible, but even here, there are experiments by Haarman [1932, 2] on minced mammalian tissues which show that glycogen is split by all these to hexose, irrespective of the extent of lactic acid formation, and hence must have entered the cells. Finally, we have to reckon with the base neutralizing the phosphorylated hexoses, but although, as Meyerhof [1927] and Meyerhof & Suranyi [1926] have shown, the lactic acid formed will not fully reveal itself as gas evolution in the manometer, their "Veresterungskorrektur" will not raise the data of Table III above what is negligible.

We therefore conclude that the distribution of availability of the various substrates anaerobically is as shown in Table III. The chick embryo during the first week of its development thus joins that class of tissues, of which tumour and mammalian brain are the most classical examples, which strongly attack glucose but leave glycogen and the phosphorylated hexoses untouched. The first reviews of these differences between tissues were those of Bumm & Fehrenbach [1930; 1931], but since that time many further facts have accumulated. Table V gives a fair picture of them. Tissues are divided by Haarman [1932, 1, 3] as follows: skeletal muscle gives, of course, a great lactic acid production from glycogen and hexosediphosphate and little from glucose; the converse is the case for smooth muscle, brain, lung, thyroid and suprarenal gland. Liver, kidney, pancreas, spleen and heart muscle [see also Cruickshank, 1936] appear to glycolyse hexoses, phosphorylated hexoses and polysaccharides all equally.

Attention may be drawn to the observation of Mündelein [1932], shown in Table IV, that white and red muscle differ in their capacity for glycolysis.

Table IV. *Relative glycolytic rates; the highest in each case being taken as 100. The table does not include experiments on extracts*

	Glucose	Man- nose	Fructose	Galactose	Glycogen	Hexose- diphos- phate	Hexose- mono- phosphate	Maltose
Chick embryo:								
Present work (intact and brei)	100	100	0	0	0	0	0	0
Dickens & Greville [1932; 1933] (intact)	100	—	0	—	—	—	—	—
Tumour:								
Warburg <i>et al.</i> [1924] (slices)	100	—	12	4	—	—	—	—
Harrison & Mellanby [1930] (slices)	100	—	52	—	—	5	2	—
Dickens & Greville [1933] (slices)	100	—	34	—	—	—	—	—
Barr <i>et al.</i> [1928] (slices)	100	—	—	—	6	—	—	—
Rosenthal [1930] (slices)	100	—	—	—	—	0	—	—
Tsuzuki [1936] (slices)	100	—	—	—	—	38	—	—
Brain:								
Loebel [1925] (slices)	100	—	14	14	7	—	—	14
Ashford [1933] (brei)	90	100	7	16	7	37	21	—
Tanko [1931] (brei)	100	—	—	—	0	—	—	—
Haarmann [1932, 1] (brei)	100	—	—	—	5	15	—	—
Dickens & Greville [1933] (slices)	100	—	5	—	—	—	—	—
Geiger [1935] (brei)	100	—	—	—	6	—	—	—
Muscle:								
Laquer & Meyer [1923] (brei)	53	46	68	1	100	100	—	—
Haarmann [1932, 1] (brei)	2	—	—	—	100	70	—	—
Mündelein [1932] (brei):								
Avian white breast	0	—	18	—	100	73	—	—
Avian red leg	19	—	5	—	65	100	—	—
Geiger [1935] (brei)	9	—	—	—	100	—	—	—
Erythrocytes:								
Irving [1926] (intact)	100	100	100	20	—	—	—	—

In this connexion Schönfelder's work [1935] is important. Schönfelder, reviewing the relevant physiological literature, shows that foetal skeletal muscle, the muscles of very young animals just after birth and denervated adult muscle have several common properties. Thus contractures are given with nicotine, coniine etc., a special type of tension curve is seen, and recovery is very slow. No histological peculiarities exist, but the muscles are characterized clearly by the difference in their utilization of substrates; thus muscle of the foetal type glycolyses glucose not glycogen, as opposed to ordinary skeletal muscle. Schönfelder studied the onset of this change, obtaining the following average figures:

Relative glycolytic rate (convention as in Table IV)

	Glucose	Glycogen
Muscle of rabbit at birth	100	18
Muscle of rabbit at 14 days	39	100
Muscle of rabbit at 36 days	4	100
Adult rabbit muscle	6	100
Adult rabbit muscle denervated	100	3

It seems, therefore, that mammalian embryos in general and mammalian embryo muscle in particular might be expected to show the same substrate preferences as are here found in the case of the chick embryo.

With Schönfelder's results may be compared those of Koschtojan & Rjabinowskaja [1935], who showed the phosphagen content of foetal mammalian muscle to be exceedingly low or even non-existent.

The question then arises as to when phosphorylating breakdown is installed in the development of the chick. Although this may form the subject of a future communication in this series, the results of experiments with older embryos may be mentioned at this point. A brei (made under ice-cold conditions) of thirteenth day embryos proved incapable of using glycogen in anaerobic glycolysis (Exp. 181); μ l. from 2.0 ml. brei in 3 hr.: autoglycolysis 189; glycogen 213; glucose 501. Again, slices were made from the leg muscle of fifteenth day embryos (Exp. 167). The Q_L^N values were: for autoglycolysis +0.05, for glucose +1.51, for glycogen +0.05 and for hexosediphosphate +0.07. This shows that even the muscular tissue glycolysis at a late stage in development partakes of the peculiarities noted in the embryo as a whole very early in development. Something similar is to be seen in the experiments of Sherman & Elvehjem [1936] on the muscles of well-grown polyneuritic chicks. Here 275 mg. lactic acid/100 g. wet wt./hr. were produced with glucose as substrate and 340–480 mg./100 g. wet wt./hr. with glycogen as substrate. The persistence of the glucose mechanism in birds must therefore be considerable, but it is true that mammalian muscle also, as Table IV shows [Laquer & Meyer, 1923], may glucolyse fairly well.

It might be suggested that the apparently non-phosphorylating character of embryo glycolysis was due to the relatively large size of the brain. From Schmalhausen's work [1926] we know that, at the fourth day of development, the brain attains its maximum relative size, 30 % of the total body weight. The point was easily tested, however, by separating the head region from the trunk and limb-buds and observing the glycolysis of these separately. The results (Table V) showed that the special type of glycolysis is found over all the body at this stage and is not confined to that part containing the large nervous system.

Table V. Q_L^N

Exp.	Autoglycolysis			Glucose		Glycogen		Hexose-diphosphate		Hexosemono-phosphate	
	Whole	Head	Trunk	Head	Trunk	Head	Trunk	Head	Trunk	Head	Trunk
9	+0.88	+0.55	+1.45	+ 7.75	+11.4	+0.07	+0.00	—	—	—	—
10	+0.69	+0.25	+2.9	+13.35	+12.4	+1.00	+0.00	—	—	—	—
11	+0.67	+1.81	+2.84	—	—	—	—	+0.0	+0.0	+0.0	+0.0

DESTRUCTION OF STRUCTURE AND GLYCOLYSIS

Still feeling that the differences between substrates reported earlier in this paper might perhaps be due to differences of permeability, we thought it necessary to check them against varying degrees of destruction of the cell structure. We began with making a brei by crushing the delicate fourth day embryos with a small pestle in a flat Syracuse watch-glass without sand. The results, which later were many times confirmed in the course of other experiments, showed that the differences persisted even so.

Thus while glucolysis and mannolysis were almost unaffected by this treatment, no sign of an action upon the other substrates manifested itself.

Table VI. $Q_L^{N_2}$

Exp.	Age	Condition	Nil	Glucose	Mannose	Glycogen	Hexosediphosphate	Hexose-monophosphate
61	4½	Brei	+2.49	+17.51	—	+0.0	—	—
62	4½	Intact	+0.99	—	—	—	—	—
		Brei	+1.29	+8.61	—	—	+0.03	+0.0
63	4½	Brei	+2.71	+5.94	+5.62	+0.22	—	—

The extent of cellular disruption must have been very considerable, because the brei was always diluted with distilled water before being placed in the manometer cups, to which extra bicarbonate was added making up the correct concentration. Microscopic control supported this opinion.

We then proceeded to the preparation of extracts, following the method of Scharles & Salter [1934] which proved successful for tumour tissue. Embryos on being removed from the eggs were dropped into an ice-cold mortar and then ground with a small quantity of sand in an equivalent amount of distilled water. The extracts were centrifuged for 15 min. before use. But by this treatment all activity had been destroyed. The acid production, whether in the absence or presence of substrates, was negligible and did not permit of a decision as to whether all were now equally used. Quotients, calculated on the basis of the weight of embryo used to make the extract, were always far below unity (Exps. 64–70).

In view of the success obtained by Geiger [1935] in reactivating dialysed extracts of mammalian brain, liver and heart muscle, we added adenylypyrophosphate and glutathione to these extracts in the hope of activating them. But it proved impossible to do so, never more than a few μ l. gas output from extra acid formation being obtained (Exps. 71, 73–75).

Dialysis experiments will be described in a succeeding paper [Needham & Lehmann, 1937].

THE POSSIBILITY OF OXIDATION WITHOUT GLYCOLYSIS

Loebel [1925] and Dickens & Greville [1933] have definitely shown that brain tissue, though unable to convert fructose into lactic acid, can nevertheless oxidize it almost as readily as glucose and with a R.Q. of unity. So also Haarmann & Stratmann [1932] found that the oxidation of xylose can be effected by muscle, though no lactic acid is formed from it. We therefore thought it of interest to investigate a few of the substrates previously mentioned from this point of view. Unfortunately, the chick embryo is a particularly difficult object for this purpose, since, as has several times been shown [Needham, 1933, 2; Dickens & Greville, 1933], the chick embryo's respiration, in the absence of glucose, is still some 85 % of what it is when glucose is present. The following experiments were carried out by the indirect method of Warburg in cubical cups, and, as Table VII shows, very little difference between the glycolysable and non-glycolysable substrates was observed.

Since for the first half-hour of Exp. 84 the autorepiration was -11.3 and the average of all three substrate respirations was -10.8 , the possibility of distinguishing in this way between sugars oxidized and sugars not oxidized was not hopeful. On the other hand, it was noted that the carbohydrates known beforehand to give lactic acid anaerobically gave in these experiments slight aerobic glycolyses, while sorbose and glucosamine did not. In the original work

Table VII

Exp.	Auto-respiration	Glucose	Mannose	Sorbose	Glucos-amine	
84 1st $\frac{1}{2}$ hr.	Q_{O_2}	- 11.3	- 12.7	- 13.2	- 6.4	—
	Q_{SCO_2}	+ 9.1	+ 16.4	+ 14.0	+ 4.1	—
	$Q_L^{O_2}$	- 2.2	+ 3.7	+ 0.8	- 2.3	—
2nd hr.	Q_{O_2}	- 8.5	- 10.8	- 9.7	- 8.5	—
	Q_{SCO_2}	+ 6.5	+ 13.4	+ 10.5	+ 6.3	—
	$Q_L^{O_2}$	- 2.0	+ 2.6	+ 0.8	- 2.2	—
3rd hr.	Q_{O_2}	- 7.4	- 9.1	- 10.3	- 9.1	—
	Q_{SCO_2}	+ 6.1	+ 12.2	+ 11.8	+ 7.4	—
	$Q_L^{O_2}$	- 1.3	+ 3.1	+ 1.5	- 2.3	—
85 1st hr.	Q_{O_2}	- 9.1	- 9.9	—	—	- 10.7
	Q_{SCO_2}	+ 6.9	+ 13.3	—	—	+ 8.2
	$Q_L^{O_2}$	- 2.2	+ 3.4	—	—	- 2.5

of Warburg *et al.* [1924] the chick embryo gave aerobic glycolyses with glucose of the order of +1.1.

An attempt was later made to exhaust the respiratory reserves of the embryo by incubating eight $3\frac{1}{2}$ -day embryos for $6\frac{1}{2}$ hr. in Ringer-bicarbonate with O_2/CO_2 gas mixture, but after this time it was found that both glycolysis and respiration were reduced to zero. It is doubtful whether it would be possible to deplete the embryo's reserves before serious decay of the enzyme systems has set in.

One respiratory quotient experiment was made in absence of substrate (117). The Dickens-Simer bicarbonate method was used.

	Q_{O_2}	$Q_L^{O_2}$	R.Q.
Exp. 117 Nil	-7.6	+0.5	0.745
Nil	-6.45	-0.48	0.76
Exp. 105 Glucose	-6.35	+1.6	1.03

The contrast here confirms the finding of Dickens & Greville [1933] that, when embryonic tissues are deprived of substrate, the R.Q. falls much more markedly than the oxygen consumption.

SUMMATION AND COMPETITION OF SUBSTRATES

One of the simplest ways of elucidating the possible presence of two ways of carbohydrate breakdown in tissues, phosphorylating and non-phosphorylating, has been to add both substrates together, e.g. glucose and glycogen, with the object of finding whether a summation of lactic acid production occurs. Thus Bumm & Fehrenbach [1931] obtained figures for the summation of glucose and glycogen breakdown in white muscle, indicating that both paths were present, and similar results were obtained for mammalian brain by Ashford [1933] who tested glycogen, hexosediphosphate and hexosemonophosphate in each case with glucose.

Our results are summarized in Table VIII. In the first place it will be seen that when mannose and glucose are being attacked together the glycolytic quotient is no higher than when each is present separately, i.e. no summation occurs. This result is exactly analogous to that of Ashford [1933] on brain, and it strongly supports the belief that the same enzyme system is at work in mannosylolysis and glucolysis. We have already seen (Fig. 1) that the decline of glycolytic intensity with embryo age is the same for these two sugars.

Table VIII. *Summation and competition experiments (intact embryos), $Q_L^{N_2}$*

All carbohydrates used at a concentration of 0.2% in the Ringer-bicarbonate

Exp.	Age	Auto-glycolysis	Glucose	Other substrate	Other substrate plus glucose	
					Observed sum	Calculated sum
Mannose						
40	3½	+ 1.92	+ 14.78	+ 17.98	+ 16.98	+ 32.76
41	3½	+ 5.0	+ 20.6	+ 15.6	+ 20.6	+ 36.2
42	4	+ 2.05	+ 16.35	+ 16.4	+ 18.15	+ 32.75
Glucosamine						
44	4½	+ 0.95	+ 12.5	+ 0.54	+ 10.85	+ 13.04
Arabinose						
47, 48	4	+ 4.09	+ 13.71	+ 0.0	+ 19.21	+ 13.71
Sorbose						
51	3½	+ 2.07	+ 26.2	+ 1.42	+ 22.5	+ 27.62
52	4½	+ 1.36	+ 11.75	+ 0.09	+ 10.28	+ 11.84
Trehalosemonophosphate						
50	4	+ 2.62	+ 17.1	+ 0.04	+ 15.67	+ 17.14
Glucosemonophosphate						
54	2½	+ 11.9	+ 14.1	+ 5.1	+ 21.35	+ 19.2
Hexosemonophosphate						
31	2½	+ 4.3	+ 19.5	+ 0.0	+ 17.9	+ 19.5
32	2½	+ 4.3	+ 18.7	+ 0.0	+ 18.3	+ 18.7
21	5	+ 0.71	+ 14.39	+ 0.45	+ 9.39	+ 14.84
35	5	+ 1.79	+ 10.21	+ 0.3	+ 9.81	+ 10.51
Hexosediphosphate						
34	2½	+ 8.65	+ 25.6	+ 0.0	+ 28.3	+ 25.6
23	4	+ 4.9	+ 14.5	+ 0.0	+ 14.6	+ 14.5
Glycogen						
27	2½	+ 1.46	+ 39.44	+ 0.0	+ 24.84	+ 39.44
28	2½	+ 1.18	+ 25.72	+ 0.0	+ 25.52	+ 25.72
27	3	+ 2.92	+ 26.58	+ 1.2	+ 19.38	+ 27.78
22	3½	+ 4.57	+ 15.48	+ 0.63	+ 21.23	+ 16.11
25	3½	+ 4.06	+ 18.44	+ 0.0	+ 18.64	+ 18.44
23	4	+ 4.9	+ 14.5	+ 0.1	+ 13.7	+ 14.6
24	4½	+ 0.95	+ 6.15	+ 0.25	+ 8.75	+ 6.4
25	4½	+ 1.42	+ 13.38	+ 0.35	+ 12.98	+ 13.73
22	3½	+ 4.6	+ 15.45	+ 3.75	+ 21.23	+ 19.2
28	3	+ 2.48	+ 25.72	+ 3.04	+ 28.32	+ 28.76

For glucosamine, arabinose, sorbose, trehalosemonophosphate, hexosemonophosphate and hexosediphosphate, which are not themselves glycolysed to any appreciable extent by the embryo, there can be no question of summation, but the figures show that their presence has no considerable or regular effect on glucolysis.

The same conclusion is to be drawn from the experiments on glycogen, which, for the most part, show that the breakdown of glucose is unaffected by the presence of glycogen (average value obtained +18.2, expected +20.2). But in two cases, placed by themselves at the bottom of the table, a small utilization of glycogen was observed, and in both of these an apparent summation was seen. The same result was found with a sample of pure glucosemonophosphate, given earlier in the table. We shall later describe experiments [Needham & Lehmann, 1937] which show that, although in general the phosphorylation path of carbohydrate breakdown is not practicable in early embryonic life, it may

occasionally function. If the exceptional cases noted here are to be interpreted as summations, we have a further indication that the paths of breakdown of glucose and glycogen are different.

GLUCOPROTEINS AND GLYCOLYSIS

Mention has already been made of the trisaccharide isolated by Levene & Mori [1929] from egg proteins. Since each egg must contain at the very least 1 g. of this substance, it seemed of interest to follow the action of the embryo upon it. A sample was prepared by a modification of the original procedure:

6.75 kg. of mixed yolk and white proteins were obtained from a sufficient quantity of yolk and white by coagulation with 10 l. of acetone and dried in a current of air. The mass was then heated with 25 l. of water and 3.5 kg. of baryta in a bath in which a steam coil was immersed. After boiling for some time, the temperature was allowed to remain constant at 75° for 16 hr. Excess baryta was removed by a stream of gaseous carbon dioxide and the supernatant fluid piped off continuously into a vacuum still at 40°. The remainder was filtered by suction and the combined distillate and filtrates were precipitated with 25% normal lead acetate. The filtrate was treated with H₂S, and after removal of the sulphide was concentrated *in vacuo*. A precipitate was then obtained by adding alternately baryta and hot saturated basic lead acetate, avoiding excess. After cooling at 0° for some days, the precipitate was suspended in warm water and decomposed with carbon dioxide, and the basic lead acetate process was twice repeated. The final filtrate from barium and lead was precipitated with 10% mercuric sulphate and the mercury removed with H₂S. After concentration to a syrup the filtrate was poured into methyl alcohol, giving a voluminous though light precipitate of the trisaccharide. The alcohol was removed in a desiccator over paraffin oil and the product further dried in air at 37°. About 200 mg. were obtained; a solution of the same strength as all other carbohydrates used was made (2.5%). Further purification was effected by reprecipitating with methyl alcohol and washing with ether in the centrifuge.

This preparation is referred to in Table IX as "L & M I"; a second sample, sent from the Rockefeller Institute, is designated "L & M II". A saccharide of similar nature was prepared from the serum proteins of ox blood by Rimington [1931], and two samples of this, already on hand in this laboratory, are termed "Rim I" and "Rim II" in the table; the former contained 2.45% N, the latter 5.29% N. In addition to these, four proteins were tried, lettered A, B, C, D, in the table.

A. A "seromucoid" fraction (119 F Pirie), prepared by full saturation with ammonium sulphate from horse serum after preliminary removal of the serum albumin. This fraction contained 4.5% total sugar by the orcinol method (excluding glucosamine) and 13.5% N.

B. Horse serum albumin, from neutral serum half-saturated with ammonium sulphate (117 Q Pirie). This fraction contained 2.3% total sugar by the orcinol method (excluding glucosamine), and 13.8% N.

C. Ovomucoid from fresh hen's eggs (Pirie).

D. Ovomucoid from 12th day of incubation [Needham, 1927].

The proteins were used in sufficient concentration to bring their known carbohydrate contents to an equivalency with that of all other carbohydrates tested.

As Table IX shows, none of the saccharides or proteins tested was available for glycolysis by the embryo, even when incubated with embryo tissue for an hour or more before the beginning of the experiment. Availability of their hydrolysis products was therefore examined. To 2.5% solutions of the saccharides enough HCl was added to bring the acid concentration to 4%, and hydrolysis was carried on in boiling-tubes with long air condensers in a boiling water-bath

Table IX. *Glycolysis of protein preparations, Q_L^N*

Exp.	Age	Auto-glycolysis	L & M I	L & M II	Rim I	Rim II	A	B	C	D	Mannose
90-96	4½-5½	+0.68	+0.04	—	+0.06	+0.18	—	—	—	—	+17.25
136-137	4½-5½	+0.83	—	+0.23	—	—	—	—	—	—	+15.6
99-100, 102, 138-140	5-6	+0.62	—	—	—	—	+0.10	+0.00	+0.40	+0.15	+9.4
After acid hydrolysis:											
97	5½	+0.34	+2.02	—	—	—	—	—	—	—	+11.9
136-137	4½-5½	+0.83	—	+4.05	—	—	—	—	—	—	—
138-140	5½	+0.5	—	—	—	—	+0.00	+0.00	+0.19	+0.24	+8.8

for 4 hr. As will be seen from Table IX, the products of the Levene-Mori trisaccharide were used to a considerable extent. No doubt the browning and destruction of carbohydrate during the hydrolysis account for the fact that two-thirds of the full value was not closely approached. On the other hand, the glucoproteins gave nothing utilizable after hydrolysis; a rather surprising result, since it has usually been assumed that the carbohydrate of ovomucoid is quite available for combustion to the embryo. According to Sørensen [1934], ovomucoid contains 9.2 % of a mixture of 3 parts of mannose and 1 part of galactose. Seromucoid, too, according to Sørensen & Haugaard [1933], contains mannose and galactose, to which Ozaki [1936] adds glucosamine. Perhaps the right conditions of hydrolysis were not found; an alkaline hydrolysis may be essential.

INHIBITION OF GLYCOLYSIS BY GLYCERALDEHYDE

The important discovery was made by Mendel [1929] that *dl*-glyceraldehyde possesses the property of completely inhibiting the anaerobic breakdown of glucose by tumour tissue even in very small concentrations. At *M*/1000 the inhibition is already nearly complete. Later, Mendel *et al.* [1931] found that the inhibition can be reversed by pyruvic acid, again in low concentrations. At *M*/1000 the reversal is complete, but the effect is perceptible as low as *M*/100,000. Ashford [1933] verified the finding of Mendel on brain, obtaining a 91 % inhibition. Conversely Holmes [1934] found that glyceraldehyde was without effect on lactic acid production from starch or glucose plus "hexokinase" ("phosphatase") in muscle extract. Since glyceraldehyde thus inhibits the glucose system but not the glycogen-hexosephosphate system, it was very inviting to try its effect upon the chick embryo. As will be seen from Table X it causes

Table X. *Inhibitions by dl-glyceraldehyde, Q_L^N*

Exp.	Age	Auto-glycolysis	Auto-glycolysis plus glyceraldehyde	% increase	Glucose	Glucose plus glyceraldehyde	% inhibition	Mannose	Mannose plus glyceraldehyde	% inhibition
55	2½	+4.6	—	—	+32.4	+1.35	96	—	—	—
55	3½	+2.68	—	—	+17.42	+0.67	96	—	—	—
56	3	+4.91	+5.41	10	—	—	—	—	—	—
57	2½	+9.7	+11.5	19	+30.49	+7.65	75	—	—	—
58	3	+6.83	+8.13	19	+19.07	+0.0	100	+18.07	+0.26	98
60	4½	+1.27	+2.03	59	+15.73	+0.77	95	+13.33	+1.28	90

inhibitions of roughly 90 % in all cases both for glycolysis and mannolysis, while its effect upon the autoglycolysis is slightly acceleratory. This is in good

agreement with the conception of embryonic glycolysis as wholly non-phosphorylating.

In these experiments concentrations somewhat higher than those of Mendel were used, $M/100$ or $M/200$. However, the inhibition was also strongly marked when $M/800$ was employed (Exp. 216). But after the first hour the inhibition passed off (see Fig. 2).

Fig. 2. Inhibition of chick embryo glycolysis by *dl*-glyceraldehyde.

	% inhibition	
	1st hr.	1st and 2nd hr.
Glyceraldehyde $M/100$	—	73
Glyceraldehyde $M/800$	41	5

Later in this series, evidence will be adduced to show [Needham *et al.* 1937] that pyruvic acid appears during the course of anaerobic glycolysis in considerable amounts, so there could be no doubt but that the temporary character of inhibition with weak glyceraldehyde was due to reversal by pyruvic acid. Another experiment indicates directly, moreover, that pyruvic acid reverses the inhibition.

Exp. 205

	μ l. from 1.8 ml. of 7th day embryo brei	% inhibition
Brei alone	289	—
Brei plus glyceraldehyde $M/100$	278	—
Brei plus glucose $M/50$	1158	—
Brei plus glucose plus glyceraldehyde	265	100
Brei plus glucose plus glyceraldehyde plus sodium pyruvate $M/20$	355	85

The restoration is probably more complete at the weak concentrations. In other experiments (237, 239) inhibition was 100 % without pyruvate, but only 45 % with it.

The glyceraldehyde effect will provide a particularly valuable opportunity of analysing the course of non-phosphorylating glucose breakdown. In preliminary experiments, using the orcinol method for total carbohydrate, the Hagedorn-Jensen method for reducing sugar, with and without copper-lime precipitation, and the Ariyama method for methylglyoxal; combining these in various ways, we obtained strong indications that the major part, at least, of the glucose, remains untouched in the presence of *dl*-glyceraldehyde. We hope to return to this point in a later communication.

Since all aldehydes, quite unspecifically, react with triosephosphate [Meyerhof *et al.* 1936], and since glyceraldehyde itself, in its phosphorylated form, is an intermediate in phosphorylating glycolysis, it is of some importance to know whether its effect upon glucose breakdown is shared with other aldehydes, or whether it is specific. The following experiment (221) shows that it is specific.

	2.0 ml. 8½-day embryo brei; glycolysis for 105 min.; all aldehydes <i>M</i> /100 μl.
Glucose	815
Glucose + glyceraldehyde	217
Glucose + acetaldehyde	755
Glucose + propaldehyde	920
Glucose + <i>n</i> -butylaldehyde	888
Glucose + benzaldehyde	730

We shall show later [Needham & Lehmann, 1937] that pyruvicaldehyde exerts no inhibitory effect.

Glyceraldehyde inhibition cannot be reversed by glutathione (Exps. 238, 239, 240) even when large amounts (20 mg./ml. brei) are used.

GLYOXALASE AND GLUTATHIONE

In the investigation of any case of apparent non-phosphorylating glucose breakdown, the classical contention of Neuberg [1925], that methylglyoxal is an intermediate in such breakdown, cannot be neglected. Neuberg *et al.* [1930], indeed, were able to isolate methylglyoxal when acetone powders of 8-day chick embryos were incubated with hexosediphosphate for 48 hr. This observation has not much significance, for we know to-day that the complete breakdown of hexosediphosphate does not go on via methylglyoxal. The methylglyoxal formed could arise from dephosphorylated triosephosphate.

We first examined the chick embryos for the presence of active methylglyoxalase, with positive results.

Table XI. Q_L^N

	Exp. 150 intact 5½-day	Exp. 151 brei 6-day
Nil	+ 0.59	—
+ MG <i>M</i> /85	+ 7.26	—
+ MG <i>M</i> /70	+ 4.84	+ 4.80
+ MG <i>M</i> /60	+ 3.11	—
+ MG <i>M</i> /45	+ 0.0	—
+ MG <i>M</i> /70 + 0.1 ml. GSH	—	+ 3.70
+ 0.2 ml. GSH	+ 6.16	+ 2.40
+ 0.3 ml. GSH	+ 5.81	—
+ MG <i>M</i> /60 + 0.3 ml. GSH	+ 0.98	—
Glucose <i>M</i> /80	+ 13.3	+ 11.2

N.B. Glutathione solution (GSH) 0.375%; mg. = methylglyoxal.

This also shows that too strong a concentration of methylglyoxal poisons the systems involved, and that neither on the intact embryos nor on the brei was any activating effect of glutathione noticeable, no doubt because sufficient was already contained in the tissues. We shall return to this subject later [Needham & Lehmann, 1937] in relation to work with dialysed embryo enzymes. We shall also show [Needham *et al.*, 1937] that very little methylglyoxal accumulates during glycolysis under whatever conditions.

That glyceraldehyde does not inhibit the action of methylglyoxalase was found in special experiments.

Exp. 204		μ l.
A dialysed brei of 6-day embryos		in $2\frac{1}{4}$ hr.
2 ml. brei + 5 mg. MG + 5.5 mg. GSH		360
2 ml. brei + 5 mg. MG + 5.5 mg. GSH in <i>M</i> /100 glyceraldehyde		408
Exp. 205		μ l.
A fresh brei of 7-day embryos		in 3 hr.
1.8 ml. brei + 5 mg. MG		740
1.8 ml. brei + 5 mg. MG in <i>M</i> /100 glyceraldehyde		660

This is in agreement with the unpublished experiment of Holmes [cit. in Ashford, 1933], that glyceraldehyde does not affect the methylglyoxalase of brain.

The above experiments with glutathione were extended to glycolysis itself, in order to test the possibility that, if glutathione was a necessary coenzyme, there might not be enough normally present in the embryos. This, however, turned out not to be the case; it was impossible to increase the glucolytic rate of intact embryos above normal by adding glutathione.

Exp. 154

		Glutathione 0.375 %		
Added to cup	...	0.05 ml.	0.1 ml.	0.2 ml.
Nil	+ 0.33	—	—	—
Glucose <i>M</i> /145	+ 12.02	—	—	—
Glucose <i>M</i> /115	+ 11.77	+ 9.97	+ 10.12	+ 14.57
Glucose <i>M</i> /90	+ 13.27	+ 14.17	+ 12.17	+ 13.22

SUMMARY

1. This paper constitutes the first of a series reporting the results of a systematic investigation into the nature of the anaerobic glycolytic mechanism in early embryonic life, the chick embryo serving as type object.

2. The autoglycolysis of the chick embryo in the first week of its development is small relatively to its maximum glycolytic intensity and, like the latter, falls with increasing developmental age. Though it is not inhibitable with glyceraldehyde, fluoride or bisulphite, a calculation shows that it does not exceed in magnitude the carbohydrate stores known to be located at the time within the embryo's tissues.

3. A study of the substrate preference of the embryo shows that besides glucose, mannose is the only carbohydrate which can be glycolysed to any substantial degree. The decline of mannolysis with age follows exactly that of glucolysis. The mannolysis of the embryo is of interest in relation to the known occurrence of mannose in egg proteins.

4. Glucosamine, fructose, galactose, sorbose, pentoses, with di- and tri-saccharides are untouched by the embryo. In general all phosphorylated

hexoses and glycogen are also completely unattacked, but occasionally an exceptional experiment may show a slight breakdown of glycogen or glucose-monophosphate.

5. When this occurs there are indications of a summation between the breakdown of the other substrate and that of glucose, if it is allowed to proceed at the same time. Conversely, mannose, which is normally vigorously glycolysed, shows, when together with glucose, every evidence of competition. Taken with the facts already mentioned, this must imply that glucolysis and mannolysis take place with the same enzyme system, but that the phosphorylation method, present only in some very weak form, involves a different enzyme system.

6. The predominance of glucolysis over the breakdown of glycogen or phosphorylated hexoses persisted when brei was used instead of intact embryo. It was never possible to obtain extracts with any significant glycolysing activity.

7. The substrate-preference picture obtained is probably not due to differences of permeability. Embryo thus joins tumour and brain as a predominantly glycolysing system. Experiments in which the trunk and limb-buds of the embryo alone were used dispose of the possibility that this property arises from the relatively large size of the nervous system in the embryo.

8. In agreement with certain observations on mammalian embryos in the literature, the phosphorylation route does not appear to be yet established, even in the musculature of chicks on the fifteenth day of development.

9. Inquiry into the possibility of oxidation of carbohydrates without glycolysis was not carried far, as in the absence of substrate the embryo has reserves of an unknown nature which permit of a long-continued aerobic respiration.

10. Although the trisaccharide of Levene & Mori yields glycolysable material on hydrolysis, acid hydrolysis of ovomucoid and seromucoid does not.

11. The glucolysis of the chick embryo is powerfully inhibited by *dl*-glyceraldehyde. This inhibition may be partially reversed by pyruvate. During the inhibition the substrate, glucose, remains in greater part intact and is not converted to accumulate as any three-carbon intermediate. The inhibition by glyceraldehyde is specific; it is not shown by other aldehydes such as propaldehyde.

12. Methylglyoxalase is present in the embryo, in fully activated form, since further additions of glutathione do not increase its activity. Its action is not inhibited by glyceraldehyde. Glucolysis by intact embryos cannot be increased by glutathione additions.

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REFERENCES

- Amberson & Armstrong (1933). *J. cell. comp. Physiol.* **2**, 381.
 Ashford (1933). *Biochem. J.* **27**, 903.
 Baldwin (1935). *J. exp. Biol.* **12**, 27.
 Barr, Ronzoni & Glaser (1928). *J. biol. Chem.* **80**, 331.
 Benzinger & Krebs (1933). *Klin. Wschr.* **12**, 1206.
 Boell (1935). *J. cell. comp. Physiol.* **6**, 369.
 Bumm & Fehrenbach (1930). *Hoppe-Seyl. Z.* **193**, 238.
 ——— (1931). *Hoppe-Seyl. Z.* **195**, 101.
 Cruickshank (1936). *Physiol. Rev.* **16**, 597.
 Dickens & Greville (1932). *Biochem. J.* **26**, 1251.
 ——— (1933). *Biochem. J.* **27**, 832.
 ——— & Šimer (1931). *Biochem. J.* **25**, 985.
 Dixon, K. C. (1935). *Biochem. J.* **29**, 973.
 Dixon, M. (1934). *Manometric Methods*. (Camb. Univ. Press.)
 Dyer & Roe (1934). *J. Nutrit.* **7**, 623.
 Fischer & Wehmeier (1933). *Naturwissenschaften*, **21**, 518.
 Frankel & Jellinek (1927). *Biochem. Z.* **185**, 392.
 Friedemann, Cotonio & Schaffer (1927). *J. biol. Chem.* **73**, 335.
 Fukahori (1933). *Nagasaki Igakkwai Zasshi*, **11**, 288.
 Geiger (1935). *Biochem. J.* **29**, 811.
 Haarmann (1932, 1). *Biochem. Z.* **255**, 103.
 ——— (1932, 2). *Biochem. Z.* **255**, 142 and 203.
 ——— (1932, 3). *Biochem. Z.* **256**, 326.
 ——— & Stratmann (1932). *Biochem. Z.* **256**, 371.
 Hanaoka (1936). *Gann*, **30**, 458.
 Harrison & Mellanby (1930). *Biochem. J.* **24**, 141.
 Heatley & Lindahl (1937). *Proc. roy. Soc. B*, **122**, 395.
 ——— Waddington & Needham (1937). *Proc. roy. Soc. B*, **122**, 403.
 Holmes (1934). *Ann. Rev. Biochem.* **3**, 395.
 Huxley & de Beer (1934). *Elements of experimental Embryology*. (Camb. Univ. Press.)
 Irving (1926). *Biochem. J.* **20**, 1320.
 Jephcott & Robison (1934). *Biochem. J.* **28**, 1844.
 Koschtojan & Rjabinowskaja (1935). *Arch. ges. Physiol.* **235**, 416.
 Krebs & Henseleit (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Kumanomido (1928). *Biochem. Z.* **193**, 315.
 Laquer & Meyer (1923). *Hoppe-Seyl. Z.* **124**, 211.
 Levene & Mori (1929). *J. biol. Chem.* **84**, 49.
 ——— & Rothen (1929). *J. biol. Chem.* **84**, 63.
 Loebel (1925). *Biochem. Z.* **161**, 219.
 Mendel (1929). *Klin. Wschr.* **8**, 169.
 ——— Bauch & Strelitz (1931). *Klin. Wschr.* **10**, 118.
 Meyerhof (1926). *Biochem. Z.* **178**, 399.
 ——— (1927). *Biochem. Z.* **183**, 178.
 ——— Lohmann & Schuster (1936). *Biochem. Z.* **286**, 301.
 ——— & Suranyi (1926). *Biochem. Z.* **178**, 440.
 Morgan & Chichester (1935). *J. biol. Chem.* **110**, 285.
 Mündelein (1932). *Biochem. Z.* **256**, 376.
 Murray (1925). *J. gen. Physiol.* **9**, 1.
 Needham, D. M. (1937). *Ann. Rev. Biol.* (in the Press).
 Needham, J. (1926). *J. exp. Biol.* **4**, 114.
 ——— (1927). *Quart. J. exp. Physiol.* **18**, 161.
 ——— (1931). *Chemical Embryology*. (Cambridge Univ. Press.)

- Needham, J. (1932, 1). *Proc. roy. Soc. B*, **112**, 98.
— (1932, 2). *Proc. roy. Soc. B*, **112**, 114.
— (1933, 1). *J. exp. Biol.* **10**, 79.
— (1933, 2). *Proc. roy. Soc. B*, **113**, 429.
— (1936). *Proc. roy. Soc. Med.* **29**, 31.
Needham & Lehmann (1937). *Biochem. J.* **31**, 1210.
Needham, Nowiński, Dixon & Cook (1937). *Biochem. J.* **31**, 1185.
Neuberg (1925). *Z. angew. Chem.* **38**, 761.
— Kobel & Laser (1930). *Z. Krebsforsch.* **32**, 92.
Nolf (1932). *Amer. J. Hyg.* **16**, 288.
Onoe (1936). *J. Biochem., Tokyo*, **24**, 1.
Ozaki (1936). *J. Biochem., Tokyo*, **24**, 73.
Parnas (1936). *Bull. Soc. Chim. biol., Paris*, **18**, 53.
— & Ostern (1936). *Bull. Soc. Chim. biol., Paris*, **18**, 1471.
Rimington (1931). *Biochem. J.* **25**, 1062.
Robison (1932). *Biochem. J.* **26**, 2191.
— & Morgan (1928). *Biochem. J.* **22**, 1277.
Rosenthal (1930). *Z. Krebsforsch.* **32**, 243.
Scharles & Salter (1934). *Amer. J. Cancer*, **20**, 613.
Schlenk (1933). *Biochem. Z.* **267**, 424.
Schmalhausen (1926). *Arch. Entw. Mech. Org.* **108**, 322.
Schönfelder (1935). *Arch. exp. Path. Pharmacol.* **180**, 24.
Sherman & Elvehjem (1936). *Amer. J. Physiol.* **117**, 142, 151.
Sørensen (1934). *C.R. Lab. Carlsberg*, **20**, no. 3.
— & Haugaard (1933). *C.R. Lab. Carlsberg*, **19**, no. 12.
Stephenson & Yudkin (1936). *Biochem. J.* **30**, 506.
Tanko (1931). *Biochem. Z.* **239**, 318.
Tsuzuki (1936). *J. Biochem., Tokyo*, **23**, 421.
Waddington, Needham, Nowiński & Lemberg (1935). *Proc. roy. Soc. B*, **117**, 289.
Warburg, Posener & Negelein (1924). *Biochem. Z.* **152**, 309.
Weiss (1935). *Physiol. Rev.* **15**, 639.