CXLIX. THE METABOLISM OF NORMAL AND TUMOUR TISSUE.

VI. THE CONVERSION OF FRUCTOSE AND GLUCOSE TO LACTIC ACID BY EMBRYONIC TISSUES.

BY FRANK DICKENS AND GUY DRUMMOND GREVILLE.

From the Courtauld Institute of Biochemistry, Middlesex Hospital, London.

(Received June 30th, 1932.)

FRUCTOSE is known to be utilised by certain animal and vegetable cells; thus it is fermented at least as rapidly as glucose by yeast-juice [Harden and Young, 1908; 1909] and certain yeasts [Slator, 1908; Willstätter and Sobotka, 1922; Fernbach, Schoen and Mori, 1927; Hopkins, 1931]; it is converted into lactic acid by tumours though to a less extent than glucose [Minami, 1923; Warburg, Posener and Negelein, 1924; Rosenthal, 1930, 1]; whilst in the summer months minced frog's muscle [Laquer and Meyer, 1923] and rat-liver [Rosenthal, 1930, 2] form lactic acid from fructose more rapidly than from glucose. On the other hand, brain tissue [Loebel, 1925] and nerve tissue [Gerard and Meyerhof, 1927] cannot convert fructose into lactic acid. The action of the other tissues has apparently hitherto not been investigated. Some preliminary experiments by the authors, however, indicate that with kidney, spleen, testis and retina there is little anaerobic attack on fructose.

Whilst fructose is not in general present in any considerable amount in the body, it has been known since the time of Claude Bernard that fructose normally occurs in the pregnant animal of several species, attaining a high concentration (up to *ca.* 1 %) in the amniotic and allantoic fluids and in the foetal blood [Bernard, 1850; Dastre, 1876; Gürber and Grünbaum, 1904; Paton, Watson and Kerr, 1907; Orr, 1924]. Therefore, it becomes of special interest to find out if fructose is utilised by embryonic tissues, which are in many cases bathed in a medium rich in this sugar. In the present paper the anaerobic conversion of fructose and glucose into lactic acid by the different embryonic tissues of several species is described.

The work of Warburg and his collaborators [Warburg, 1926, 1930] has shown the extraordinary ability of embryonic tissues to form lactic acid from glucose, the foetal membranes of the rat resembling the most active tumours in this respect. It may be noted, however, that almost all these investigations refer to the rat, and therefore the figures which we give for other species are of interest.

It will be shown that while glucose is converted readily into lactic acid by all the embryonic tissues, the attack on fructose depends both on the tissue and on the species, varying from zero to a value equal to that observed with the same tissue on glucose.

The discussion of the results follows the experimental section.

Nomenclature. For the sake of brevity, we shall use the following terms.

- Glycolysis.* General expression denoting the breakdown of carbohydrate to lactic acid [cf. Warburg, 1925].
- Glucolysis.* The glycolysis in the presence of added glucose.
- Fructolysis.* The glycolysis in the presence of added fructose.
- Autoglycolysis.* The glycolysis when no substrate is added.

This distinction between glycolysis and glucolysis is in conformity with the officially approved nomenclature now adopted for the glycosides [Armstrong and Armstrong, 1931].

EXPERIMENTAL.

PREPARATION OF MATERIAL AND METHODS USED.

The smaller animals were killed by a blow on the neck; the larger ones were killed under ether anaesthesia. The animal was immediately immersed in warm, dilute lysol, and after a few minutes was washed thoroughly with running water. The uterus was then removed and dissected with sterile instruments.

After dissection, portions of the embryonic membranes of suitable size were cut with scissors: such material is particularly suitable for *in vitro* experiment since it is sufficiently thin to be used directly without further cutting. The placenta was used intact when it was very small, otherwise it was cut with a razor into thin slices. In all cases the material was rinsed in a dish containing Ringer solution before being transferred to the manometer vessels, where the tissues were suspended in Ringer solution containing 25 *mM*. per litre of sodium bicarbonate and saturated with nitrogen containing 4.5–5 % CO₂ by volume (*p*_H 7.45–7.50). Sugar was added to the Ringer solution in freshly prepared 10 % solution to give in all cases a final concentration of 0.2 %. The fructose used was Schering-Kahlbaum "Laevulose cryst. for scientific purposes." Recent analyses of this product have been made by Wierzychowski *et al.* [1931]; polarimetric measurements on the specimen used by us agree with those given by these authors.

The glycolysis was measured manometrically at 38° by the method of Warburg [1926; 1930]. The first readings were taken 10 to 15 minutes after putting the vessels in the bath, and were usually continued for a period of 2 to 3 hours. These prolonged experiments were desirable since we have found that the autoglycolysis of embryonic tissues is often large in the first hour of the experiment. The general procedure was to make simultaneous observations of the glycolysis of pieces of the same tissue in glucose, fructose and sugar-free Ringer solutions.

In typical cases (rat-yolk-sac and cat-amnion) the lactic acid formation

measured manometrically was confirmed by chemical estimation by Clausen's method. The results show that the action of the embryonic membranes on fructose is a pure lactic acid fermentation.

The glycolysis is expressed throughout by Warburg's quotient $Q_M^{N_2}$, which is defined as the number of mm.³ of CO₂ at N.T.P. liberated per hour per mg. dry weight of tissue. Since 1 gram-equivalent of lactic acid liberates 22.4 litres of CO₂, the lactic acid per hour formed is expressed as a percentage of the dry weight of the tissue when these figures are multiplied by 0.4.

The disposition and development of the embryonic membranes in the several species are so varied that in the following text we quote references to standard works for the description of the individual membranes as they arise. The experiments are numbered with respect to the individual animals; experiments bearing the same number thus refer to tissues of the same animal throughout.

GLYCOLYSIS OF THE FOETAL MEMBRANES.

Outer sac.

The results are collected in Table I.

Although for the sake of convenience we describe together the outer membranes of the various species, it must be remembered that the outer sac of Rodentia consists of the inverted inner half of the yolk-sac, the outer half of the yolk-sac having disappeared together with the greater part of the chorion laeve during the second half of pregnancy [Grosser, 1927, p. 122]. On the other hand, the outer sac of Carnivora [Grosser, 1909, pp. 131-2] consists of the chorion laeve in close contact with the outer wall of the allantois.

Rodentia.

Rat (albino). (For description of membranes see Grosser [1927, p. 135].) The inner and outer membranes were dissected from the embryo and placenta, and the yellowish yolk-sac carefully freed from the fine white amnion. Pregnancies of known date are readily obtained in this species from observations of the occurrence of vaginal plugs. Observations on the glucolysis, fructolysis and autoglycolysis of the yolk-sac of rats 13 to 20 days pregnant (*i.e.* in the second half of pregnancy) are given. The glucolysis remained practically constant for the 3 hours of the experiment, the Q values agreeing well with those obtained in Ringer solution by Kumanomido [1928]. The fructolysis is of the same order of magnitude as the glucolysis (in Exp. 7 the values were identical for the 2 hours of the experiment), but in no case did the fructolysis exceed the glucolysis. In Exps. 4 and 7 the fructolysis and glucolysis ran parallel, in the remainder the fructolysis fell off more rapidly than the glucolysis. The autoglycolysis was sometimes appreciable in the first 30 minutes, but afterwards fell to a negligible value.

Mouse [Jenkinson, 1913, p. 232]. Whilst the glycolysis of the mouse-yolk-

Table I. *Outer membrane.* $Q_M^{N_2}$.

Exp. no.	Glucolysis			Fructolysis			Autoglycolysis			Embryo		Relative fructo- lysis 2nd hr. %	
	1	2	3	1	2	3	1	2	3	Wt. g.	Age days		
hours													
<i>Rat (albino)</i>													
4	35.5	32.5	31.5	27.0	26.5	27.5	1	0	0	0.065	13	82	
3	32.0	27.5	27.0	30.0	22.0	20.0	4	1	0.5	0.175	15	79	
				28.0	22.0	19.0	—	—	—	—	—	79	
				30.0	22.0	—	—	—	—	—	—	79	
8	24.2	24.0	21.4	21.2	18.8	13.7	—	—	—	0.34	16	—	
35	18.0	17.0	17.5	11.5	9.0	8.5	4	1	1	2.6	20	50	
1	24.0	19.0	18.0	18.0	13.0	11.0	7	1.5	0	3.2	20	66	
7	19.0	16.0	—	20.0	15.5	—	2.5	0	—	3.2	20	97	
				20.0	15.0	—	—	—	—	—	—	94	
				20.0	16.0	—	—	—	—	—	—	100	
												Mean	75
<i>Mouse</i>													
27	26.2	22.8	—	7.0	4.0	—	7.2	2.3	—	0.425	15	8	
33	24.2	20.3	—	5.8	3.7	—	6.7	1.1	—	0.62	16	13.5	
42	26.0	19.0	—	9.5	4.5	—	9.0	2.0	—	1.0	17	15	
												Mean	12
<i>Guinea-pig</i>													
18	11.2	11.2	—	11.9	11.6	—	8.4	3.2	—	30	50	105	
29	11.0	11.0	—	10.0	9.5	—	2.0	0.5	—	31.5	50	86	
												Mean	96
<i>Rabbit</i>													
26	25.5	25.0	—	12.0	10.0	—	6.0	1.0	—	0.07	13	37	
24	17.0	15.0	—	9.5	7.5	—	4.5	1.0	—	30.2	27	46	
22	22.4	23.1	—	5.5	4.4	—	3.0	1.1	—	31.6	27	15	
												Mean	33
<i>Cat</i>													
11	41.0	24.0	—	6.0	5.0	—	5.0	5.0	—	19.5	—	0	
38	16.5	13.5	13.0	3.0	2.5	1.5	2.0	1.0	1.0	45.5	—	12	
13	17.0	15.0	—	4.8	2.8	—	2.9	1.8	—	46.0	—	7.5	
36	17.0	14.4	—	4.1	2.2	—	3.3	1.4	—	58.5	—	6	
10	17.0	10.5	—	3.0	2.5	—	3.5	2.0	—	109	—	6	
												Mean	6
<i>Ferret</i>													
23	34.5	31.5	—	—	—	—	4.0	1.3	—	1.4	—	—	
19	29.0	27.0	—	6.0	5.0	—	6.0	4.0	—	2.9	—	4	
34	29.0	23.0	19.5	8.0	7.0	6.0	3.0	0.5	0.3	6.0	—	29	
				8.5	6.0	5.5	—	—	—	—	—	24	
												Mean	15.5
<i>Human peripheral membrane</i>													
Maternal side													
17	9.3	8.0	—	6.3	4.6	—	2.9	0.8	—	10 months	—	53	
21	8.0	6.3	—	4.2	2.4	—	2.3	1.0	—	—	—	26	
28	8.2	7.9	—	7.2	6.0	—	2.1	0.8	—	—	—	73	
												Mean	51
Foetal side													
17	3.8	1.9	—	4.1	0.6	—	3.8	0	—	—	—	32	
20	3.9	3.0	—	3.6	2.6	—	2.5	1.4	—	—	—	75	
28	6.5	6.0	—	4.3	2.9	—	1.2	0.7	—	—	—	42	
												Mean	50

The figures given in italics in these Tables are the values of $Q_M^{N_2}$ calculated from half-hour experimental periods. The ages given are calculated from the weights, using the tables of Needham [1931, Appendix I]: in the rats the ages were known, and agreed with those calculated.

sac is shown to be comparable with that of the rat, the fructolysis on the other hand is negligible, barely exceeding the autoglycolysis.

Guinea-pig [Grosser, 1927, pp. 38, 148]. Values for two late pregnancies are given. Although the glucolysis and fructolysis are less than those of the rat at a correspondingly late stage (Exps. 1 and 7, Table I), the fructolysis resembles that of the rat in being practically identical with the glucolysis.

Rabbit [Grosser, 1927, p. 122]. Results for two very advanced pregnancies and for one half-way through gestation are included in Table I. The glucolysis is of the same order as that of the rat-yolk-sac, but the membrane differs from the yolk-sac of the other rodents investigated in that it attacks fructose at about one-third of the rate at which it converts glucose to lactic acid. The fructolysis as well as the glucolysis remains constant over the experimental period.

Carnivora.

Cat. The allanto-chorion of the cat was used without attempting to separate it into its component layers, although it was noted that such a separation occurred partially during the course of the experiment. The membrane is not inferior to that of the rat in attacking glucose, but the fructolysis is negligible, being only slightly greater than the autoglycolysis.

Ferret. The allanto-chorion of the ferret was used in the same way as that of the cat. The glucolysis is at least as high as that of the rat; the fructolysis, while steady and relatively greater than that of the cat, is still small.

Man. The human material used in this paper was obtained from the full-time afterbirth only, and was used within half an hour of its expulsion, while the placenta was still warm. It was kindly supplied by the Obstetric Department of the Middlesex Hospital.

The membrane attached peripherally to the placenta of the afterbirth consists, after separation of the amnion, of chorion laeve together with decidua [Grosser, 1909, pp. 269, 212]. As this membrane was thick, it was divided. On pulling apart, it separated fairly easily along a plane nearer to the foetal side into foetal and maternal portions, which were used separately in the manometers. Histological examination indicated that the cleavage had occurred through the "Zwischenzone" [Grosser, 1909, p. 212] which consists of chorionic epithelium and the remains of villi, decidua capsularis and decidua compacta. The foetal portion thus consisted largely of chorionic connective tissue together with a small amount of the "Zwischenzone"; the maternal part consisted largely of the latter. As was to be expected from the known low metabolic activity of connective tissue, it was found (Table I) that the metabolism of the maternal portion was higher than that of the foetal. Both attacked fructose, but to about half the extent to which they attacked glucose.

Amnion.

The glycolysis of the amnion of the rat, rabbit, guinea-pig, cat and ferret in glucose, fructose and sugar-free solutions is shown in Table II. The preparation of the membrane presented no difficulties; in the rabbit, cat and

Table II. *Amnion.* $Q_M^{N_2}$.

Exp. no.	Glucolysis			Fructolysis			Autoglycolysis			Embryo		Relative fructo- lysis 2nd hr. %	
	1	2	3	1	2	3	1	2	3	Wt. g.	Age days		
	hours			hours			hours						
<i>Rat (albino)</i>													
32	16.0	16.0	16.0	5.0	3.0	2.0	6.0	0	0	1.6	19	19	
35	15.0	14.5	14.5	7.0	3.5	4.0	—	—	—	2.6	20	Not > 24	
7	17.0	11.5	—	7.0	2.0	—	—	—	—	3.2	20	Not > 17	
												Mean	19
<i>Rabbit</i>													
37	17.5	13.5	14.0	5.5	4.5	4.5	1.5	0.5	0	36.5	28	31	
				6.5	5.0	5.5	—	—	—	—	—	35	
												Mean	33
<i>Guinea-pig</i>													
18	5.1	5.2	—	3.8	2.6	—	2.8	0.2	—	30.0	50	48	
29	5.8	6.0	—	4.0	2.5	—	2.6	0.4	—	31.5	50	38	
												Mean	43
<i>Cat</i>													
11	17.0	11.0	—	10.0	8.0	—	4.0	4.0	—	19.5	—	57	
13	15.7	15.0	—	6.9	7.5	—	3.8	1.9	—	46.0	—	43	
41	—	—	—	10.6	8.2	8.0	—	—	—	57.0	—	—	
				14.6	10.3	9.8	—	—	—	—	—	—	
36	12.5	12.5	—	6.5	7.5	—	6.0	4.5	—	58.5	—	37	
												Mean	46
<i>Ferret</i>													
23	15.5	13.5	13.0	13.5	13.0	12.0	10.5	1.0	0	1.4	—	96	
19	18.4	13.2	—	12.8	10.4	—	8.2	1.6	—	2.9	—	76	
												Mean	86
<i>Rabbit-allantois</i>													
24	22.0	14.2	—	16.0	11.5	—	12.0	2.5	—	30.2	27	77	
37	19.0	20.0	20.0	13.0	11.5	11.5	1.0	0.5	0	36.5	28	56	
				12.0	12.5	11.5	—	—	—	—	—	62	
												Mean	68
<i>Cat-yolk-sac</i>													
38	21.0	19.5	17.5	11.5	9.5	8.5	5.0	2.5	1.5	45.5	—	41	
				11.0	9.5	10.5	—	—	—	—	—	41	

ferret, dorsal portions were taken in order to avoid free allantois. The membrane in the cat and ferret consists of fused allantois and amnion [Grosser, 1909, p. 132]. The glucolysis was in all cases steady over the course of the experiment. It should be noted that the values obtained for the rat were from late pregnancies, and therefore were lower than the value in horse-serum obtained from an early pregnancy by Negelein [1925]. The fructolysis of amnion of the rat is small compared with the glucolysis; that of the ferret approaches the glucolysis; whilst in the rabbit, guinea-pig and cat the amnion is intermediate in its activity towards fructose.

The metabolism of the amnion from the human afterbirth at term was

found to be very low. This is scarcely surprising since the amnion undergoes a hyaline degeneration at the end of pregnancy, which Belosor [1925] considers to be a physiological preparation for its rupture.

Allantois.

Measurements on the allanto-chorion and allanto-amnion of the Carnivora have been described above. Free allantois can be obtained in sufficient quantity from the rabbit. In the later stages the allantoic sac stretches between the placenta and the ventral surface of the embryo, over which it is fused with the amnion [Grosser, 1927, p. 129]. The side portions consist of pure allantois and are easily dissected. The results are given in Table II, from which it will be seen that the glucolysis is high and almost identical with that of the yolk-sac of the same animal (Exp. 24). The allantois readily attacks fructose, to a greater extent than either the amnion or the yolk-sac of the same animal. The good agreement between the duplicates in fructose shows that the results are highly reproducible and the membrane homogeneous.

Yolk-sac.

The modified yolk-sac of Rodentia, which takes on the special nutritive function of the primary chorion laeve [Grosser, 1927, p. 84] and even develops villi in the later stages [Robinson, 1892], has been studied above. In Carnivora, the yolk-sac persists throughout pregnancy as a small intact sac, presumably with very different function from that of the rodents. The cat was the most convenient source of this type of yolk-sac. In this animal it is found as a yellow sac lying between embryo and placenta and drawn out into two long horns in the direction of the long axis of the uterus. It was easily dissected and freed from allantois. The results are given in Table II. It was found to attack glucose slightly more readily than the allanto-chorion or allanto-amnion from cat embryos of the same age. The fructolysis was a little less than half the glucolysis, and remained constant. Here again the uniformity of the material is shown by the equal fructolysis of two portions from different parts of the membrane.

PLACENTA.

Previous studies have shown that the placenta of the rat [Murphy and Hawkins, 1925; Loeser, 1927], mouse [Fujita, 1928], rabbit [Bell *et al.*, 1928] and man [Loeser, 1927; Bell *et al.*, 1928; Kumanomido, 1928] readily converts glucose into lactic acid. These workers all used either the whole organ or slices cut from it; placenta *Brei* has been studied by Ishikawa [1928] (rabbit) and Hayashi [1928] (human), who differ as to whether added hexoses are converted into lactic acid at all by the placenta.

We have studied the glucolysis, fructolysis and autoglycolysis of the placenta of the rat, mouse, guinea-pig, rabbit, cat, ferret and human being (Table III).

Table III. *Placenta*. $Q_M^{N_2}$.

Exp. no.	Glucolysis			Fructolysis			Auto- glycolysis			Embryo		Relative fructo- lysis %	Remarks	
	1	2	3	1	2	3	1	2	3	Wt. g.	Age days			
Rat														
16	17.2	12.8	—	12.8	5.6	—	9.8	5.2	—	0.03	13-14	5	Whole placenta	
8	13.2	11.3	11.7	6.5	3.5	1.6	4.1	2.9	1.8	0.34	16	7		
32	19.5	14	14	8	3	2.5	7.5	1.5	2	1.6	18-19	12		
1	16.5	15.5	15	4.0	2.5	2.0	3.5	2.0	2.0	3.2	20	4		
											Mean	7		
Mouse														
27	7.0	6.5	—	4.8	3.4	—	5.3	3.1	}	0.425	15	9	Materna half	
27	8.3	8.0	—	2.6	2.1	—	3.5	2.0			—	16		2
33	7.2	7.3	—	2.4	1.7	—	2.7	1.3			—	0.62	16	7
											Mean	6		
Guinea-pig														
18	5.1	4.2	—	3.3	1.4	—	2.5	1.2	—	30	50	7	—	
29	4.25	3.15	—	2.1	0.9	—	1.8	0.8	—	31.5	50	4	—	
											Mean	6		
Rabbit														
26	9.6	7.2	—	4.5	2.2	—	6.6	4	—	0.072	14	0	—	
24	4.6	3.4	—	1.9	1.5	—	1.95	1.0	—		30.2	27	21	—
22	4.6	3.9	—	2.3	1.5	—	1.7	0.9	—		31.6	27	20	—
											Mean	14		
Cat														
11	6.0	5.6	—	3.8	3.0	—	2.6	1.4	—	19.5	—	26	—	
40	7.2	5.8	5.6	4.5	3.4	3.3	2.6	0.9	0.8	—	—	51	Foetal Mid Maternal	
	3.8	3.0	3.1	5.0	3.6	3.4	1.8	0.4	0.3	69	—	(120)		
	5.2	4.5	3.8	3.4	2.2	1.6	1.0	0	0	—	—	49		
43	4.4	4.1	3.8	2.4	1.6	1.6	1.0	0.7	0.6	—	—	26	Foetal	
	3.6	3.1	3.2	2.3	1.5	1.4	1.5	0.6	0.6	85	—	33	Mid	
	5.3	4.5	4.4	2.8	1.8	1.5	—	—	—	—	—	—	—	
10	4.4	3.7	3.6	2.0	1.4	1.0	1.0	0.4	0.4	—	—	30	Maternal	
	6.4	5.6	—	4.1	2.5	—	2.2	0.6	—	109	—	38	—	
											Mean	36		
Ferret														
23	22	14	—	8.3	5.5	—	5.4	1.5	—	1.4	—	32	—	
19	21.4	19.8	—	8.8	6.2	—	4.7	1.4	—	2.9	—	26	—	
											Mean	29		
Human														
0	3.7	2.6	—	3.6	2.1	—	1.6	0.4	—	—	—	77	Maternal	
28	5.7	4.5	—	4.7	2.9	—	1.7	0.4	—	—	—	61	Maternal	
28	4.0	2.5	—	2.3	0.9	—	1.3	0.4	—	—	—	24	Foetal	
17	4.6	4.0	—	4.5	2.8	—	—	—	—	—	—	—	Foetal	
21	2.9	1.5	—	2.5	1.3	}	2.3	0.6	—	—	—	62	Foetal	
				2.5	1.0									
											Mean	57		

Rodentia (placenta haemochorialis labyrinthica).

Although the placentae examined were from different stages of pregnancy, inspection of the figures shows that the glucolysis is highest in the case of the rat, the other three animals having much lower Q values. In Exp. 16 whole placentae were used; in Exp. 27 the placenta was divided horizontally into maternal and foetal halves which agreed with one another and with the slices of Exp. 33 in their metabolism.

The placentae of the four rodents hardly attacked fructose, the fructolysis being little larger than the autoglycolysis.

Carnivora (placenta endotheliochorialis).

The placenta of the cat is zonary, that of the ferret zono-discoidal [Strahl, 1906, p. 301] consisting of two discs separated on the antimesometrical side by a blood extravasation. The ferret placenta showed a very high glucolysis: fructose was also attacked but only to about one-third the extent of glucose. In the cat the glucolysis was lower, but fructose was attacked relatively to about the same extent as in the ferret.

As both maternal and foetal tissues enter into the placenta of *Carnivora*, we have compared the activity towards glucose and fructose at different depths through the placenta (Exps. 40 and 43, Table III). The placenta was divided horizontally into three portions, after rejecting thin layers from the outer surfaces. Slices were cut from the foetal, middle and maternal portions thus prepared and were used separately for manometric measurements. Histological examination of the portions used in Exp. 40 showed that whilst the foetal and mid parts consisted entirely of placental labyrinth, the maternal portion contained labyrinth together with the mixed maternal and foetal tissues of the "invasion-zone" [Grosser, 1909, p. 128; 1927, p. 119]. The glucolysis remains approximately constant as we pass through the placenta, although an apparently anomalous low result was obtained for the middle portion in Exp. 40. The fact that the duplicates in Exp. 43 show rather a high divergence illustrates the variable nature of the material, which makes accurate comparison difficult. The fructolysis falls off with the autoglycolysis as the maternal side is approached.

Man (placenta haemochorialis olliformis).

Full-time human placentae were obtained as already described. Slices were cut from the warm placenta, the larger white infarcts being avoided and the surface layers rejected. The *Q* values for full-time human placentae show a greater variation among themselves than that shown by the placental tissue of the other animals; this is no doubt due to the large amount of metabolically inert fibrinoid tissue in the human placenta. There was a relatively large autoglycolysis in the first hour, which fell to a small value in the second. In all cases the glucolysis was considerably higher than the autoglycolysis, particularly in the second hour. The figures given show no systematic difference between the metabolism of maternal and foetal sides. Both sides definitely attack fructose but to a less extent than glucose, the fructolysis falling off rather more rapidly than the glucolysis. These experiments show clearly, in contradistinction to those of Hayashi [1928] on *Brei*, that the human placenta converts glucose more readily than fructose to lactic acid, under our experimental conditions.

EMBRYO AND FOETAL TISSUES.

Embryos were taken of a size suitable for use without cutting, and were carefully freed from membranes. Table IV shows that the rat embryo of the

Table IV. *Embryo and foetal tissues.* $Q_M^{N_2}$.

Exp. no.	Glucolysis			Fructolysis			Autoglycolysis			Embryo		Relative fructo- lysis %
	1	2	3	1	2	3	1	2	3	Wt. Dry wt. mg.	Age days	
	hours			hours			hours					
Embryo.												
Rat												
14	14	10	—	4	2.5	—	3	1.5	—	3.4	13	10
30	—	—	—	3.9	2.5	1.8	4.6	1.8	0	3.8	13	(7)
5	—	—	—	4.6	2.2	2.6	2.8	1.6	1.3	4.7	13	(6)
16	—	—	—	3.7	—	—	3.9	—	—	5.3	13-14	—
Rabbit												
26	15.9	12.9	11.2	2.8	1.4	1.3	—	—	—	12.0	14	11
Chick												
9	15.5	—	—	—	—	—	—	—	—	} 3.8	4	— (4)
	12.0	—	—	—	—	—	—	—	—			
	22.5	—	—	2.3	—	—	1.7	—	—			
15	—	—	—	3.5	1.2	—	2.9	1.2	—	5.2	4½	0
2	—	—	—	3.0	1.0	1.0	2.5	1.0	1.0	10.5	5	0
6	—	—	—	3.6	0.8	0.4	3.6	0.8	0.4	10.7	5	0
Foetal tissues.												
Cat-liver										Moist wt. g.		
11	5.2	—	—	2.6	—	—	—	—	—	19.5	—	—
Guinea-pig-liver												
18	3.4	2.6	—	2.6	1.8	—	2.0	0.6	—	—	50	60
Mouse-liver												
42	12.5	9.6	8.9	10.4	9.2	8.8	11.6	10.2	8.3	1.0	17	—
Mouse-skin												
42	18.5	17.0	16.5	6.5	3.0	2.5	6.5	3.5	2.0	1.0	17	0
Cat-lung												
41	17	14	—	4.0	3.5	—	4.5	2.5	—	} 57	—	0
Cat-brain												
41	19	15	—	2.4	2.0	—	1.8	1.4	—	4		

13th to 14th day and the rabbit embryo of the 14th day do not fructolyse. These embryos are in the mid-period of gestation. The chick embryo of 4 to 5 days' incubation shows a similar inability to attack fructose.

For the investigation of the later embryo, the large size necessitates the use of individual tissues. The experiments on the foetal brain, liver, lung and skin given in Table IV were designed to find out if these tissues develop any special fructolytic power in the terminal stages of gestation. The liver of the cat and guinea-pig was used in slices; that of the mouse was small enough to be used as a whole organ. In the guinea-pig there was a definite attack on glucose (contrast adult rat-liver [Rosenthal, 1930, 2]); and the fructolysis lay

between the glucolysis and autoglycolysis. In the mouse all three coincided, the autoglycolysis being extraordinarily high. The skin of the mouse embryo and slices of embryonic brain and lung of the cat, all failed to attack fructose, though their glucolysis, like that of most embryonic tissues, was high.

CHEMICAL ESTIMATION OF LACTIC ACID FORMED FROM FRUCTOSE.

The manometric measurements of the fructolysis in two representative cases, rat chorion and cat amnion, were controlled by simultaneous lactic acid determinations by Clausen's method. For this purpose the contents of the vessel were removed and acidified immediately after taking the last manometer-reading; the proteins were precipitated by tungstic acid, and sugar was removed from the filtrate by the copper-lime reagent. The filtrations were carried out through Jena glass filters. The lactic acid was determined by the method of Friedemann and Kendall [1929]; instead of aerating, however, washed carbon dioxide from a cylinder was used. Duplicate specimens of tissue were placed in the bath at the same time as those above, and were removed at the time of the first reading for determination of the preformed lactic acid. In the last experiment it was found that the extent of this correction could be greatly reduced by suspending the tissue in warm oxygenated Ringer solution for 10 minutes before the experiment. Variations in the preformed lactic acid are the principal source of error. The figures given in Table V confirm, however, that the manometric findings accurately represent lactic acid formation.

Table V.

Exp. no.	Dry wt. of tissue mg.	Time mins.	Lactic acid			CO ₂ mm. ³	Lactic acid mg.	Chemical Manometric %
			Chemical estimation		Manometric			
			Preformed mg.	Total mg.				

<i>Rat-yolk-sac</i>								
3	3.7	0	0.16	—	—	—	—	—
3	2.7	180	0.11	0.81	0.70	195	0.78	90
3	3.3	180	0.14	0.95	0.81	228	0.91	89
7	3.8	0	0.14	—	—	—	—	—
7	4.3	140	0.16	0.89	0.73	169	0.68	107
39	3.2	0	0.10	—	—	—	—	—
39	5.1	0	0.18	—	—	—	—	—
39	2.3	270	0.07	0.35	0.28	77	0.31	90
39	3.6	270	0.12	0.62	0.50	133.5	0.53	94
<i>Cat-amnion</i>								
41	3.9	0	0.04	—	—	—	—	—
41	3.8	0	0.02	—	—	—	—	—
41	6.2	0	0.08	—	—	—	—	—
41	4.5	240	0.04	0.67	0.63	158	0.63	100
41	5.1	240	0.04	0.90	0.86	228	0.91	95

SURVEY OF RESULTS.

Nature of the medium. It is difficult to say what medium most nearly approaches the physiological for these membranes. In the living animal the membranes may enter into relationship with foetal blood, with the foetal

fluids, or with both of these, whilst both maternal and foetal blood enter the placenta. This question is of importance since the glycolysis is to some extent dependent on the nature of the medium used. Our glucolysis values in Ringer solution for rat outer membrane fall between those obtained by Negelein [1925] in inactivated horse-serum and those of Kumanomido [1928] in rat-serum. Indeed, the latter considers that serum exerts an inhibiting action on the glucolysis of embryonic tissues. Our experiments have therefore been carried out entirely in Ringer solution.

It should be emphasised that the above results were all obtained from experiments in 0.2 % solutions of the sugars. The arbitrary concentration of 0.2 % was adopted for glucose by Warburg; this concentration does not depart widely from the physiological level, while giving a sufficient sugar reserve, and for tumour tissue there is little fall in glucolysis when the concentration is decreased from 0.2 % to 0.1 % [Warburg, Posener and Negelein, 1924]. It should be noted, however, that 0.2 % is not necessarily the optimum concentration for tissues other than tumours or for sugars other than glucose. For our experiments such a concentration may not even be physiological, since considerably higher concentrations of fructose have been reported in the foetal fluids. It is, therefore, realised that a somewhat different picture might be obtained with other fructose concentrations. Again, the experiments were carried out at the p_H of blood, from which the p_H of the various foetal fluids may differ widely [Needham, 1931, p. 1543]. However, our use of the blood- p_H and of 0.2 % sugar concentration enables our measurements in glucose to be compared directly with those of other authors, whilst the fructolysis figures allow a comparison to be made of the attack on glucose and fructose under identical conditions.

Relative fructolysis. Since the autoglycolysis is often fairly large in embryonic tissues, direct comparison of the glucolysis and fructolysis does not always give a true indication of the relative extent to which the added sugars are attacked. When sugar is present in the medium the glycolysis is not always equal to the sum of the autoglycolysis and the lactic acid formed from the added sugar. This is shown by the fact that when in the later stages of an experiment the autoglycolysis falls to a low value, glycolysis in the sugar solution frequently does not fall by the same amount and may even remain constant at its initial rate throughout (see the Tables). We can only say that a sugar is attacked, when its addition causes the autoglycolysis to be exceeded, but for the reasons given above, the excess of glycolysis over autoglycolysis does not accurately represent the amount of the added sugar converted to lactic acid. This error, however, decreases with the autoglycolysis, so that we can best estimate the lactic acid formation from added sugar in the later period of the experiments when the autoglycolysis has fallen to a low value. The relative fructolysis is then described by the percentage

$$\frac{\text{Fructolysis} - \text{Autoglycolysis}}{\text{Glucolysis} - \text{Autoglycolysis}} \times 100.$$

The values of the relative fructolysis for the second hour of the various experiments are set out in the last columns of Tables I-IV, and are represented diagrammatically for the different tissues in Figs. 1 and 2, where the

Fig. 1. Relative fructolysis of outer membrane.

Fig. 2. Relative fructolysis of placenta.

values and their means are represented by continuous and dotted vertical lines respectively. Duplicates on the same tissue are shown as one. In a few experiments where observations over the second hour were not available, those of the second half-hour have been taken.

It will be seen that in some cases where the relative fructolysis is considerable the individual values are widely spread. Since the figures are obtained from tissues at different stages of pregnancy it might be supposed that the relative fructolysis is related to the age of the embryo. This, however, is not the case, for although the fructolysis falls with the glucolysis as pregnancy advances, the relative fructolysis shows no systematic variation. The spread of the relative fructolysis is not surprising with mixed tissues such as human and cat-placenta, but it is remarkable when it occurs in an apparently homogeneous membrane such as the rat-yolk-sac. However, even this latter membrane is by no means simple, since in the later stages it becomes villous [Robinson, 1892]. In view of the complexity of the tissues and the diverging results, it is impossible from our limited number of observations to make any far-reaching generalisations, but we wish to point out one or two inferences which are rendered probable by our findings.

Outer membrane. Considering first the yolk-sac of the rodents, it appears that the various species differ dramatically in their relative attack on fructose, rat and guinea-pig having high, and rabbit and mouse low, relative fructolysis. In contrast with this, the morphological appearances are almost identical, so much so that Grosser [1927] states: "Die Embryonalhüllen dagegen besitzen bei allen Nagern so viele auffällige gemeinsame, nur bei ihnen vorkommende Züge, dass die ganze Ordnung auf Grund der Hüllen ebensogut hätte aufgestellt werden können wie auf Grund des Zahnbaues."

Nevertheless, the previous history of the rabbit membranes is very different from that of the other three, and some difference in behaviour might be understood on this account; but the entirely different behaviour of rat and

mouse towards fructose is most surprising in view of their absolute similarity of development.

The allanto-chorion of Carnivora (cat and ferret) seems to attack fructose but little.

Placenta. In the later stages of pregnancy in the guinea-pig, rat and mouse the discoidal placenta is attached to the decidua over only a small portion of its surface. The conditions are therefore favourable for obtaining homogeneous material and consistent results. Such results indeed were obtained, the relative fructolysis being consistently low. The form of the placenta is rather different in the rabbit, and the relative fructolysis is possibly higher. It appears to be certain that the relative fructolysis is higher in the placenta of the cat and ferret than in that of the guinea-pig, rat and mouse. It is also seen from Exps. 40 and 43 (Table III) that the relative fructolysis is constant through the whole depth of the placenta, if we neglect the mid portion in Exp. 40 where the glucolysis is anomalously low.

The attack of the placenta on fructose does not depend on the extent of the erosion of the maternal uterine tissues effected by the trophoblast, for the haemochorial human placenta resembles in its relative fructolysis the syndesmochorial placenta of the carnivores rather than the haemochorial placenta of the rodents. We cannot therefore refer the fructolysis of the placenta of the carnivores to the retained maternal elements, nor can we say that the trophoblast itself behaves similarly in the placentae of the different species.

DISCUSSION.

"The fructose question," says Needham [1931, p. 1054], "is the most enigmatic aspect of embryonic carbohydrate metabolism." Whilst the appearance of fructose in pregnancy has been recorded, no way in which it may be utilised has so far been suggested. Its source is unknown, and in any case its presence in the amniotic and allantoic fluids cannot be explained until the vexed question of their origin has been settled. But the appearance of this sugar in pregnancy led us to look for a fructolysis of the foetal membranes and placenta, and this we have actually found to occur. At least one of the three tissues, outer membrane, amnion, or placenta, of the pregnant animal of every species examined (with the possible exception of the mouse in which the amnion was not investigated) was found to convert fructose into lactic acid. It would not be possible to correlate our findings with the occurrence of this sugar, which varies greatly in the different species, until complete data were available giving the amounts of fructose and glucose in the foetal fluids at different stages of pregnancy for the various species examined. The information which we have is slight, for only now are reliable methods for the determination of small quantities of fructose being developed. However, if in spite of this we examine our metabolic findings from the physiological standpoint, they are found to present several surprises. Paton, Watson and Kerr [1907]

found that among the animals we have used, fructose is a constant constituent of the allantoic and amniotic liquids of the cat and of the mixed fluids of rabbit, ferret and guinea-pig. Information on rat and mouse is lacking. We may note that in all four animals in which fructose is observed during gestation the amnion definitely fructolyses. This membrane has therefore to some extent adapted itself to the fructose-containing *milieu* which surrounds it. On the other hand, the allanto-chorion of the cat does not fructolyse, although the allantoic face of it is bathed in a medium which definitely contains fructose. Further, the placenta of the cat attacks fructose, so that chorion and placenta behave differently although they are both of trophoblastic origin.

We have shown that the whole embryo half-way through pregnancy does not utilise fructose for the formation of lactic acid, and that no special fructolytic power is developed in embryonic liver, lung or brain. Although they glucolyse readily, these tissues do not adapt themselves to the presence of the fructose in the circulating blood. It is clear also that the power to convert fructose into lactic acid is not a property always possessed by rapidly growing tissues. We have therefore neither shown a universal adaptation of embryonic tissues to the presence of fructose nor adduced a reason for its appearance during gestation. It may be that fructose is a by-product in embryonic metabolic processes of whose occurrence we are at present quite ignorant. It may be said that the fructose mystery deepens.

In seeking an explanation of the great variation in the relative attack on fructose and glucose shown by different tissues the following two main hypotheses might be advanced.

(1) Difference due to a different permeability of the tissues for fructose and glucose.

(2) Variation in the action of the "glycolytic enzyme"; or more exactly, of the system of enzymes and co-enzymes responsible for the conversion of added hexose into lactic acid. This might be due either to different modes of attack in the various tissues (different or partially different enzyme systems) or to a different reactivity towards different forms of the sugars, so that with one tissue one form (*e.g.* *h*-fructose) might have a higher affinity for the enzyme system than the other (*e.g.* *n*-fructose), whilst in another tissue the converse might hold (nomenclature of Schlubach [1932]).

Similar problems in the selective fermentation of sugar solutions by yeast have been much investigated [Willstätter and Sobotka, 1922; Hopkins, 1931; Fernbach, Schoen and Mori, 1927; Dawson, 1932]. Here most workers do not favour the permeability theory, though direct evidence is difficult to obtain. In animal tissues observations on the relative permeability of rat intestinal canal to fructose and glucose have been made by Cori [1925] who found that this tissue was freely permeable to fructose, the ratio of permeabilities of glucose and fructose being approximately 2 : 1.

It appears unlikely that a different permeability is the main factor in

deciding the extent of attack on fructose and glucose by the tissues described in the present paper. If this were so it would be necessary to assume that several morphologically and embryologically almost identical membranes (such for example as the yolk-sac of rat and mouse) which show a high and almost equal rate of attack on glucose, differ so widely in their permeability to fructose that the relative fructolysis is caused thereby to differ from nearly 0 to 100 % of the glucolysis. It is true that this possibility has not been excluded by direct experiment, and that this might be possible by a study of the oxidative reactions, as was done by Loebel [1925] for brain tissue. If those tissues which do not fructolyse should be found to oxidise fructose this would be a definite proof of its diffusion into the cells. Experiments on this point are at present in hand but some difficulty is caused since we find that the respiration of these embryonic tissues, unlike that of brain, often does not fall off markedly in the absence of added substrate, and the clear demonstration that fructose is being oxidised is thus rendered more difficult.

It should be remarked that the possibility that permeability regulates the attack on the sugar has not as yet been definitely excluded even for glucose as substrate. It has yet to be established how far the gradation in the ability of the various tissues to glucolyse [Warburg, 1927], which coincides with that in their power to oxidise, carbohydrate [Dickens and Šimer, 1930], is to be attributed to enzymic rather than diffusion differences. Nevertheless, it is usual to attribute the differing ability of various types of tissue to convert glucose into lactic acid, or to oxidise it, not to their differing permeabilities to the sugar but to the different activities of the respective enzyme systems in them. It is therefore at least as justifiable to exclude permeability questions as the deciding factor in the present problem.

It is noteworthy that in the normal tissues previously observed to convert fructose into lactic acid (muscle, liver) several apparent differences from the other body tissues are evident. These are the only tissues in which resynthesis through the Meyerhof cycle has been experimentally demonstrated. Muscle tissue attacks preferentially polysaccharides such as glycogen, starch and trihexosan, whilst the simple hexoses are less affected. The other tissues, on the other hand, react, often vigorously, with glucose and mannose converting these sugars into lactic acid, whilst fructose is hardly attacked by them. Loebel [1925] found that brain tissue could not form lactic acid from fructose though it oxidised this sugar as readily as glucose or lactic acid, thus showing that it is not impermeable to fructose. According to Ashford and Holmes [1929] the lactic acid formation in brain is of two types, the one without and the other with preliminary interaction of the carbohydrate with phosphoric acid; of these the former greatly predominates, whilst the latter, which involves glycogen, accounts for only a small fraction of the total anaerobic glycolysis. Thus in brain inability to fructolyse is associated with the absence of phosphorylation. On the other hand, Meyerhof [1930, p. 155] has shown that fructose is broken down to lactic acid rapidly by his glycolytic muscle-

extract, provided that hexokinase be added to the system. During the initial phosphorylation period the attack on fructose was twice as rapid as that on glucose. In view of these observations, Meyerhof [1930, p. 194] has suggested that the special suitability of fructose for lactic acid formation in muscle and muscle-extract is analogous to the behaviour of these two sugars in alcoholic fermentation and can be attributed to the fact that fructose is more readily phosphorylated than is glucose. "Mann kann vielleicht das abweichende Verhalten der übrigen Gewebe darauf beziehen, dass bei der Zuckerspaltung hier intermediär keine Phosphorsäureester gebildet werden. Denn ebensowenig wie Glykogen wird die Hexosephosphorsäure von diesen Geweben gespalten, mit alleiniger Ausnahme der Leber, wo ja auch Glykogen abgebaut wird und der Spaltungsmechanismus mit dem des Muskels näher übereinstimmt." To this example (liver) we may probably add a number of embryonic tissues which are also able to break down fructose to lactic acid, but possess the advantage of attacking both glucose and fructose strongly. It is probable that these tissues, showing every degree of fructolysis from nearly 0 to 100 % of the glycolysis, will provide a peculiarly suitable material for the investigation of the different mechanisms by which carbohydrate is attacked by animal tissues.

At the present time we would, therefore, merely indicate the nature of the problem. The embryonic tissues described in this paper appear to resemble muscle and liver in little but that some of them may contain fairly high concentrations of glycogen [Grosser, 1927, pp. 86, 391]. However, if Meyerhof's explanation should prove to be applicable to the different behaviour of glucose and fructose in our embryonic tissues, we should expect to find the two types of attack—with and without preliminary phosphorylation of the sugar—occurring to different extents in tissues whose behaviour towards glucose apparently gives no indication of any difference in metabolism. A striking example of this would be given by the yolk-sac of the rat and mouse, the former showing purely phosphorylation and the latter none. On the other hand, the two mechanisms might be combined in any one tissue in any proportion, resulting in an attack on fructose intermediate between the autoglycolysis and the glycolysis. This fundamental question of carbohydrate utilisation by animal tissues, which for lack of suitable material has not yet received any systematic investigation, obviously cannot be decided on the evidence at present available. In view of its importance to carbohydrate metabolism in general, we propose to continue investigations on the suitable tissues provided by the embryonic material whose behaviour has been described in the present paper.

SUMMARY.

1. A study has been made of the anaerobic conversion of fructose and glucose into lactic acid by the foetal membranes (outer membrane, amnion, yolk-sac, allantois), the placental tissues, the whole embryo and the tissues

of the later embryo (liver, lung, brain and skin). The measurements were made throughout at p_H 7.5 in bicarbonate-Ringer solution containing 0.2 % of the sugar.

2. Tissues of the rat, mouse, guinea-pig, rabbit, cat and ferret at different stages of pregnancy were examined. Whilst glucose is converted into lactic acid with great readiness by most embryonic tissues, the attack on fructose depends on both the tissue and the species, varying from zero to a value equal to that observed with the same tissue in glucose.

3. For a given tissue the figures for the relative attack on fructose and glucose may show a considerable spread. In view of this some of the results cannot be adequately summarised, but the following are the most probable conclusions.

Tissues with attack on fructose approaching or equalling that on glucose. Outer membrane of rat and guinea-pig; allantois of rabbit.

Tissues with intermediate attack on fructose. Placenta of ferret and cat; yolk-sac of cat.

Tissues with little attack on fructose. Placenta of rabbit, guinea-pig, rat and mouse; outer membrane of mouse, cat and ferret. Tissues of the embryo.

4. The amnion attacks fructose, though to a different degree in different species.

5. The placenta and peripheral membrane from the full-time human after-birth both attack fructose, though to a less extent than glucose.

6. Neither the whole embryo at mid-term (rat, rabbit) nor the isolated tissues of the later embryo mentioned under 1 above, are able to convert fructose into lactic acid, although fructose is known to be present in the foetal blood.

7. It is concluded that in all species in which fructose is known to be present in the foetal fluids, it is a possible source of energy to at least one of the extra-embryonic foetal tissues.

8. There is no apparent correlation between the fructolytic power and the development and morphology of the tissues examined.

9. In view of Meyerhof's suggestion that ability or inability to fructolyse is connected with the existence or absence of preliminary phosphorylation, the tissues examined may well prove of value in the elucidation of the early stages of intermediate carbohydrate metabolism.

We wish to express our thanks to Prof. E. C. Dodds for his interest in these experiments, and to Dr J. H. Woodger and Dr L. W. Proger of this Hospital for their advice, and for histological examination of some of our material.

One of us (F. D.) is a member of the Scientific Staff of the Medical Research Council, and the other (G. D. G.) wishes to make acknowledgments to the Halley Stewart Trust Fund for a scholarship.

REFERENCES.

- Armstrong and Armstrong (1931). The glycosides, p. 1. (Longmans, Green and Co., London.)
- Ashford and Holmes (1929). *Biochem. J.* **23**, 748.
- Bell, Cunningham, Jowett, Millett and Brooks (1928). *Brit. Med. J.* **1**, 126.
- Belosor (1925). *Zentr. Gynäkol.* **49**, 644.
- Bernard (1850). *Leçons de physiologie expérimentale*, 401.
- Cori (1925). *J. Biol. Chem.* **66**, 691.
- Dastre (1876). Inaug. Dissertation, Paris.
- Dawson (1932). *Biochem. J.* **26**, 531.
- Dickens and Šimer (1930). *Biochem. J.* **24**, 1301.
- Fernbach, Schoen and Mori (1927). *Compt. Rend. Acad. Sci.* **184**, 168, 551.
- Friedemann and Kendall (1929). *J. Biol. Chem.* **82**, 23.
- Fujita (1928). *Biochem. Z.* **197**, 175.
- Gerard and Meyerhof (1927). *Biochem. Z.* **191**, 125.
- Grosser (1909). Vergleichende Anatomie der Eihäute u. der Placenta. (Braumüller, Wien u. Leipzig.)
- (1927). Frühentwicklung, Eihautbildung u. Placentation. (Bergmann, München.)
- Gürber and Grünbaum (1904). *Münch. med. Woch.* **51**, 377.
- Harden and Young (1908). *Proc. Chem. Soc.* **24**, 115.
- (1909). *Proc. Roy. Soc. Lond. B* **81**, 336.
- Hayashi (1928). *Biochem. Z.* **196**, 323.
- Hopkins (1931). *Biochem. J.* **25**, 245.
- Ishikawa (1928). *Biochem. Z.* **195**, 469.
- Jenkinson (1913). Vertebrate embryology. (University Press, Oxford.)
- Kumanomido (1928). *Biochem. Z.* **193**, 315.
- Laquer and Meyer (1923). *Z. physiol. Chem.* **124**, 211.
- Loebel (1925). *Biochem. Z.* **161**, 219.
- Loeser (1927). *Klin. Woch.* **6**, 587.
- Meyerhof (1930). Die chemischen Vorgänge im Muskel. (Springer, Berlin.)
- Minami (1923). *Biochem. Z.* **142**, 334.
- Murphy and Hawkins (1925). *J. Gen. Physiol.* **8**, 115.
- Needham (1931). Chemical embryology. (University Press, Cambridge.)
- Negelein (1925). *Biochem. Z.* **165**, 122.
- Orr (1924). *Biochem. J.* **18**, 171.
- Paton, Watson and Kerr (1907). *Trans. Roy. Soc. Edin.* **46**, 71.
- Robinson (1892). *J. Anat. Physiol.* **26**, 308.
- Rosenthal (1930, 1). *Z. Krebsforschung*, **32**, 220.
- (1930, 2). *Biochem. Z.* **227**, 354.
- Schlubach (1932). *Naturwiss.* **20**, 273.
- Slator (1908). *J. Chem. Soc.* **93**, 238.
- Strahl (1906). In Hertwig's Entwicklungslehre der Wirbeltiere (Fischer, Jena), **1**, ii, 301.
- Warburg (1925). *Biochem. Z.* **160**, 307—footnote.
- (1926). Stoffwechsel der Tumoren. (Springer, Berlin.)
- (1927). *Biochem. Z.* **184**, 484.
- (1930). Metabolism of tumours. (Constable, London.)
- Posener and Negelein (1924). *Biochem. Z.* **152**, 309.
- Wierzechowski, Pieskow and Owsiany (1931). *Biochem. Z.* **230**, 146.
- Willstätter and Sobotka (1922). *Z. physiol. Chem.* **123**, 170.