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Regulation of Development of Hepatic Glucokinase in the Neonatal Rat by the Diet

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1. Feeding a high-glucose diet to weanling rats showed that high hepatic glucokinase activities could be induced at 18 days of age, i.e. 2 days after development of the enzyme begins. 2. The normal development of glucokinase activity can be retarded by weaning rats on to carbohydrate-free, high-fat and high-protein diets. 3. Precocious development of the enzyme before 16 days of age cannot be induced by oral glucose administration. 4. It is concluded that the ability to synthesize glucokinase develops very rapidly and that the nature of the diet determines the normal developmental pattern.

The development of hepatic glucokinase (ATP–D-glucose 6-phosphotransferase, EC 2.7.1.2) in the suckling rat between 16 and 28 days after birth, like the increase in activity that occurs on re-feeding starved animals with glucose or treating alloxan-diabetic rats with insulin (reviewed by Walker, 1966*a*), appears to represent the synthesis of new protein (Walker & Holland, 1965). When neonatal rats were starved for 3 days during the period of normal glucokinase development and then re-fed with glucose overnight, quite high glucokinase activities were recorded, suggesting that the ability to synthesize the enzyme continued to develop during the period of starvation and that this potential was rapidly expressed when the animals were re-fed. In addition to a requirement for both glucose and insulin (Walker & Holland, 1965) therefore it seemed probable that physiological factors such as the diet influence the development of hepatic glucokinase. The present paper shows that the ability to synthesize glucokinase develops very rapidly and that the nature of the diet determines the rate of appearance of activity. A preliminary report has been given (Walker, 1966*b*).

METHODS

Animals. The rats were of the Wistar strain maintained as described by Walker & Rao (1964). Birth dates of all litters were recorded after twice-daily inspections. Litters were culled to ten 2 days after birth by eliminating weakling animals. Whenever young animals were removed from their mother they were kept in a well-ventilated incubator at 28°.

Because of the size and immaturity of the newborn rat, completely artificial rearing requires very elaborate procedures not available to us and impracticable to arrange for a limited number of experiments. Compromise procedures were therefore developed and studied whereby, when it was wished to feed neonatal animals with an artificial liquid diet before normal weaning, the animals remained for 12hr. of the day (overnight) with a lactating female and were fed artificially during the daytime. Further details of this regimen are given in the legends to the appropriate Tables. The liquid diets (details below) were administered by intubation into the oesophagus with a ball-tipped serum needle (made in the Departmental workshop) as described by Miller & Dymsza (1963). The volume of diet administered was gradually increased with age as follows: 8-day-old rats received three feeds of 0·5ml. during the day period; for 12-day-old rats the volume was 0·8–1·0ml.; 15-day-old animals received 1·2–1·5ml. per feed. At the time of each feed the rats were stroked in the perianal area to stimulate urine excretion. Animals weaned at 16 days were usually able and willing to drink the liquid diet from an open dish, but a teat-pipette was used to encourage milk intake when necessary during the first 2 days after weaning.

The solid diets (see below) were offered to weanling rats in a crumbled form. Most weanling animals readily ate this diet; failure to do so was readily indicated by a marked loss of weight. The groups of weanling animals were kept together in one cage for practical reasons; this meant that individual food intakes could neither be regulated nor assessed. Much food was found scattered in the cages, and the supply was replenished twice daily until the animals were old enough for the diet to be pelleted and put in a wire hopper in the normal manner.

These procedures were adopted after preliminary trials that showed that suckling rats aged 8–15 days continued to show regular normal weight increases during the daily normal–artificial feeding regimen and that weanling rats aged 16 days upwards, after a short break of about 1 day immediately after weaning from the mother, continued to increase in weight regularly, if not always at the full normal rate. Details of weight increases are recorded in the Tables.

Diets. The normal liquid diet was based on a recommendation (S. A. Miller, personal communication) of a synthetic milk suitable for rearing rat pups and containing, in a total volume of 100 ml.: 7 g. of protein (casein, Hammarsten quality, British Drug Houses Ltd.), 15 g. of corn oil B.P., 2 g. of dextrin, 0·5 g. of salt mixture (U.S.P. XIV), 0·08 g. of choline and 1 g. of vitamin mixture (see below); these components were thoroughly mixed in an MSE homogenizer. The vitamin mixture was based on that recommended by Dymsza, Czajka & Miller (1964) and contained: 35 mg. (325000 units) of vitamin A, 200 mg. of vitamin E acetate (25%, w/v), 0·2 mg. (32500 units) of vitamin D_2, 1 mg. of vitamin K, 0·0106 g. of thiamine hydrochloride, 88·5 mg. of riboflavine, 220 mg. of nicotinamide, 525 mg. of ascorbic acid, 7·6 mg. of pyridoxine hydrochloride, 1 mg. of *p*-aminobenzoic acid, 0·8 mg. of biotin, 83·6 mg. of calcium pantothenate, 0·3 mg. of folic acid, 6·23 g. of inositol and 0·3 mg. of vitamin B_{12}; all these vitamins were triturated with 92·6 g. of dextrin to make 100 g. of mixture.

Glucose feeding in liquid form was achieved by modifying the above liquid diet by the addition of 15% (w/v) glucose and decreasing the corn oil content to 10% (w/v) for animals aged 8–12 days; the respective amounts for animals aged 13–17 days were 30% and 5%.

The composition and method of preparation of the solid diets are given in Table 1; these diets are approximately isocaloric and they were available *ad lib.*

Determination of glucokinase and hexokinase. The procedures used followed the general pattern described by Walker & Rao (1964) and Walker & Holland (1965). One modification was introduced: to ascertain that in all the various conditions of animals assayed 2 moles of $NADP^+$ were reduced/mole of glucose 6-phosphate formed, 0·2 unit of partially purified 6-phosphogluconate dehydrogenase, which was prepared by the method of Glock & McLean (1953) and was free of hexokinase activity, was added to the incubation mixture. The difficulties of measuring low glucokinase activities have been discussed (Walker & Holland, 1965). One of the primary difficulties is now known to be due to the occurrence of isoenzyme forms of the hexokinase (González, Ureta, Sánchez & Niemeyer, 1964; Katzen & Schimke, 1965; Grossbard & Schimke, 1966) and, in particular, to the presence of one form of hexokinase (type III) that is inhibited by high glucose concentrations (Grossbard & Schimke, 1966). For these reasons, glucokinase activities lower than 0·1 μmole/min./g. wet wt. are subject to considerable error.

Rates of glucose phosphorylation have been calculated per g. wet wt. of liver, per whole liver, per 100 g. body wt. and on a protein basis, but, because no further information can be deduced from the other data, are usually expressed in the Results section on the first basis only. Statistical significance of means and differences were calculated by a *t* test. Protein was determined by the method of Gornall, Bardawill & David (1949) with bovine serum albumin as a standard.

RESULTS

The results of three types of experiments are presented showing that (*a*) high glucokinase activities can be induced by high-glucose feeding of rats older than 16 days, (*b*) the normal development of glucokinase activity is retarded by diets lacking glucose or rich in fat or protein or both, and (*c*) high-glucose feeding orally does not bring about precocious development of the ability to synthesize glucokinase.

Induction of high glucokinase activites in rats from 18 days onwards. The normal pattern of glucokinase development (Walker & Holland, 1965) shows a very low rate of increase over the period 16–20 days. When neonatal rats were weaned prematurely on to a solid diet consisting of either 30% glucose–70% laboratory diet or an artificial diet containing 60% of glucose, each supplemented with milk (Table 2), hepatic glucokinase activities of over

Table 1. *Composition of solid diets*

The mixes (in g./100 g.) together with 0·25 g. of vitamin mixture (as described by Baker, Chaikoff & Schusdek, 1952) were moistened with 0·5% non-nutrient agar that had been liquefied by warming and allowed to set to a cake as described by Kornacker & Lowenstein (1965).

	Normal (A)	High-glucose (B)	High-fat (C)	High-protein (D)	Carbohydrate-free (E)
Glucose	—	60	—	—	—
Dextrin	60	—	—	—	—
Solkafloc, cellulose (Johnsen, Jorgensen and Wettre Ltd., London, E.C. 4)	6	6	36	14	25
Liver, desiccated (Oxoid, London, E.C. 4)	2	2	2	2	2
Salt mixture (U.S.P. XIV)	6	6	6	6	6
Casein, low-vitamin content (Genatosan Division, Fisons Pharmaceutical Ltd., Loughborough, Leics.)	22	22	32	74	53
Corn oil B.P. (Boots Pure Drug Co., Nottingham)	4	4	24	4	14

Table 2. *Effect of glucose in inducing high activities of hepatic glucokinase precociously in weanling rats*

The animals were weaned from their mother on the first day of the period and given one of two diets. In Expts. 1, 2 and 4 the animals were offered *ad lib.* a crumbled diet containing 30% glucose+70% normal laboratory diet. In Expts. 3 and 5 the food offered was a synthetic high-glucose diet (diet B, Table 1). In all cases the animals had free access to both water and the normal liquid diet, as described in the Methods section. Assays were performed as described in the text and the results are given as means ± S.E.M.

Expt. no.	Age period on special diet (days)	No. of animals	Mean wt. increase over period on diet (g.)	Body wt. (g.)	Liver wt. (g.)	100 × Mean liver wt./mean body wt.	Supernatant protein (mg./g. of liver)	Glucose-phosphorylating activity (μmoles of glucose phosphorylated/min./g. of liver)		
								Total	Glucokinase*	Hexokinase
1	16–18	8	1·8	30·0 ± 2·2	1·40 ± 0·07	4·7	84·7 ± 2·3	2·10 ± 0·15	2·01 ± 0·14	0·09 ± 0·01
2	16–20	6	3·6	35·3 ± 1·2	1·36 ± 0·06	3·9	99·5 ± 3·7	2·97 ± 0·19	2·79 ± 0·16	0·19 ± 0·04
3	16–20	4	2·2	30·2 ± 0·5	1·41 ± 0·07	4·7	—	2·33 ± 0·20	2·21 ± 0·23	0·12 ± 0·06
4	19–23	4	3·5	21·9 ± 1·5	1·13 ± 0·03	3·5	114·0 ± 3·8	2·51 ± 0·11	2·33 ± 0·11	0·18 ± 0·02
5	19–23	12	4·2	29·2 ± 2·1	1·48 ± 0·09	5·1	—	2·50 ± 0·15	2·39 ± 0·16	0·11 ± 0·04

* These glucokinase activities may be compared with those quoted by Walker & Holland (1965) and also with random control assays performed at the same time as these determinations, which gave activities 0·06 ± 0·02 (6), 0·14 ± 0·05 (6) and 0·86 ± 0·05 (8) for 16-, 20- and 23-day-old animals respectively.

2 μmoles of glucose phosphorylated/min./g. of tissue were recorded when the animals were killed 2 or 4 days later. These activities are higher than found in adult rats on the laboratory diet (Walker & Rao, 1964) and are approaching those for adult rats on a high-glucose diet (Walker, Khan & Eaton, 1966). The weaned animals did not put on as much weight as normal but litter mates weaned at the same time on to ordinary laboratory diet plus milk gained even less in weight and showed only very low glucokinase activities when killed and assayed. The latter animals are not, however, appropriate controls in these experiments. The important finding was that it is possible to induce high glucokinase activities in rats from 18 days of age onwards.

Retardation of glucokinase development by weaning on to carbohydrate-free diets. A group of experiments was performed in which rats were weaned at 16 days of age on to various types of solid diets, supplemented by milk made available by bottle. These clearly showed that carbohydrate-free diets resulted in much lower glucokinase activities at 23 days than normal, but were not entirely satisfactory because the animals did not gain very much weight and the liver wt./body wt. ratios suggested an inadequate dietary intake. A procedure was therefore devised (see the Methods section and the legend to Table 3) that permitted weaning the animals on to artificial solid diets while remaining with the lactating mothers and thus receiving the normal gradually declining milk supply. Table 3 shows that under these conditions all the animals made substantial growth with liver wt./body wt. ratios within the normal range. The animals weaned on to the high-protein diet (Expts. 2 and 3) did not increase in body wt., especially during the early stages, as did the controls or those on the high-fat diet. This suggests that the high-protein diet could not be utilized so successfully as an energy source by the weanling rats. The animals weaned on to the high-fat diet only developed very low glucokinase activities typical for adult animals on a high-fat diet (Niemeyer, Clark-Turri, Garcés & Vergara, 1962; Pérez, Clark-Turri, Rabajille & Niemeyer, 1964). The high-protein diet (Expt. 5) permitted the development of an appreciable activity but in all cases (Table 3) the various types of carbohydrate-free diets resulted in significantly ($P < 0·01$) lower glucokinase activities than those of the control litter mates.

Inability to induce precocious glucokinase development. Earlier attempts (Walker & Holland, 1965) to induce the appearance of glucokinase activity before the normal time by a variety of means, including intraperitoneal infusions of glucose, were unsuccessful. In view of the results in Table 2, a further series of experiments was designed to determine whether oral administration of glucose

Table 3. *Effect of carbohydrate-free, high-protein and high-fat diets on the development of hepatic glucokinase activity during the weaning period*

Each experiment involved eight litter-mates from each of two litters that had been culled to eight soon after birth. The two halves of each litter, taken at random when the animals were 16 days old, were pooled with half of the other litter to make the sets of eight animals, which were then placed in two cages containing either the normal synthetic or the other diet (see Table 1) as indicated below and described in detail in the Methods section. The mothers of the two litters were interchanged between the two cages at 9 a.m. each day during the experimental period. The neonatal animals thus were weaned on to a fixed diet while the mothers had the two diets on alternate days. Assays were performed as described in the text and the results are given as means +S.E.M.

Expt. no.	Diet	Age period on diet (days)	No. of animals	Body wt. when 16 days old (g.)	Body wt. when killed (g.)	Mean wt. gain (g.)	Liver wt. (g.)	100× Mean liver wt./ mean body wt.	Super-natant protein (mg./g. of liver)	Glucose-phosphorylating activity (μmoles of glucose phosphorylated/min.) Glucokinase (per g.)	Glucokinase (per whole liver)	Glucokinase (per 100g. body wt.)	Hexokinase (per g.)
1	Normal synthetic (A)	16–23	8	31·8±0·8	46·7±1·4	14.8	1·93±0·04	4·1	117±2·6	1·12±0·10	2·15±0·19	4·7±0·46	0·10±0·02
	Carbohydrate-free (E)		8	29·0±0·6	42·4±1·4	13·4	1·96±0·09	4·6	156±5·6	0·25±0·03	0·50±0·07	1·2±0·14	0·19±0·09
2	Normal synthetic (A)	16–23	8	26·5±0·8	39·6±1·5	13·1	1·82±0·12	4·6	110±4·5	0·67±0·03	1·27±0·23	3·1±0·50	0·13±0·01
	High protein (D)		8	26·0±0·8	23·4±1·1	−2·6	1·10±0·07	4·7	137±5·5	0·07±0·02	0·08±0·02	0·3±0·07	0·08±0·03
3	Normal synthetic (A)	16–23	8	27·1±0·6	39·1±0·8	12·0	1·91±0·04	4·9	146±15·4	0·80±0·05	1·56±0·14	3·8±0·26	0·08±0·01
	High protein (D)		8	25·9±0·8	29·1±0·9	3·2	1·39±0·04	4·8	153±6·6	0·11±0·02	0·13±0·04	0·5±0·11	0·05±0·01
4	Normal synthetic (A)	16–23	8	30·8±2·7	45·3±2·6	14·5	1·93±0·11	4·3	129±6·6	1·06±0·07	2·07±0·18	4·6±0·29	0·22±0·02
	High fat (C)		8	32·3±2·8	50·6±3·6	18·3	2·26±0·17	4·5	127±3·6	0·26±0·03	0·58±0·09	1·2±0·11	0·15±0·02
5	Normal synthetic (A)	16–30	8	26·4±0·5	68·8±2·1	26·0	3·16±0·14	4·6	121±2·5	1·81±0·07	5·67±0·22	8·3±0·27	0·14±0·02
	High protein (D)		8	25·9±0·5	53·0±1·6	18·3	2·82±0·13	5·3	128±2·2	0·56±0·10	1·63±0·32	3·0±0·56	0·10±0·01
6	Normal synthetic (A)	16–30	8	24·7±0·5	67·4±1·5	27·5	3·27±0·07	4·9	107±1·9	1·71±0·14	5·60±0·48	8·3±0·61	0·15±0·03
	High fat (C)		8	24·7±0·5	63·7±1·5	26·1	3·08±0·07	4·8	132±3·5	0·26±0·03	0·79±0·09	1·2±0·13	0·10±0·02

Table 4. *Effect of feeding glucose to neonatal rats on the hepatic glucose-phosphorylating activity*

The animals from two litters were mixed into two groups, as in the experiments described in Table 3, on the day before a special diet was started and left with the two mothers in separate cages. For the period on the special diet all the suckling animals were removed from the mothers at 6 a.m. and placed in an incubator at 28°. At 10 a.m., 2 p.m. and 6 p.m. they were given either the synthetic normal liquid diet (controls) or the high-glucose synthetic liquid diet by intubation into the oesophagus as described in detail in the Methods section. They were returned to one of the lactating mothers overnight, the two groups being alternately placed with the two mothers. Assays were performed as described in the text and the results are given as means ±S.E.M.

Expt. no.	Diet	Age period on diet (days)	No. of animals	Mean wt. increase during period on special diet (g.)	Body wt. (g.)	Liver wt. (g.)	100 × Mean liver wt./body wt.	Supernatant protein (mg./g. of liver)	Glucose-phosphorylating activity (μmole of glucose phosphorylated/min./g. of liver)		
									Total	Glucokinase	Hexokinase
1	Control	8–11	3	3·5	18·7±1·2	0·53±0·11	2·8	112±6·2	0·12±0·01	0·02±0·01	0·10±0·01
	High glucose		5	3·5	17·1±0·8	0·51±0·05	3·0	101±8·8	0·14±0·03	0·08±0·03	0·06±0·03
2	Control	9–12	6	4·8	19·2±0·9	0·62±0·04	3·2	106±1·1	0·18±0·02	0·03±0·01	0·15±0·02
	High glucose		5	5·7	19·9±0·5	0·60±0·04	3·0	110±4·7	0·22±0·03	0·07±0·03	0·15±0·03
3	Control	10–13	7	4·8	22·5±1·1	0·86±0·06	3·8	108±4·3	0·16±0·04	0·03±0·02	0·13±0·03
	High glucose		6	4·7	22·2±0·2	0·82±0·03	3·7	117±7·1	0·24±0·05	0·13±0·04	0·11±0·05
4	Control	12–15	7	5·2	27·2±0·7	0·99±0·05	3·6	84±3·5	0·18±0·02	0·09±0·02	0·09±0·02
	High glucose		8	4·8	27·1±0·4	0·93±0·03	3·4	91±3·5	0·22±0·03	0·15±0·03	0·07±0·02
5	Control	14–17	8	3·9	29·9±1·4	1·16±0·05	3·9	99±6·0	0·29±0·03	0·06±0·02	0·23±0·04
	High glucose		7	3·4	29·4±1·4	1·07±0·04	3·6	104±5·6	0·83±0·10	0·61±0·10	0·23±0·03

to neonatal rats would induce precocious development. The normal concentration of carbohydrate in rat milk is very low (2–3%, w/v; Dymsza *et al.* 1964) and it appears that the newborn rat cannot deal with high concentrations of low-molecular-weight nutrients such as monosaccharides and free amino acids owing to an inability to tolerate the osmotic effects. Pancreatic amylase and the intestinal disaccharidases (except β-galactosidase) do not develop until about the same time as glucokinase (for review see Walker, 1967), thus making administration of polysaccharide inappropriate. The feeding of a completely artificial diet was also impracticable for the reasons given, so a procedure was used in which the animals were given an artificial liquid diet by intubation during the day and fed naturally by their mothers overnight (see the Methods section and legend to Table 4).

By using this procedure it was found possible to induce a higher activity in treated animals over their litter mates similarly treated but with a glucose-free artificial diet in only that group of animals which were 17 days old at the time of killing (Expt. 5, Table 4). In all the experiments the animals gained weight adequately and there were no indications of inadequate diet. The glucose-fed animals in Expts. 1–4 (Table 4) did not show significant glucokinase activities, if allowance is made for the inherent difficulties of measuring very low activities accurately.

DISCUSSION

The importance of glucokinase in the control of glucose utilization by the liver (Walker, 1966*a*) has been further emphasized by experiments on the effect of glucose concentration on the incorporation of glucose into lipid by rat liver (Ballard & Hanson, 1967). In spite of differences in adaptive behaviour in certain species (Lauris & Cahill, 1966) and doubts as to its presence and hence significance in some species (Lauris & Cahill, 1966), including man (Boxer & Shonk, 1966), recent work suggests that glucokinase is present and is adapted to diet in several species, including man (Brown, Miller, Holloway & Leve, 1967; D. G. Walker, unpublished work), and that in the rhesus monkey glucokinase is present in adult liver but absent from the foetal liver (M. J. Parry & D. G. Walker, unpublished work). The postnatal appearance of the enzyme therefore represents an important aspect of hepatic maturation in many species.

The dependence of hepatic glucokinase synthesis on glucose and insulin in the adult rat applies also to its development in the weaning period in the rat (Walker & Holland, 1965). The present work shows that the effect of carbohydrate-free, high-fat and high-protein diets on the developmental

pattern follows closely that predicted from the effects on adult animals (Pérez *et al.* 1964; Blumenthal, Abraham & Chaikoff, 1964). A high-glucose diet can, however, induce high glucokinase activities from 18 days onwards (Table 2). The competence to synthesize glucokinase thus matures rapidly over a period of about 2 days and the normal slower developmental curve (Walker & Holland, 1965) results from the increasing carbohydrate and decreasing fat intake as the rats are weaned from the milk diet to normal laboratory diet. Rat milk is 74% water and contains on average (per 100ml.) 9·2g. of protein (73% of which is casein), 12·3g. of lipid and 3g. of carbohydrate (lactose), so that lipids provide 69% and carbohydrates only 8% of the calorie intake (Dymsza *et al.* 1964). Normal laboratory diet contains approx. 60% (w/w) digestible carbohydrate (as polysaccharide, not as free glucose) and only 3½–4% (w/w) lipid.

The nature of the carbohydrate in the diet is of further importance because the enzymes of digestion also develop during the weaning period. A high-glucose diet containing a similar percentage of total carbohydrate as in the normal laboratory diet induces a highly significant increase in hepatic glucokinase activity over normal in adult rats (Walker *et al.* 1966). Procházka, Hahn, Koldovský, Nohýnek & Rokos (1964) reported that large increases in the synthesis of rat pancreatic amylase occur between 15 and 23 days after birth. Significant quantities of many intestinal disaccharidases (except β-galactosidase) are not found until the third week of life (Doell & Kretchmer, 1963, 1964; Rubino, Zimbalatti & Auricchio, 1964). The implication of these facts is that, even if it was possible to wean the rat directly on to a high-polysaccharide or high-oligosaccharide diet, high hepatic glucokinase activities would not be seen unless premature development of the digestive hydrolytic enzymes also occurred.

Earlier negative attempts to induce precocious development of hepatic glucokinase included administration of glucose intraperitoneally with or without insulin and also with an insulinogenic drug (Walker & Holland, 1965). The possibility that intestinal factors may control insulin secretion (McIntyre, Holdsworth & Turner, 1965) and result in a greater stimulation of pancreatic insulin secretion after oral glucose administration over that for intravenous administration prompted renewed attempts (Table 4) to induce premature glucokinase appearance. That these experiments were unsuccessful supports our earlier conclusions, but could also be because the amount of glucose that can be administered is restricted by the gross immaturity of the newborn rat and the slow ontogeny of physiological regulations; these include several stress reactions, such as the inability to control water balance (Aldolph, 1957; McCance, 1961). Further examination of the question of precocious induction of hepatic glucokinase by glucose *in vivo* does not seem possible because the animal cannot deal with the osmotic effects of the glucose. No procedure for examination of induction of the enzyme *in vitro* is available.

Hormonal control of enzyme development is important in many cases and it is possible that hormones other than insulin may be involved in the control of glucokinase induction. The adrenocorticosteroids do not affect observed glucokinase activities either in adult or neonatal rats (Sharma, Manjeshwar & Weinhouse, 1964; Walker & Rao, 1964; Walker, 1965). Recent evidence (e.g. Unger & Eisentraut, 1964; see also Sokal, 1966) that there is increased secretion of both glucagon and growth hormone during glucose lack and the low dietary supply of carbohydrate before weaning taken together provide the basis of a situation that may lend support to the suggestion (Niemeyer, Peréz & Rabajille, 1966) that glucokinase induction may be repressed by glucagon. There is no information about the concentrations of many hormones, including glucagon, either in adult rats on a high-fat carbohydrate-free diet or in the neonatal rat. Glucagon has been shown to induce release of insulin by organ cultures of foetal rat pancreas (Vecchio, Luyckx, Zahnd & Renold, 1966) and to result in increased concentrations of plasma insulin after injection into man (Samols, Marri & Marks, 1966). Levine (1964) has stressed the 'anti-insulin' effects of growth hormone.

In view of the continuing discussion about the relative roles of glucose and insulin in the induction of glucokinase either directly or permissively (Sols, Sillero & Salas, 1965; Niemeyer *et al.* 1966) and the possible roles of glucose as a stabilizer of glucokinase degradation (Parry & Walker, 1967) and in blocking glucagon release from the pancreatic α-cells (Unger & Eisentraut, 1964), further analysis of the more complex situation in the weanling rat is premature.

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