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GLUCOSE METABOLISM DURING ONTOGENY OF INTESTINAL ACTIVE SUGAR TRANSPORT IN THE CHICK

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SUMMARY

1. Glucose transport, uptake, utilization, and lactate production by intestinal slices from embryos and young chicks have been determined by means of the *in vitro* tissue accumulation method. Changes in these parameters with age, after feeding, and in the presence of phlorrhizin have been measured, in most cases, under both aerobic and anaerobic conditions.

2. The embryonic intestine, 3 days before hatching, took up and utilized only a negligible quantity of glucose from the incubating medium and net lactate production was limited. The transport and utilization of exogenous sugar thus seem to be minimal at this age.

3. On the day of hatching, the intestine concentrated and utilized glucose, and lactate production was significant as was the inhibition of glucose uptake by phlorrhizin. This capacity to metabolize exogenous sugar appears to be consequent to onset of function of the sugar transport mechanism just before hatching.

4. At 2 days of age intestinal slices concentrated and utilized more glucose than at 0 days of age, if the chicks were not fed. After eating, glucose transport was decreased while lactate production was enhanced. Feeding schedules thus influence sugar transport and metabolism by the young chick intestine.

5. The metabolic parameters measured showed essentially the same relationships in intestinal slices from 8- and 36-day-old chicks as in 2-day-old birds. Although there were indications that intestinal maturation continues well into post-natal life, the most striking changes in functional capacity, observed in these studies, occurred during the several days around hatching.

INTRODUCTION

During the hatching period the chick intestine undergoes intensive differentiations which prepare it, presumably, for the onset of feeding (Argeseanu & May, 1938; Moog, 1950; Moog & Wenger, 1952). Coincident

with this final maturation of the intestine, the active transport mechanism for sugars becomes functional. This conclusion is based on both *in vitro* (Bogner & Haines, 1964) and *in vivo* evidence (Bogner, 1961; Bogner & Haines, 1961). Three days before hatching, embryonic intestinal slices do not accumulate galactose against a concentration gradient. Furthermore, glucose absorption from the intestinal tract of intact embryos proceeds at the same slow rate as passively absorbed sugars. By the day of hatching the intestine actively transports sugars. At this stage intestinal slices concentrate galactose and the *in vivo* absorption of glucose is depressed by phlorrhizin. Between 0 and 2 days of age the concentrating power of intestinal slices increases and *in vivo* glucose and galactose absorption rates rise sharply to a high level. The sugar transport mechanism in the chick gut thus appears to become active between -3 and $+2$ days around hatching.

The aim of experiments reported here was to determine whether or not intestinal glucose metabolism alters in conjunction with development of the sugar transport function. Information bearing on this question was sought by simultaneously measuring glucose transport, utilization and lactate production in embryonic and chick intestinal slices before, during and following onset of sugar transport. The observed indications of changes in glucose metabolism and their relation to development of the sugar transport system constitute the subject of this communication.

METHODS

Animals

Partially incubated white Leghorn chick embryos (Keystone cross) were obtained from a local hatchery. Incubation was completed in the laboratory in a Sears Roebuck cabinet electric incubator. The ages of the embryos and hatched chicks used in this study were -6 (days before hatching or 15 days incubated), -3, 0, 2, 4, 8 and 36 days. Sex of the chicks was not established. Hatched chicks were fasted before experiments for 48 or 24 hr or not at all (0 hr fasted group). Since newly hatched chicks possess an intra-abdominal yolk sac, withholding food from them is probably not equivalent to the fasting of adults.

Procedure

The *in vitro* tissue accumulation method (Agar, Hird & Sidhu, 1954) as described by Crane & Mandelstam (1960) was used. After severing the bird's cervical spinal cord, the entire small intestine (not everted) was removed and cut serially into small rings. These intestinal slices were added one at a time and in rotation to small beakers containing Krebs–Henseleit bicarbonate buffer (1932) gassed with either O_2:CO_2 (95:5) or N_2:CO_2 (95:5). Tissue samples consisted of pooled slices from at least two birds although as many as twelve intestines were sometimes required to achieve the desired wet tissue weight of 250–300 mg. The intestinal slices were incubated in buffer containing 0·002 M glucose for 15 min at 37° C in a Dubnoff incubator-shaker oscillating 100 times/min. Recovery and preparation of the tissues and media, as well as other procedural details, have been described elsewhere (Bogner & Haines, 1964).

Chemical estimations

D-glucose (Fisher Scientific Co.) was assayed by means of the glucose oxidase-peroxidase reaction as modified by Washko & Rice (1961). D-galactose (Fisher) was estimated as the difference between total reducing substances (according to Somogyi, 1952) and glucose oxidase positive material. Lactic acid was determined by the Barker & Summerson method (1941) as outlined by Barker (1961). Phlorrhizin was obtained from the Nutritional Biochemical Corp.

Calculation of results

The following terms are used in expressing the results:

T/M is the ratio of the sugar concentration in the tissue water to that in the medium at the end of the incubation.

Glucose uptake is the amount of glucose lost from the medium during incubation. It provides only an approximate estimate of the amount of sugar extracted from the medium by the tissues since it does not take into account back diffusion of glucose from tissue to medium.

Glucose utilization is the quantity of free sugar which disappears, as such, during the incubation. It is estimated by the difference between sugar added and that recovered from both the tissue and medium.

Net lactate refers to the quantity of lactate produced by tissues incubated in sugar in excess of that formed by controls (tissues incubated in a sugar-free medium).

The conversion of wet tissue weights to dry weights was based on values obtained in an earlier study of the per cent water content of intestines from different-aged embryos and chicks (Bogner & Haines, 1964).

RESULTS

Background observations

The embryonic intestine 6 days before hatching contains little stored glycogen. It has been shown that a transient accumulation of polysaccharide begins about 15 days after the start of incubation which reaches a peak at 18 days and then disappears by the day of hatching (Moog & Richardson, 1955; Moog & Thomas, 1957).

The aim of the present experiment on the -6-day-old embryonic intestine was to determine the sugar transport and utilization capacity before the appearance of the glycogen stores. Accordingly, pooled tissue slices from twelve embryos were incubated with either glucose or galactose for 15 and 30 min intervals in an O_2:CO_2 (95:5) atmosphere. In all cases the tissue water to medium sugar concentration ratios (T/M) fell between 0·2 and 0·3, suggesting that sugar entry into the tissue was not through an active transport process. The uptake and utilization of glucose by these slices was, nevertheless, significant in that it ranged between 25 and 35 μ-moles/g dry wt. or about one half that observed in 0-day-old slices.

Endogenous glucose in the -3-day-old embryonic intestine. Three days before hatching the embryonic intestinal slices contain substantial amounts of reducing substances of which some 23–44 % appears to be free glucose (Bogner & Haines, 1964). The source of the glucose is probably the glycogen stored in the duodenal epithelium at this age. The quantity of

free glucose can be reduced by incubating the tissues in a sugar-free medium and its disappearance rate is greater in N_2 than in an O_2 atmosphere. In some cases, however, the glucose content begins to rise again after a prolonged incubation. The results of a typical experiment are presented in Fig. 1. The fluctuations in the endogenous glucose content of the embryonic intestine near term constitute an important variable to be considered in sugar transport studies on this age group.

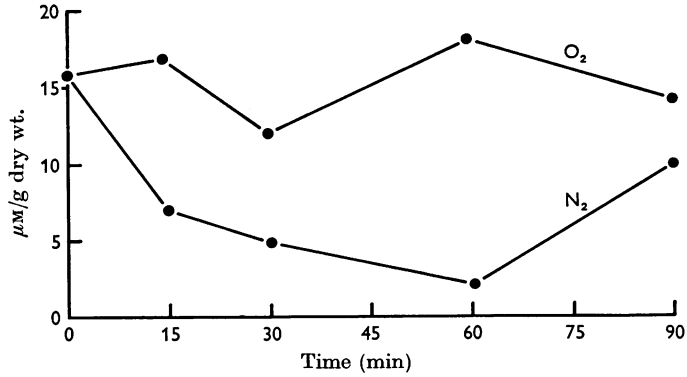

Fig. 1. Change in endogenous glucose content of −3-day-old embryonic intestinal slices incubated in sugar-free buffer for varying time periods. Gaseous atmospheres were O_2:CO_2 (95:5) or N_2:CO_2 (95:5). All data presented were collected during a single experiment in which each tissue sample consisted of pooled intestinal slices from twelve embryos.

Lactate production by intestinal slices incubated in a sugar-free medium for varying periods of time is presented in Fig. 2. In all cases in which the tissues were oxygenated the total lactate content of the incubating flasks decreased with time. These data suggest that sufficient O_2 was available to these tissues to permit aerobic metabolism to keep pace with glycolysis. The accumulation of lactate in vessels containing slices exposed to a N_2 atmosphere varied with both the age of birds and the feeding regimens before experiments. Intestinal slices exhibiting the most rapid and best sustained glycolytic rates were those from the −3-day-old embryos and the fed chicks. The intestines which produced the least lactate were those from the 0-day-old non-fed and 48 hr fasted chicks. These data suggest that the glycogen stored in the intestinal epithelium of the late embryos and the eating of exogenous food both act to promote glycolysis.

Experiments with glucose in the incubating medium

Tissue to medium concentration gradients (T/M) developed by intestinal slices during a 15 min incubation with glucose are reported in Table 1 and Fig. 3. In an O_2 atmosphere all age groups—with the possible excep-

tion of the −3-day-old embryos—demonstrated a capacity for actively transporting glucose. The question with regard to the apparent transport by the embryonic intestine arises from the presence of free endogenous glucose in these tissues which serves to elevate the T/M. If this glucose is sequestered in only a few cells, or parts of cells, then it should possibly be excluded from the calculation. An observation in line with this is that

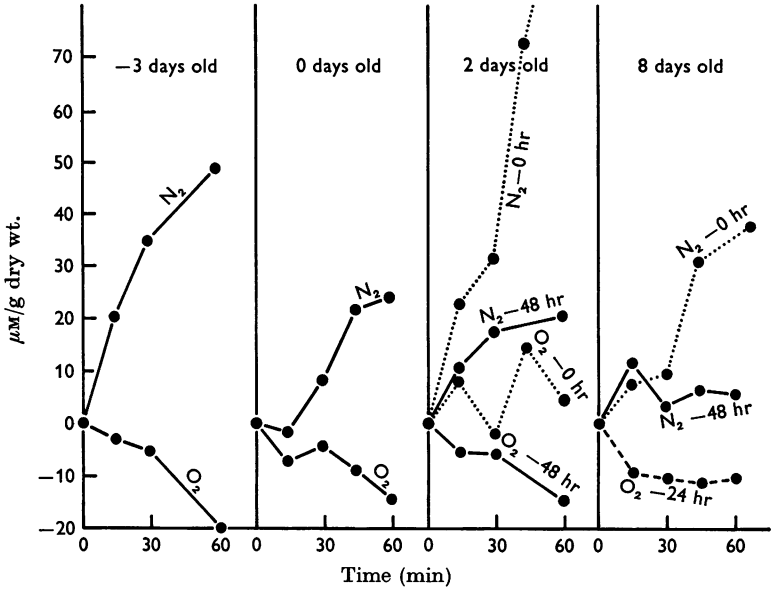

Fig. 2. Lactic acid formed by intestinal slices incubated in sugar-free buffer for varying time periods. Gaseous atmospheres were O_2:CO_2 (95:5) or N_2:CO_2 (95:5). The numbers of hours chicks were fasted before experiments are indicated by the notations −0 hr, −24 hr, −48 hr.

reducing the glucose content of the tissue leads to a lowering of the T/M value toward one. It is also pertinent that galactose is not accumulated by these tissues. Active sugar transport by the −3-day-old intestine must therefore be limited—if it occurs at all.

After hatching, active transport of glucose by the chick intestine depends upon the presence of O_2 and appears greatly influenced by the eating of food. In general the concentrating power of the intestine was increased by prolonging the fasting period before experiments. Similar effects of fasting on intestinal sugar transport have been reported by others (Crane & Mandelstam, 1960; Kershaw, Neame & Wiseman, 1960).

None of the intestinal slices incubated in a N_2 atmosphere transported the glucose against a concentration gradient. Nevertheless, tissues from the 48 hr fasted chicks contained more free glucose after incubation (or

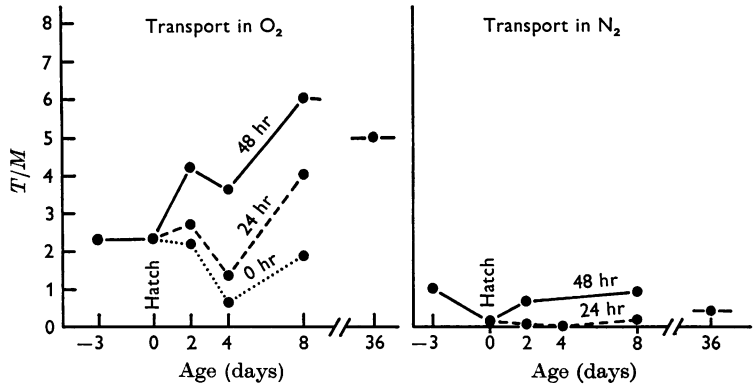

Fig. 3. Ratio of the glucose concentration in the tissue water to that in the bathing medium (T/M) after a 15 min incubation period at 37° C. Initial glucose concentration in medium was 0·002 M. Gaseous atmospheres were O_2:CO_2 (95:5) or N_2:CO_2 (95:5). The numbers of hours chicks were fasted before experiments are noted as 0 hr, 24 hr or 48 hr.

TABLE 1. Glucose transport, uptake, utilization and lactate production by chick intestinal slices

Age in days	Hr fasted	Phlor. concn.	No. of expt.	T/M	Glucose uptake	Glucose utilized	Net lactate
−3	0		8	2·3±0·2	5±1	0	6
	*0		3	1·0±0·2	19±7	14	30 (1)
0	0		6	2·3±0·6	67±8	50	48 (5)
	*0		5	0·1±0·1	47±9	46	69 (3)
	0	1×10^{-5} M	2	0·8±0·4	26±9	20	35
	0	2×10^{-4} M	2	0	12±12	12	3
2	48		5	4·2±0·5	81±7	59	52 (3)
	*48		3	0·6±0·3	74±2	71	60
	24		5	2·7±0·3	70±7	55	75 (2)
	*24		5	0·1±0·1	64±7	64	99 (3)
	0		2	2·2±0	65±3	58	—
4	48		2	3·6±0·1	81±10	63	—
	24		3	1·4±0·2	61±3	52	—
	*24		3	0	52±2	52	69 (1)
	0		2	0·6±1·7	66±4	64	—
8	48		6	6·0±1·1	84±5	53	41 (4)
	*48		4	0·9±0·2	65±6	59	56
	24		10	4·0±0·5	61±7	47	82 (5)
	24	1×10^{-5} M	2	1·0±0·1	46±5	41	40
	24	2×10^{-4} M	2	0·2±0·1	26±3	26	16
	*24		3	0·2±0·1	57±16	57	107 (1)
	0		2	1·9±0·5	62±26	53	—
36	48		1	5·0	71	45	26
	48		1	0·4	65	63	34

Incubations were 15 min at 37° C in 0·002 M glucose. A random selection of intestinal slices from no less than two birds were pooled for each experiment. An asterisk marks the anaerobic experiments. All values are means; the standard errors are included for some data. Glucose uptake, utilization, and net lactate are in terms of μ-moles/g dry wt. Numbers in parentheses show the no. of expts. upon which a value is based when it differed from other data in the same line of the table.

filled to a greater extent) than did the slices from the 24 hr fasted birds. This enhanced 'filling' in response to the more prolonged fast may be an example of an increased activity of the Na-dependent, energy-independent sugar entry process proposed by Bihler, Hawkins & Crane, 1962. These authors visualize the process as one which mediates the rapid equilibration of certain sugars between tissue and medium. Another possibility, however, is that the transport mechanism was activated by the extended fast but not to an extent capable of bringing about the accumulation of a sugar as rapidly utilized as glucose. The latter alternative is suggested by the finding that anaerobic active transport of galactose occurs in the young chick intestine if a 48 hr fast precedes experiments (Bogner, Haines & McLain, 1962).

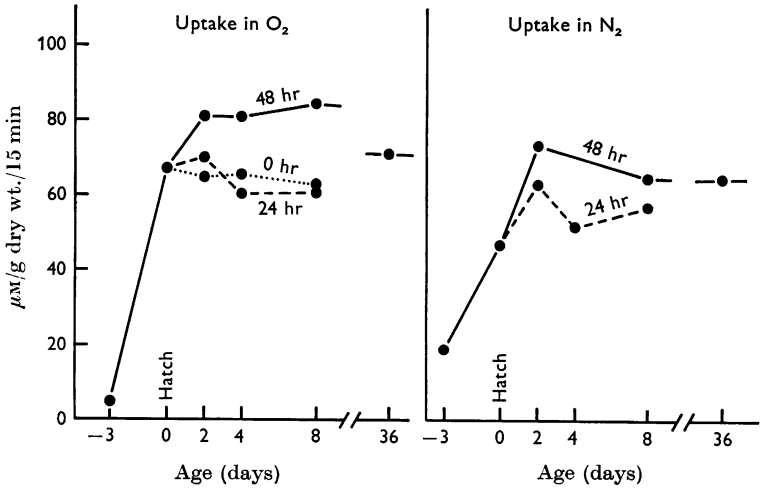

Fig. 4. Glucose uptake from the medium by intestinal slices during a 15 min incubation period. Other experimental conditions were as described in Fig. 3.

The glucose uptake by intestinal slices from the bathing medium (equal to the free sugar retained within the tissue plus the amount metabolized) is reported in Table 1 and Fig. 4. One of the most striking features of these data is the relative inability of the −3-day-old intestine to extract glucose from the medium. This characteristic must represent a transient phenomenon in view of the demonstrated capacity of the −6 and 0-day-old intestines to both take up and utilize glucose. The rise in glucose uptake between −3 and +2 days of age, although more pronounced in oxygenated tissues, also occurred in the absence of O_2. These data are entirely consistent with evidence that an active transport mechanism—capable of functioning under both aerobic and anaerobic conditions—develops in the chick intestine during the hatching period (Bogner & Haines, 1964;

Bogner *et al.* 1962). Finally, the data show that glucose uptake after fasting is greater than glucose uptake after feeding. Since the concentrating power of the intestine also increases after fasting it would appear that the transport mechanism for sugars becomes more active when food is withheld. A similar effect has been observed in studies with the poorly utilized sugar, galactose (P. H. Bogner, unpublished observations).

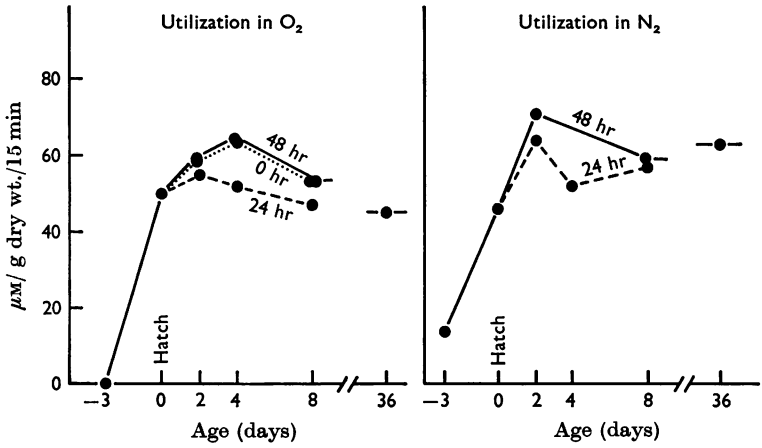

Fig. 5. Glucose utilization by intestinal slices during a 15 min incubation period. Other experimental conditions were as described for Fig. 3.

Glucose utilization by intestinal slices (equal to glucose uptake less the free glucose retained in the tissues) is reported in Table 1 and Fig. 5. The negligible utilization of glucose from the medium by the −3-day-old embryonic intestines is an expected consequence of their inability to take up glucose. The rise in glucose utilization between −3 and +2 days of age most likely reflects the increasing capacity to transport sugar which occurs at this time. However, the possibility that metabolic pathways for glucose appear or are activated during this period may also be a contributing factor. Certainly the rise in utilization between 0 and 4 days of age, in the presence of an excess of free sugar intracellularly, is suggestive of such a change. The influence of fasting on utilization, as measured here, is unclear since similar curves were obtained for oxygenated tissues from both the 0 and 48 hr fasted birds.

Net lactate production by intestinal slices incubated with glucose (over and above the lactate formed by controls) appears in Table 1 and Fig. 6. The small amount of net lactate produced by the −3-day-old embryonic intestine is in accord with the finding that these tissues take up very little glucose from the medium. The increased production of lactate between −3 and 0 days of age indicates that substantial quantities of the

medium glucose gained access to the intracellular site due, presumably, to onset of function of the sugar transport mechanism. After hatching the glycolytic rate of the chick intestine appears to be promoted by the intake of exogenous food. The data clearly show a more rapid glycolysis after a 24 hr fast than after a 48 hr fast. A similar effect on the inherent glycolytic rate of controls was noted in an earlier section. This enhancement in lactate production is in contrast to the depression of glucose uptake and accumulation which occurs after feeding.

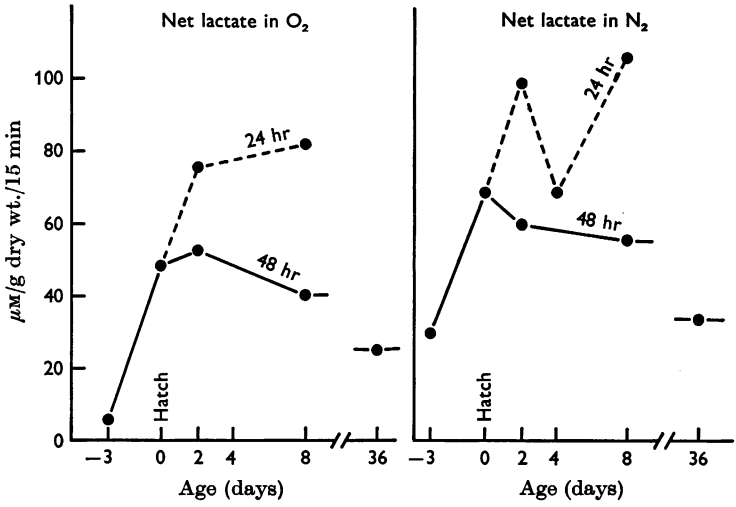

Fig. 6. Net lactate production by intestinal slices incubated under conditions described in Fig. 3.

The proportion of the glucose utilized which was converted to lactate is presented in Fig. 7. By graphing the data in this manner it becomes clear that the intestinal glycolysis was promoted more by feeding than by anaerobic conditions in these experiments. The inability of the 48 hr fasted intestines to increase their lactate production in a N_2 atmosphere suggests that the glycolytic potential of these tissues is seriously limited.

Another observation worthy of note is the depressed utilization and lactate production of the 4-day-old intestines incubated in N_2. Tissue accumulation of glucose is also less at this age (see Fig. 3) as is the *in vivo* sugar absorption rate (unpublished data). The possible significance of these findings will be considered in the discussion.

Phlorrhizin inhibition studies. Glucose uptake, utilization and net lactate production by intestinal slices incubated with phlorrhizin, in O_2, are compared with the normal experiments in Table 1 and Fig. 8. The older chicks were fasted for 24 hr before experiments.

With the lower concentration of phlorrhizin (1×10^{-5} M) the most

profoundly affected parameter measured, in the 0-day-old chicks, was glucose uptake from the medium. This inhibition was of the order of 60%. The accompanying small reduction in glucose utilization and lactate formation was an expected consequence of the decreased entry of sugar into the cells. Similar results and conclusions have been reported by others using comparable concentrations of phlorrhizin (Parsons, Smyth & Taylor, 1958). The 25% inhibition of glucose uptake by the 8-day-old intestine, associated as it was with a 50% reduction in lactate production, suggests that the phlorrhizin interfered with glycolysis as well as uptake in these tissues. With the higher concentration of phlorrhizin (2×10^{-4} M) both glucose uptake and lactate formation were further suppressed. The

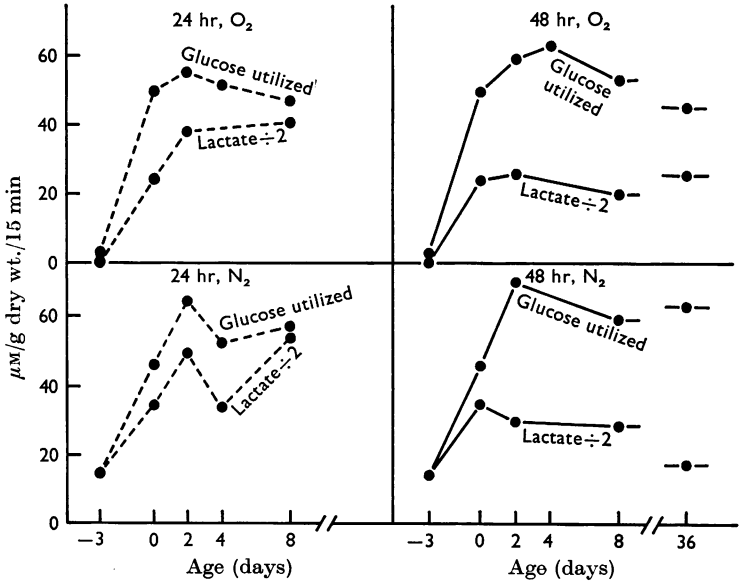

Fig. 7. The amount of glucose from the incubating medium which was metabolized to lactic acid by the chick intestinal slices. The lactate data are presented as equivalents of glucose to aid in visualizing the extent of the glucose to lactic acid conversion. Other experimental conditions were as described for Fig. 3.

magnitude of the effect on the lactate suggests that the phlorrhizin may have exerted a direct effect on glycolysis. High concentrations of phlorrhizin have been found to inhibit intestinal endogenous metabolism (Parsons *et al.* 1958).

The suspected inhibition of glycolysis in three of the four above experimental groups opens up the possibility that it was this action of phlorrhizin—and not the blocking of active transport sites—that led to the reduced entry of sugar into the cells. This criticism does not apply, however, to the experiment on the 0-day-old intestine incubated with

1×10^{-5} M phlorrhizin. In this one case, at least, the action of phlorrhizin appears to have been exerted at the cell surface on the sugar transport system.

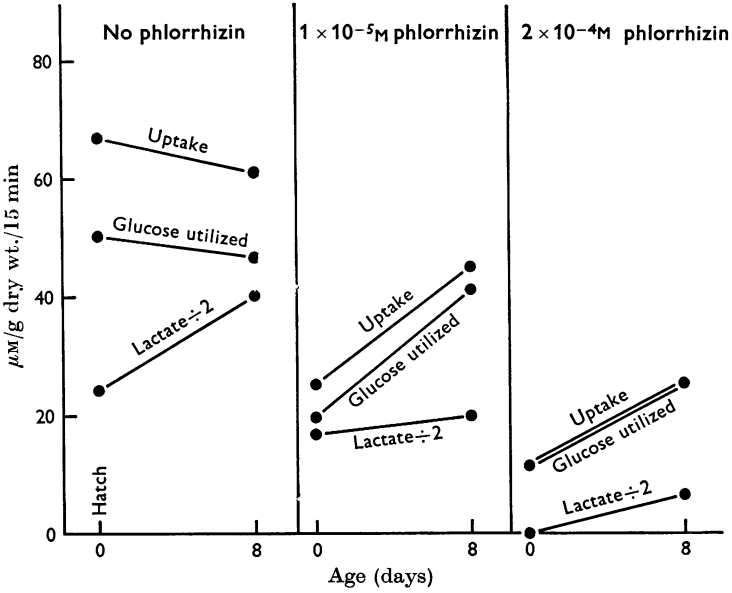

Fig. 8. Glucose uptake, utilization and net lactate production (in equivalents of glucose) by intestinal slices incubated in buffer containing phlorrhizin. Other experimental conditions were as described for Fig. 3.

DISCUSSION

Substantial evidence indicates that the active transport mechanism for sugars in the chick intestine becomes functional between -3 and $+2$ days around hatching (see introduction for references). Some metabolic changes associated in time with maturation of this process, as revealed by experiments with glucose, will be discussed below by age groups.

Embryonic intestine. The most outstanding characteristic of the -3-day-old embryonic intestinal slices was their inability, under the conditions of these experiments, either to take up or to utilize glucose from the incubating medium. As a consequence, little or no net lactic acid was produced in either the O_2 or N_2 atmosphere despite a considerable glycolytic potential, as indicated by the continued formation of lactic acid over a 90 min period by control slices. The only suggestion that these tissues might have a capacity for active glucose transport was the final tissue to medium concentration ratio (T/M) of about 2·0. The content of free endogenous glucose in these cells appears to be largely responsible, for this T/M value for preincubation to reduce its quantity lowers the T/M

toward 1·0. Furthermore, somewhat younger embryonic slices, which contained no free glucose and probably little glycogen, gave T/M values between 0·2 and 0·3 for both glucose and galactose after both 15 and 30 min incubation intervals. All of these observations taken together suggest that active transport, if it occurs at all in −3-day-old slices, is minimal at best. The absence of uptake and utilization of exogenous glucose seems therefore to be the result of both a slow entry of sugar into the cells and an already maximal glycolytic rate due to the glycogen stores in these tissues. The endogenous substrate would appear to be glycolysed in preference to the exogenous glucose, perhaps by keeping the enzymes of the pathway saturated.

The 0-day-old intestine. Between −3 and 0 days of age the chick intestinal slices acquired a marked capacity for transporting extracellular glucose into the intracellular site. In an O_2 atmosphere glucose accumulated within the tissue in spite of considerable utilization of the sugar, thus providing direct evidence that active glucose transport had occurred. This is precisely the time during which active transport of the non-utilizable sugar galactose begins (Bogner & Haines, 1964). The inhibition of sugar uptake by phlorrhizin at this age both *in vivo* (Bogner & Haines, 1961) and *in vitro* provides additional supporting evidence that active sugar transport is indeed underway by the day of hatching.

The rise in glucose utilization and lactate production observed in oxygenated intestinal slices between −3 and 0 days of age most probably reflects the onset of function of sugar transport. A similar explanation may apply to the tissue incubated in N_2 even though utilization kept pace with sugar uptake in these experiments. This latter conclusion is based on evidence that the transport mechanism functions under anaerobic conditions in the young chick intestine (Bogner *et al.* 1962). A capacity for anaerobic active transport is not a unique feature of the chick; it has been shown to occur in the new-born intestine of other species as well (Wilson & Lin, 1960).

In order to understand better the functional status of the newly hatched chick intestine, it is helpful to keep in mind the following: The 0-day-old intestine, unlike that of the late embryo, contains no measurable free glucose or glycogen (Moog & Thomas, 1957); several intracellular enzymes are in the process of accumulating in the mucosa (Moog, 1950; Richardson, Berkowitz & Moog, 1955; Nunnally, 1962) and the microvilli are differentiating at this time (Argeseanu & May, 1938; Moog, 1950; Moog & Wenger, 1952); at hatching the R.Q. of the chick is about 0·7 but rises to 1·0 within a few days thereafter (Needham, 1942), and there is a large intra-abdominal yolk sac which presumably provides the bird with some lipid and protein nutrients for a short while after birth. It is therefore reasonable to suppose

that the biochemical and morphological differentiations of the intestine at hatching underlie the functional development of the sugar transport. Furthermore, the simultaneous rise in exogenous glucose uptake, depletion of intestinal glycogen stores, and the transition to carbohydrate as a primary energy source seem likely to be more than coincidental events. All of these changes may represent, to some degree, a series of co-ordinated preparations within the organism for the onset of feeding.

Chicks 2-days-old never on food. These chicks rather closely resembled the 0-day-old birds in that they (1) received no food between hatching and experiments, (2) had similar body weights, and (3) possessed a substantial yolk sac. Nevertheless, the T/M values obtained in the O_2 experiments show that the concentrating power of the older intestines was approximately twice that of the 0-day-old slices. These data are consistent with the rise in intestinal sugar absorption between 0 and 2 days of age observed *in vivo* (Bogner & Haines, 1961) and suggest that the latter is consequent to an increase in the activity of the sugar transport mechanism.

A small but demonstrable rise in glucose utilization by the oxygenated intestinal slices accompanied the increase in concentrating power discussed above. Since this change occurred in tissues containing an excess of free intracellular glucose, there is reason to believe that the capacity of the intestine to metabolize glucose also improves during the 2 days after hatching.

In the N_2 experiments the intestinal slices did not concentrate the medium glucose. However, glucose uptake and utilization increased substantially between 0 and 2 days of age. Since the T/M value also increased it appears that the rise in glucose uptake somewhat exceeded the simultaneous rise in utilization. One explanation of these data derives from evidence that the sugar transport mechanism in the young chick's intestine functions under anaerobic conditions (Bogner *et al.* 1962). It is therefore possible that the anaerobic transport activity also increased after hatching but not to an extent capable of bringing about the accumulation of a rapidly metabolized sugar.

Fed 2-day-old chicks. The intestinal slices from this group of chicks showed a distinctly lower level of glucose uptake and accumulation than did intestines from 2-day-old non-fed birds. In contrast, glucose utilization remained relatively constant while lactate production was considerably elevated. The eating of food seems therefore to depress glucose transport but not utilization by the intestine. The increased lactate production further suggests that intestinal glycolysis becomes more active after feeding. This apparent suppression of active sugar transport in the presence of a well maintained, if not increased, utilization of glucose may be an expression of a shift in available energy from transport to the more

vegetative activities of cells well fortified with nutrients. This speculation suggests that the local needs of the intestinal tissue may be cared for primarily during periods of adequate nutrition. A corollary to this is that fasting promotes the transport of glucose into the bloodstream. In this situation, the nutritional needs of the organisms as a whole would appear to have priority over those of the intestinal cells.

Chicks 4 *days of age.* Although our observations on this age group were limited, they were sufficiently consistent to suggest that sugar transport and lactate production by the intestine are reduced at this age. However, glucose utilization (as we measured it) appears to be well maintained. A possible interpretation of these findings stems from evidence that maturation of the chick intestine, although largely completed during the hatching period, continues for perhaps a week thereafter. The differentiation of an oxidative pathway appears to be in progress during the 4 or 5 days after hatching (Nunnally, 1962), while recent results in this laboratory indicate that transport activity in the lower intestine increases between 2 and 9 days of age (Bogner, Haines & McLain, 1963*a*, *b*). If the 4-day-old chick intestine is involved in differentiations which divert energy supplies away from transport and glycolysis, then our results become meaningful.

Chicks 8 *and* 36 *days of age.* The intestinal characteristics of these birds were grossly similar to those of 2-day-old chicks. The only notable changes which occurred with increasing age were a rise in T/M values and a decrease in lactate production after a 48 hr fast. The increased concentrating power of the intestine may be due to the increase in transport capacity of the lower two-thirds of the intestine which occurs between 2 and 9 days of age (Bogner *et al.* 1963*a*, *b*). This change was not apparent, however, when random slices from the whole small intestine were used, as in these experiments (Bogner & Haines, 1964). The drop in lactate formation with age may be associated with the well known post-natal shift from an anaerobic to a more aerobic type of metabolism.

With these rather delayed effects, notwithstanding, it does seem clear that the most striking changes in intestinal function do occur in the hatching period when intestinal differentiations are maximal and the tasks of digestion and absorption are about to begin.

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