Characterization of Glucose Metabolism in the Isolated Rat Heart During Fetal and Early Neonatal Development

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SUMMARY

Uptake of 1-14-C glucose, lactate production, 14-C-CO_2 production, glycogen content, and the incorporation of 1-14-C glucose into glycogen, protein, and lipid have been studied in isolated fetal and neonatal rat hearts from the sixteenth day of gestation to the fifth neonatal day. The effects of insulin (5,000 μU./ml.) on these processes was also studied. Glucose uptake fell during gestation from 28.4 $\pm$ 2.2 to 10.0 $\pm$ 1.1 μmoles per gram wet weight tissue per two hours and remained constant after birth. At all ages insulin significantly increased glucose uptake. Lactate production fell from 77.6 $\pm$ 11.5 to 51.5 $\pm$ 2.3 μmoles per gram wet weight per two hours between the sixteenth day of gestation and the first day after birth and was not influenced by insulin. Production of 14-C-CO_2 from glucose was small (range: 2 to 7 per cent of glucose uptake) and was also unaffected by insulin. Hot KOH extractable cardiac glycogen content rose between the sixteenth and twenty-first day and then fell. The change in hot KOH extractable cardiac glycogen followed similar changes in 1-14-C glucose incorporation into glycogen. Whereas 1-14-C glucose incorporation into hot KOH extractable glycogen was unaltered by insulin until after birth, insulin did significantly augment the incorporation of 1-14-C glucose into the cold TCA extractable fraction. This fraction, which includes oligosaccharides destroyed by the hot KOH, also had a higher specific activity than the hot KOH extractable glycogen. The majority of the intracellular counts were in this fraction; the remainder of the counts were in the lactate and acetate or acetoacetate fraction. Insulin did not augment incorporation of 1-14-C glucose into these fractions. Incorporation of 1-14-C glucose into both protein and lipid was less than 2 per cent of glucose uptake and was not influenced by insulin.

The data suggest that fetal myocardial development is characterized by a progressive decline in glucose uptake which can be stimulated by insulin from the sixteenth day onward. The conversion of 14-C glucose to 14-C-CO_2, protein, and lipid was not influenced by insulin. Incorporation of 1-14-C glucose into hot KOH extractable glycogen was augmented by insulin only after birth under the experimental conditions described. The cold TCA extractable glycogen had a higher specific activity and insulin stimulated the incorporation of 1-14-C glucose into this fraction. It is concluded that insulin augments glucose uptake and incorporation into a glycogen fraction turning over rapidly in the fetal rat heart. DIABETES *22*:41-49, January, 1973.

Glucose serves as the major energy substrate in the course of fetal development.[1,2] During the latter third of gestation the fetal myocardium stores glycogen, which then serves as an energy source during birth and the early neonatal period.[3] It has been demonstrated recently that the isolated fetal heart in vitro can serve as a useful experimental model to observe developmental changes in the tissue uptake of glucose in rats[4] and chicks[5] and of amino acid in chicks.[5] The present studies were undertaken to characterize further carbohydrate metabolism in the isolated fetal rat heart during the latter third of gestation and the neonatal period. Glucose uptake, lactate and CO_2 production, and the incorporation of 14-C-labeled glucose into glycogen, lipid, and protein were studied. Additional studies were performed as to the nature of the 14-C-labeled intermediates which accumulated in the fetal hearts after incubation with C-14 glucose.

METHODS

Animals. Pregnant rats of known gestational age were obtained from the Charles River Breeding Laboratory and maintained on Purina Rat Chow. Gestational age of the fetuses was confirmed by their weight.

Incubation. The mothers were anesthetized with ether

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and the fetuses were removed by cesarean section. The fetal hearts were then removed and placed in 0.9 per cent sodium chloride at room temperature. After all hearts were collected they were blotted free of blood, weighed, and placed in separate 12 x 75-mm. disposable glass tubes (Bellco Glass, Vineland, N.J.) containing 0.5 ml. of Krebs-Ringer bicarbonate buffer to which had been added 0.0625 ml. of 16 per cent gelatin (Armour Lot No. K188151) (final concentration 2 mg./ml.), 0.2 μc. of 1-14-C glucose (New England Nuclear, lot number 522-29, 6.4 mc./mmole), and 3.5 μmoles of unlabeled glucose (final concentration 7 mM). "Glucagon-free" beef insulin was added in .01-ml. volumes in buffer, whereas 0.01 ml. buffer was added to the control tubes. The tubes were then gassed with 95 per cent oxygen to 5 per cent carbon dioxide and sealed with air-tight caps (Capall, Scientific Products, Evanston, Illinois). Incubations were carried out at 37° C. in a Dubnoff metabolic incubator (100 cycles per minute). In the 14-C-CO_2 experiments, the incubations were carried out as described below. One heart per flask was used except for hearts removed on the sixteenth day of gestation, which were so small that four hearts were incubated in each flask. A two-hour incubation was used in all experiments.

Glucose Uptake. Medium in the amount of 0.01 ml. was placed in 10 ml. of scintillation mixture made up as follows: 333 ml. Triton-X 100, 4 gm. 2,5-Diphenyloxazole (PPO), 40 mg. 1,4-bis-2-(4-methyl-5-Phenyloxazolyl)-Benzene (dimethyl POPOP) (Packard Instrument Co., Downers Grove, Illinois) and 667 ml. of toluene. The samples were counted in a Packard Model No. 4322 liquid scintillation counter at 56 per cent efficiency and with a counting error of 1 per cent or less. Glucose uptake was estimated by the following formula:

$$\text{Glucose uptake } (\mu\text{mole/gm.}) = \frac{(\text{Initial cpm/ml. media} - \text{Final cpm/ml. media}) \times (\text{volume of incubation media})}{(\text{Tissue wet weight in gm.} \times (\text{cpm}/\mu\text{mole glucose in the initial media})}$$

The largest absolute amount of glucose taken up was less than 8 per cent of the total glucose available, and the glucose concentration was considered to be constant throughout the experiment. Recycling of 14-C glucose metabolites could result in an underestimate of glucose uptake calculated in this manner. That this does not occur is suggested by the similarity between 14-C glucose and 14-C-2-deoxy-glucose uptake.[4] In addition, chromatography of the media after incubation reveals no significant accumulation of nonglucose label.

Production of 14-C-CO_2. In experiments measuring 14-C-CO_2 production from 14-C glucose, two hearts were incubated in 2 ml. of media in 1-oz. polyethylene bottles (Nalgene Co., Rochester, N.Y.) that were sealed with serum stoppers. The 14-C-CO_2 was collected on filter paper placed in disposable plastic wells (Kontes Glass Co., Vineland, N.J.) by addition of 0.25 ml. of approximately 1 M triethanolamine (triethanolamine diluted 1:7 (V/V) with distilled water) to the well and 0.2 ml. of 2.5 M H_2SO_4 to the medium at the termination of the incubation. The sealed bottles were then incubated at 37° C. and shaken for one and one-half hours to insure complete trapping. The wells were then placed in a scintillation mixture containing 4 gm. PPO and 40 mg. dimethyl POPOP per liter of toluene and counted in the same scintillation counter at a counting efficiency of 38 per cent and a counting error of 2 per cent.

Lactate production. Lactate production was measured in a 0.1-ml. aliquot of media by an enzymatic method.[6]

Glycogen. After incubation, the hearts were digested in a solution of hot 30 per cent (W/V) KOH and 0.5 per cent (W/V) Na_2SO_4. Glycogen was isolated by the method of Good, Kramer, and Somogyi.[7] Glycogen was also extracted by cold trichloroacetic acid (TCA).[8] This fraction contains oligosaccharides destroyed by the hot KOH.[7,8] Briefly, the hearts were blotted and homogenized in cold (4° C) 10 per cent TCA. After centrifugation the supernatant was decanted. Subsequent alcohol precipitation and isolation were identical. The alcohol precipitate was dissolved in 2.5 M H_2SO_4 and the solution was neutralized to a phenolphthalein end point with 2 M sodium hydroxide. Radioactivity was measured by adding a 1-ml. aliquot of this solution to 10 ml. of the previously described Triton scintillation mixture. Samples were then assayed in the manner described for 1-14-C-glucose (see above). Glucose was measured by a glucose oxidase method (Glucostat, Worthington Biochemical, Freehold, N.J.). The concentration of glycogen is expressed as μmoles of glucose equivalent per gram wet weight.

Total lipids. After incubation, hearts were placed in 10 ml. of chloroform:methanol (2:1 V/V) and extracted by the method of Folch, Lees, and Sloane-Stanley.[9] The chloroform was transferred to counting vials, evaporated overnight under nitrogen, and counted in 10 ml. of the scintillation mixture described above. Counting efficiency was 70 per cent and counting error was less than 2 per cent.

Protein. After incubation, hearts were placed in 0.5

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ml. of 10 per cent (W/V) trichloroacetic acid (TCA) and boiled for five minutes.[10] The mixture was centrifuged, the supernatant was decanted, and the precipitate was washed with 1.0 ml. of 5 per cent (W/V) TCA. One-half ml. of 5 per cent (W/V) TCA was again added to the precipitate and the tubes were heated to 90° C. for fifteen minutes. After centrifugation, the supernatant was removed and the precipitate was dissolved in 0.12 ml. performic acid (0.1 ml. 88 per cent (V/V) formic acid and 0.02 ml. of H_2O_2) and left standing for twenty minutes at room temperature. The protein was reprecipitated with 0.6 ml. of 10 per cent (W/V) TCA, centrifuged at 3,000 rpm for ten minutes, and the TCA was removed. The protein was dissolved in 0.1 ml. of 88 per cent (V/V) formic acid and counted in 20 ml. of the previously described Triton scintillation mixture. Counting efficiency was 43 per cent and the counting error was less than 2 per cent.

Calculation of per cent of glucose conversion. All samples were converted to disintegrations per minute (dpm) by addition of internal standards. The dpm values were then divided by the initial specific activity of the glucose to obtain μmoles of glucose converted:

μmoles of C-14 from glucose in product per gram weight of tissue =

$$\frac{\text{dpm of product per gram wet weight}}{\text{dpm of glucose per } \mu\text{mole glucose}}$$

This calculation assumes that the specific activity of the glucose in the cells was the same as that of the glucose in the medium. This assumption appears to be valid since there is little, if any, gluconeogenesis in the mature rat heart[11] and none in the fetal rat.[12] The only source of unlabeled glucose, therefore, would be from the breakdown of glycogen. Since the concentration of glycogen has been shown to change very little during the incubation of fetal rat hearts[4] this does not appear to be a significant source of glucose.

Intracellular metabolic fate of 1-14-C from 1-14-C glucose. In separate experiments twelve hearts were incubated in the presence or absence of 5,000 μU./ml. of insulin in 2 ml. of the previously described medium. In these experiments the specific activity of the 1-14-C glucose was increased by the addition of 8 μc. of the labeled compound. At the end of a two-hour incubation, the hearts were removed, carefully blotted, placed in 2 ml. of water, and boiled for ten minutes. Ten-microliter aliquots were then plated on four separate chromatography systems. Chromatography was also performed on the original media and on the media at the end of the incubation. Standards were chromatographed as follows: 1-14-C glucose, 1-14-C glucose-6-phosphate, U-14-C fructose-6-phosphate, U-14-C fructose-1,6-diphosphate, 1-14-C lactate, 1-14-C pyruvate, and U-14-C alpha-glycerol phosphate. Ten-lambda aliquots of standards or unknowns were placed on precoated thin layer chromatography cellulose sheets (Merck AG, Darmstadt, Germany). Standards and unknowns were all spotted on a single 20 x 20-cm. plate which was then placed in an ascending chromatography chamber and run for approximately 15 cm. The chromatographic systems used were secondary butanol: formic acid: water (75:15:15 V/V);[13] isopropanol:pyridine:acetic acid: water (8:8:1:4 V/V);[12] isobutyric acid: ammonium hydroxide: water (66:1:33),[14] and t-amyl alcohol: water:p-toluenesulfonic acid (65:33:2) (V/V/W). At the end of chromatography, the chromatograms were allowed to dry overnight in a hood. They were then cut in 1-cm. segments, placed in 10 cc. of the previously described Triton scintillation mixture, and counted at 28 per cent efficiency with less than 1 per cent counting error.

Separate aliquots were placed on a Dowex 1—X8 (Cl), 200 to 400 mesh (AG grade, Bio-Rad Laboratories) column and eluted with HCl.[15]

All determinations were performed in duplicate. Differences were determined by Student's *t* test.

RESULTS

Heart weights vs. fetal weights vs. gestational age. The average fetal weights for the experiments herein reported are summarized in table 1. They are in excellent agreement with results reported by other investigators.[16] The relationship between fetal and neo-

TABLE 1

The effects of age on the weight of fetuses and neonates and fetal and neonatal hearts

Age in days	Fetal and neonatal weight in grams*	Heart weight in milligrams†
Fetal		
16	0.14	2.7
18	0.75	4.0
20	3.02	11.7
21	3.27	15.8
22	5.29	25.9
Neonatal		
1	5.72	31.2
2	7.29	37.0
5	10.40	52.3

* Average wet weight of 10 to 12 fetuses (less hearts).
† Average wet weight of 10 to 12 hearts before incubation.

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natal weight and heart weight was linear ($r = 0.995$, $N = 10$, $p < 0.001$).

The effects of age on glucose uptake and insulin responsiveness in the rat heart in vitro. Glucose uptake was studied in hearts ranging in age from sixteen days of gestation to five days post partum. There was a stepwise decline in glucose uptake before birth; glucose uptake remained constant after birth (table 2). In all tissues insulin caused a significant increase in glucose uptake. Whereas the insulin concentration was 5,000 μU./ml. in these experiments, similar results (which were maximal) have been obtained with insulin concentrations as low as 5 μU./ml.[4]

TABLE 2
The effects of age and insulin on 1-14-C glucose uptake in vitro by fetal rat hearts

Age in days	1-14-C glucose uptake: μmole/gm. wet wt./2 hrs.*		n	P value‡
Fetal	Control	Insulin†		
16	28.4 ± 2.2	51.9 ± 3.2	4-5	< 0.001
18	25.7 ± 3.9	42.5 ± 4.8	6	< 0.05
21	10.0 ± 1.1	15.6 ± 1.1	6	< 0.02
Neonatal				
1	5.6 ± 0.2	9.6 ± 0.7	3	< 0.01
2	6.0 ± 0.2	6.8 ± 0.2	7	< 0.02
5	4.7 ± 0.5	7.1 ± 0.4	6	< 0.01

* Results are expressed as mean ± S.E.
† 5,000 μU./ml.
‡ Insulin versus control.

The effects of age and insulin on cardiac lactate and 14-C-CO_2 production in vitro. Mean lactate production fell from 77.6 μmoles per gram per two hours at sixteen days of gestation to 51.5 μmoles per gram per two hours on the first day after birth (table 3). It fell further between the first and second day after birth and did not change thereafter. Insulin did not significantly alter lactate production at any age studied. There was very little conversion of 1-14-C glucose to 14-C-CO_2 at any age studied (table 4). Production of 14-C-CO_2 was not related to age and was not altered by insulin.

TABLE 3
The effects of age and insulin on cardiac lactate production in vitro

Age in days	Lactate production: μmole/gm. wet wt./2 hrs.*		n
Fetal	Control	Insulin†	
16	77.6 ± 11.5	87.8 ± 3.7‡	6
18	70.8 ± 6.2	76.8 ± 7.4‡	5-6
21	52.8 ± 3.1	52.8 ± 2.9‡	6
Neonatal			
1	51.5 ± 2.3	57.1 ± 2.4‡	6
2	40.2 ± 3.8	45.8 ± 2.2‡	6
5	44.0 ± 1.8	45.1 ± 1.1‡	6

* Results are expressed as mean ± S.E.
† 5,000 μU./ml.
‡ Not significantly different from control.

TABLE 4
The effects of age and insulin on cardiac 14-C-CO_2 production in vitro

Age in days	14-C-CO_2: μmole/gm. wet wt./2 hrs.*		n
Fetal	Control	Insulin†	
18	0.5 ± 0.1	0.6 ± 0.1‡	5-6
21	0.2 ± 0.1	0.2 ± 0.1‡	4-5
Neonatal			
1	0.4 ± 0.1	0.5 ± 0.1‡	4
2	0.1 ± 0.1	0.1 ± 0.1‡	6
5	0.1 ± 0.1	0.1 ± 0.1‡	6

* Results are expressed as mean ± S.E.
† 5,000 μU./ml.
‡ Not significantly different from control.

The effects of age and insulin on cardiac glycogen and 1-14-C glucose incorporation into glycogen in vitro. Hot KOH extractable cardiac glycogen rose from the sixteenth to the twenty-first day of gestation (table 5 and figure 1). It had fallen by the twenty-second day of gestation. It had reached even lower values by the fifth day after birth. The rate of 1-14-C glucose conversion to hot KOH extractable glycogen had increased markedly between the sixteenth and the eighteenth day of gestation (table 5 and figure 1). The rate of incorporation

TABLE 5
The effects of age on cardiac glycogen and 1-14-C glucose incorporation into hot KOH extractable glycogen in vitro

Age in days	Glycogen content: μmoles/gm. wet wt.*	n	1-14-C glucose incorporation into glycogen: μmole/gm. wet wt./2 hrs.*	n
Fetal				
16	42.9 ± 4.0	3	0.5 ± 0.1	5
18	137.8 ± 5.3	5	6.6 ± 1.0	6
21	181.4 ± 11.0	8	3.6 ± 0.4	6
22	103.4 ± 5.7	4	2.4 ± 0.3	4
Neonatal				
1	113.2 ± 6.1	6	2.4 ± 0.2	7
2	97.9 ± 7.2	6	2.3 ± 0.3	6
5	41.6 ± 10.1	6	1.9 ± 0.2	6

* The results are expressed as mean ± S.E.

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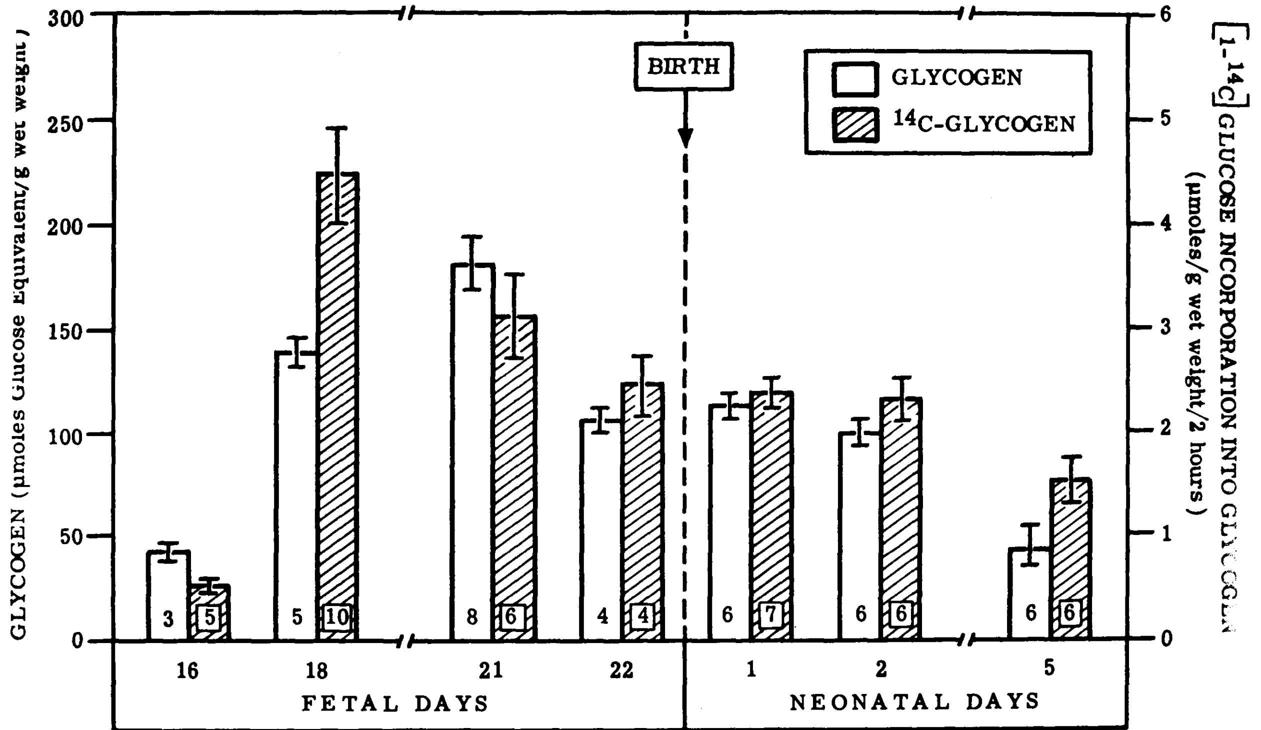

FIG. 1. The effects of age on cardiac glycogen and 1-14-C glucose incorporation into glycogen in vitro. The means (boxes) and S.E. (vertical bars) are presented. The number at the base of each box is the number of observations.

of 1-14-C glucose into hot KOH extractable glycogen correlated well with glycogen content. The peak rate of incorporation occurred on the eighteenth day; glycogen content was highest on the twenty-first day. The rate of incorporation of 1-14-C glucose into hot KOH extractable glycogen had fallen before the decrease in glycogen content, which occurred on the twenty-second day (table 5 and figure 1). Insulin did not alter 1-14-C glucose incorporation into hot KOH extractable glycogen until after birth (table 6).

The cold TCA extracted less glycogen than the hot KOH (table 7). This fraction had a higher specific activity. Insulin increased 1-14-C glucose incorporation into cold TCA extractable glycogen.

The effect of age and insulin on 1-14-C glucose incorporation into cardiac protein and lipid in vitro. Only a small amount of 1-14-C glucose carbons were found in either protein or lipid (tables 8 and 9). Both tended to fall during gestation along with glucose uptake. Insulin did not significantly alter incorporation of 14-C glucose into either protein or lipid.

Chromatographic analysis of 14-C-counts after incubation with 1-14-C glucose. Whereas the media contained a small amount of C-14 labeled compound chromatographing with lactate, the vast majority of the counts chromatographed with 1-14-C glucose, substantiating the impression, derived from experiments with 1-14-C-2-deoxy-glucose,[4] that there was not significant recycling of 14-C counts into the media. In all systems, the counts derived from the hearts remained at the origin and

TABLE 6

The effects of age and insulin on cardiac 1-14-C glucose incorporation into hot KOH extractable glycogen in vitro

Age in days	1-14-C glucose incorporation into glycogen µmole/gm. wet wt./2 hrs.*		n	P value
Fetal	Control	Insulin†		
16	0.5 ± 0.1	0.4 ± 0.1		N.S.‡
18	4.4 ± 0.4	4.8 ± 0.4	6-10	N.S.
21	3.1 ± 0.4	3.2 ± 0.6	9-10	N.S.
22	2 4 ± 0.3	2.2 ± 0.2	4	N.S.
Neonatal				
1	2.4 ± 0.2	2.9 ± 0.3	7	N.S.
2	2.3 ± 0.3	2.2 ± 0.3	6	N.S.
5	1.5 ± 0.2	2.2 ± 0.2	6	< 0.05

* Results are expressed as mean ± S.E.
† 5,000 µU./ml.
‡ N.S. means not significant.

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TABLE 7

The effect of extraction method on cardiac glycogen and 1-14-C glucose incorporation into glycogen in vitro

Insulin: μU./ml.	Glycogen content μmole/gm. wet wt.*	N	1-14-C glucose incorporation into glycogen: μmole/gm. wet wt./2 hrs.*	N	Glycogen specific activity: cpm/μmole*	N
		Hot KOH extraction				
None	102.9 ± 3.5	6	1.7 ± 0.2	5	654 ± 81	5-6
5,000	104.0 ± 6.0	6	1.9 ± 0.2	6	699 ± 70	6
	(N.S.)†		(N.S.)†		(N.S.)†	
		Cold TCA extraction				
None	25.1 ± 4.5	6	0.9 ± 0.1	6	1,427 ± 106	6
5,000	33.0 ± 4.8	6	1.9 ± 0.3	6	2,248 ± 314	6
	(N.S.)†		($p < 0.01$)		($p < 0.05$)	

* The results are expressed as mean ± S.E.
† N.S. means not significantly different from control.

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there did not appear to be significant qualitative differences in the chromatograms between those hearts incubated in the presence or absence of insulin. This peak at the origin was not identical to any standard substance in all four systems.

The elution pattern of the intracellular counts from the Dowex column is shown in figure 2. Three peaks are present. Peak B elutes identically with acetate and acetoacetate. Peak C elutes identically with lactate. Peak A is the largest fraction. The counts from peak A elute with the void volume of a G25 sephadex column (figure 3). This suggests a molecular weight equal to or greater than 5,000. The counts from peak A are precipitated by adding ethyl alcohol to a final concentration of 50 per cent (V/V). Insulin increased the counts in peak A. Peaks B and C were unaltered (figure 2).

DISCUSSION

It has been observed previously that glucose uptake by the isolated fetal rat heart falls progressively during the latter third of gestation and that glucose uptake by the isolated fetal rat heart can be increased by the addition of insulin in physiologic amounts to the incubation medium.[4] A similar fall in glucose uptake has been observed in the isolated chick heart and in cardiac tissue isolated from human abortuses.[5,17] The mechanism whereby glucose uptake falls with age has yet to be elucidated. This fall does not appear to be a phenomenon of surface area since similar changes with age are observed when isolated cells from fetal rat hearts are utilized.[4] The present observations reveal that glucose uptake by the isolated fetal rat heart remains relatively constant during the interval immediately after birth (table 2).

Lactate production by the isolated fetal rat heart exceeded that which could be accounted for by the glucose taken up (table 3). This high rate of lactate production by the isolated fetal rat heart may be explained, in part,

TABLE 8

The effects of gestational age and insulin on 1-14-C glucose incorporation into cardiac protein in vitro

Age in days	1-14-C glucose incorporation into protein: μmole/gm. wet wt./2 hrs.* Control	Insulin†	n
Fetal			
18	0.59 ± 0.05	0.57 ± 0.03‡	6
20	0.34 ± 0.13	0.30 ± 0.10‡	6
22	0.15 ± 0.11	0.16 ± 0.05‡	6
Neonatal			
1	0.08 ± 0.01	0.07 ± 0.01‡	6
2	0.11 ± 0.01	0.10 ± 0.01‡	6
5	0.06 ± 0.01	0.07 ± 0.01‡	6

* Results are expressed as mean ± S.E.
† 5,000 μU./ml.
‡ Not significantly different from control.

TABLE 9

The effect of age and insulin on 1-14-C glucose incorporation into cardiac total lipids in vitro

Age in days	1-14-C glucose incorporation into total lipids: μmole/gm. wet wt./2 hrs.* Control	Insulin†	n
Fetal			
18	0.41 ± 0.01	0.39 ± 0.01‡	6
20	0.18 ± 0.08	0.17 ± 0.11‡	6
22	0.11 ± 0.01	0.11 ± 0.01‡	6
Neonatal			
1	0.12 ± 0.1	0.11 ± 0.1‡	6
2	0.10 ± 0.01	0.09 ± 0.01‡	6
5	0.05 ± 0.01	0.05 ± 0.01‡	6

* Results are expressed as mean ± S.E.
† 5,000 μU./ml.
‡ Not significantly different from control.

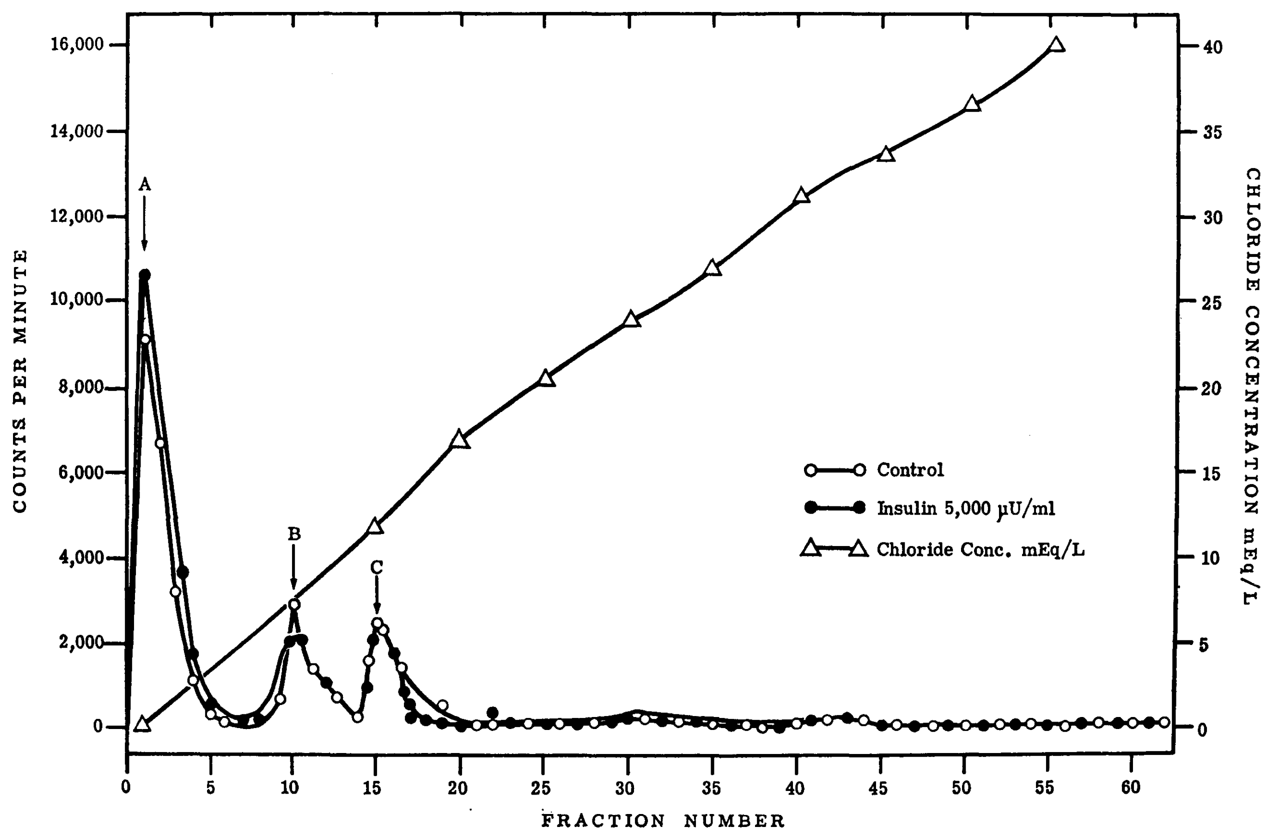

FIG. 2. Elution pattern of intracellular 14-C counts after incubation in the presence or absence of insulin, 5,000 μU./ml. The intracellular 14-C counts were placed upon a 1 by 14 cm. Dowex 1—XB (Cl), 200 to 400 mesh column. The counts were eluted with 0.1 molar HCl. Aliquots of 2 ml. were collected.

by the augmented glycogenolysis which occurs between death and incubation.[4] This may be the consequence of hypoxia resulting from the technical procedure of obtaining the specimens. Glycogen does not fall during the period of incubation.[4] This high glycolytic capacity which appears characteristic of certain fetal tissues, including heart, of several species (chick,[5] human,[18] and rat[19]) may be one means whereby fetal and neonatal animals can survive prolonged periods of anoxia.[16]

The production of 14-C-CO_2 from 1-14-C glucose by the isolated fetal rat hearts was less than 2 per cent of the 1-14-C glucose taken up at all ages studied except the one-day-old newborn where it was 7 per cent of uptake (table 4). The fetal rat liver contains a small number of mitochondria. Immediately after birth there is a rapid proliferation of mitochondria.[18] A lack of mitochondria could explain low conversion of 1-14-C glucose into 14-C-CO_2. However, an estimate of the number of fetal heart mitochondria has not yet been made. Insulin had no effect on 14-C-CO_2 production from glucose (table 4).

It has been demonstrated previously that certain fetal rat tissues store glycogen during development.[3] These glycogen stores are then utilized during the neonatal period while glucose homeostasis is being established.[3] Fetal survival under anoxic conditions is largely influenced by cardiac glycogen content.[3] The pattern of a peak in cardiac glycogen prior to birth has been previously observed.[3] As regards hot KOH extractable glycogen, at sixteen days gestation both glycogen content and 1-14-C glucose incorporation into glycogen were low; there was then a sharp rise in 1-14-C glucose incorporation into glycogen with a peak at eighteen days gestation and a decline thereafter (table 5 and figure 1). Cardiac glycogen content, in contrast, was highest at twenty-one days and then decreased (table 5 and figure 1). To the extent that the rate of incorporation of 1-14-C glucose into glycogen reflects the rate of glycogen synthesis, it appears that glycogen content may be regulated by glycogen synthesis. It is of note that the changes in glycogen content reported here parallel the

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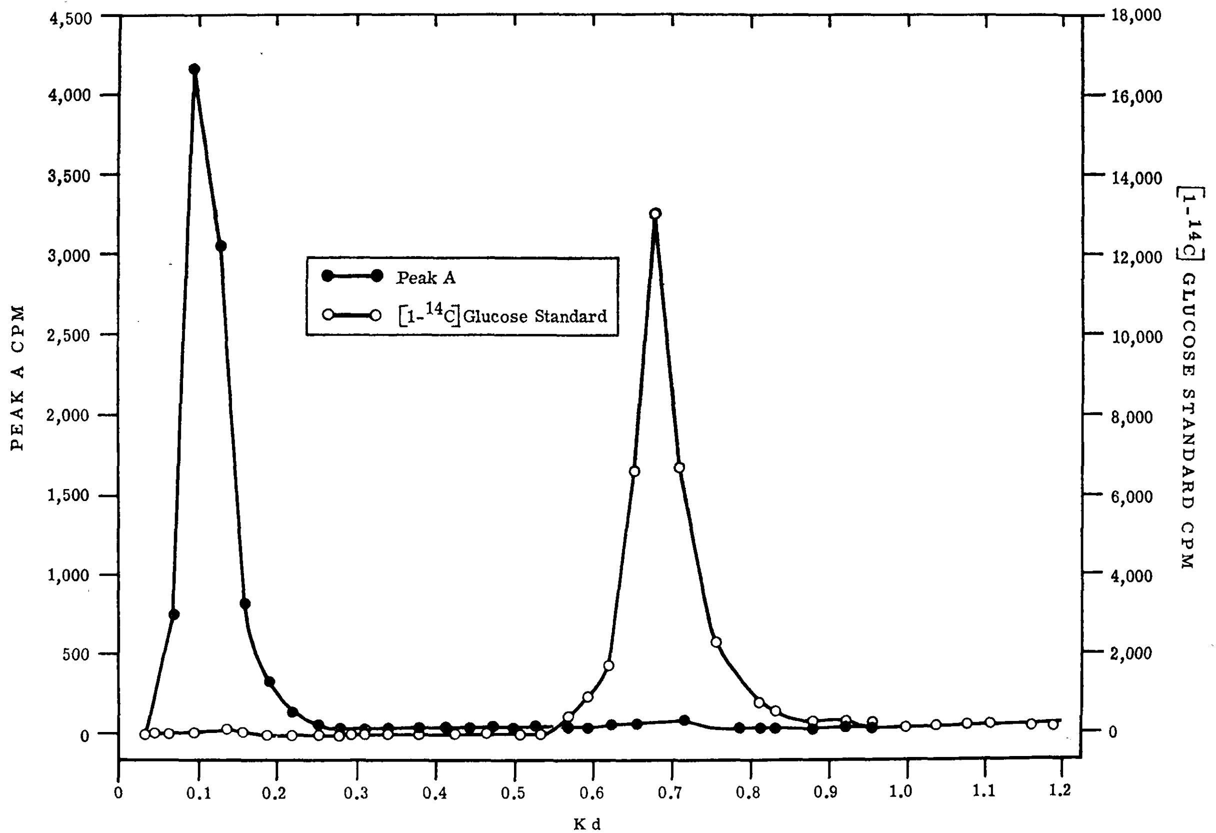

FIG. 3. Elution pattern of peak "A" from Dowex column and standard 14-C labeled glucose. The counts were placed upon a 1 by 80 cm. G-25 Sephadex column in a 25-millimolar phosphate buffer at pH 7. Fractions of 1 ml. were collected.

changes in insulin concentration in fetal rat plasma over the same interval.[19]

Insulin did not stimulate the conversion of 1-14-C glucose into hot KOH extractable glycogen until after birth. It has been previously observed that insulin did not augment either glycogen content or 14-C glucose incorporation into glycogen until after birth in developing rat skeletal muscle and diaphragm.[20-22] In these studies glycogen was also extracted by hot KOH. It has been concluded from these observations that insulin has no effect on glycogen metabolism in fetal muscle prior to birth.[20-22] The present data suggest that this interpretation may be incorrect.

Glycogen exists in the cell in a macromolecular state which contains not only the glycogen molecule but the enzymatic machinery for its synthesis and breakdown.[24,25] Hot alkali treatment results in complete extraction of muscle glycogen but destroys the outer tiers of the glycogen molecule, which are most involved in glycogen synthesis.[8,23,24] Cold acid treatment results in a less complete extraction but does not destroy these outer tiers; this fraction has a higher specific activity and contains the more rapidly synthesized portion of the glycogen molecule.[23-25] Insulin augments incorporation of glucose into this fraction in the adult diaphragm[23] and, according to our experiments, in the fetal rat heart. These observations suggest that a major portion of C-14 glucose taken up by the isolated fetal rat heart is incorporated into the outermost tiers of the glycogen molecule and that this incorporation is augmented by insulin.

The remainder of the intracellular C-14 counts were chromatographically similar to lactate and acetate plus acetoacetate. Insulin failed to augment incorporation of glucose molecules into this fraction. The failure of insulin to augment glycolysis also has been observed in fetal rhesus monkey skeletal muscle.[26]

In conclusion, it appears that insulin increases glucose uptake by the fetal rat heart from at least the sixteenth day of gestation onward. The majority of the C-14 carbons derived from glucose were incorporated into glycogen, and insulin augmented this process.

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