Hormonal Regulation of Incorporation of Alanine-U-^{14}C into Glucose in Human Fetal Liver Explants

Effect of Dibutyryl Cyclic AMP, Glucagon, Insulin, and Triamcinolone

Alan L. Schwartz, Ph.D., and Theodore W. Rall, Ph.D., M.D.(Hon.), Cleveland, Ohio, and Helsinki, Finland

SUMMARY

Incorporation of alanine-U-^{14}C into glucose and liver glycogen increased linearly over sixty-five hours in culture of human fetal liver explants. This rate of incorporation was stimulated two- to tenfold by incubation with $N^{6,2'}O$-dibutyryl adenosine 3'-5':cyclic monophosphate (dibutyryl cyclic AMP) (0.1 mM) plus theophylline (0.5 mM) or glucagon (7.5 μg./ml.) plus theophylline. No apparent lag period was detected, and the hormonal effect continued throughout the observation period. Insulin (1 U./ml.) significantly decreased both the basal rate of incorporation and the stimulated rate resulting from dibutyryl cyclic AMP or glucagon incubation. These effects were observed at both high (10 mM) and low (2.8 mM) media glucose and from both 2.3 μM and 5 mM alanine-U-^{14}C. Triamcinolone (20 μg./ml.) alone stimulated the rate of alanine-U-^{14}C incorporation into glucose, whereas triamcinolone in the presence of dibutyryl cyclic AMP produced an increase in incorporation greater than the sum of the individual effects.

The basal incorporation of alanine-U-^{14}C into glucose by these human fetal liver explants provide a rate of approximately 4 nmoles glucose/gm. min, which is discussed in relation to the physiologic needs of the fetus and newborn. DIABETES *24*:650-57, July, 1975.

Newborn human infants, similar to the newborns of other mammalian species, undergo a period of hypoglycemia immediately after birth.[1,2] This hypoglycemia may be a result of a decreased glucose production or increased utilization or some combination. Blood glucose production is mainly controlled at the hepatic level and involves both glycogenolysis and gluconeogenesis. In the fetus and neonate the control of glucose production is largely unexplored. Studies have focused on the regulation of these processes in the neonatal rat,[3-8] sheep,[9] and dog.[10,11] These studies demonstrate that glycogen accumulation to very high levels (18 mg. per cent) takes place in the fetal liver and that the mobilization of these stores associated with birth (hormonal-mediated?) can account for initial hepatic glucose output.[10]

However, sustained hepatic glucose output requires a sufficient rate of gluconeogenesis (on the order of 25-40 μmoles glucose/kg./min.).[12] Overall gluconeogenesis in the fetal rat liver is virtually absent and develops only some hours after birth,[3,4] whereas the fetal sheep liver performs gluconeogenesis at approximately 30 per cent that found in the postnate or adult.[9] It is difficult to place the human fetus in perspective to these very different models. As a result of limited tissue availability only a few studies have examined this process in the human fetus.

Villee[13] first demonstrated gluconeogenesis in slices of human fetal liver. More recently, Adam et al.[14] have quantified glucose output and gluconeogenesis in the isolated perfused human fetal liver. However, they were unable to demonstrate alterations in gluconeogenesis after addition of cyclic AMP, glucagon, or insulin.

The organ culture system for fetal liver explants allows one to examine gluconeogenesis in a steady-state in vitro environment. This system maintains liver explants in a chemically defined medium for a period of days and thus allows both short- and long-term determinations, quantitative as well as qualitative. With this technic we have recently examined other aspects of the regulation of hepatic glucose output in the human fetus, including the hormonal regulation of amino acid uptake,[15] glucose-6-phosphatase activity,[16] and glycogen metabolism.[17]

From the Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, and Department of Medical Chemistry, University of Helsinki, Helsinki, Finland.

Present address: Department of Pharmacology, Case Western Reserve University.

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MATERIALS AND METHODS

L-Alanine-U-^{14}C (specific radioactivity: 173 mCi/mole) and glucose-1-^{14}C (specific radioactivity: 2.8 mCi/mmole) were purchased from Amersham Radiochemical Centre, Amersham, Great Britain. Hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and NADP were purchased from Boehringer Biochemical Co., Mannheim, Germany. Dibutyryl cyclic AMP* was obtained from Calbiochem, Los Angeles, Calif. Glucagon was from Eli Lilly, Indianapolis, Ind.; insulin (porcine monocomponent, lot number MC-S-821506) was a gift of Novo Research Institute, Copenhagen, Denmark; and triamcinolone (Kenacort) was from Squibb of Sweden. All other chemicals were of reagent grade and were purchased from Sigma Chemical Co., St. Louis, Mo.

Organ Culture

A complete description of the organ culture system for fetal liver has been presented earlier.[18] Tissues were obtained at therapeutic abortion performed by hysterotomy† and immediately placed into ice-cold culture medium. Explants were prepared and placed into culture dishes, which were preincubated twenty-four hours before experiments began. Additions to the culture medium were made at appropriate times in volumes of less than 0.125 ml. (less than 1 per cent of the total volume), so as not to alter the composition of the medium. Furthermore, most agents were prepared in culture medium before addition. The concentrations of hormonal agents are much in excess of physiological concentrations as has been seen previously with these explants,[15-18,21] perhaps due to rapid degradation of the agents by the fetal liver or more probably due to limitations in the diffusion of these agents into the cultured explants.

Since the availability of tissue dictated the range of sample sizes examined and since no two specimens were obtained at the same time, each series of culture dishes prepared from one tissue had to constitute a complete experiment. Thus, many factors, including the various tissue ages as well as unaccountable variations occurring during pregnancy and surgery, make it difficult to compare absolute values from one culture tissue to another (e.g. dose-response curves from two tissues demonstrate the same pattern, although the absolute values are different).

Determination of Glucose Specific Radioactivity

Determination of ^{14}C-glucose was performed according to the description of Adam et al.,[19] in which a sample of the culture media (generally 25-100 μl.) is incubated at room temperature in a system containing 0.2 M Tris (pH 7.6), 7 mM EDTA, 1 mM NADP, 10 mM $MgCl_2$, 2 mM ATP, 1 U./ml. hexokinase, and 1 U./ml. glucose-6-phosphate dehydrogenase until completion. The change in optical density was measured at 340 mμ in a Zeiss PMQII spectrophotometer. The entire sample was then transferred into a Warburg-style flask on ice. Additions of alpha-ketoglutarate and NH_4Cl were made to yield final concentrations of 3 mM of each. A cup containing 250 μl. hyamine hydroxide (Packard Corp.) was included within the flask. Glutamate dehydrogenase (3 U./ml.) and 6-phosphogluconate dehydrogenase (0.2 U./ml.) were then added, and the flask sealed and incubated for thirty minutes (completion) at 37° C. The hyamine was then counted for radioactivity in a Packard-Tri-carb Scintillation counter using a standard toluene-PPO-POPOP scintillant.[18] Counting efficiency was 80 per cent. Appropriate alanine-U-^{14}C blanks were run and their values were subtracted from the glucose determinations. In some culture dishes, the entire tissue was analyzed for radioactivity in glycogen by digesting the tissue homogenate in 30 per cent KOH, followed by ethanol precipitation as previously described.[21]

Glucose radioactivity is expressed as media ^{14}C-glucose c.p.m. per culture dish. This is six times the radioactivity detected in the C-1 position, based upon the assumption of a uniform distribution of the alanine-U-^{14}C label: Each value reported in figures or tables is based on the average of duplicate determinations, which differed from each other by no more than 9 per cent. The amount of tissue per dish as reflected in the total protein content of each dish is very constant (within ± 5 per cent), and hence these changes in protein content (see figure 2) would only slightly affect the rates of incorporation expressed per culture dish.

Protein

Tissue protein concentrations were determined by the method of Lowry et al.,[22] as modified by Hartree,[23] using bovine serum albumin as a standard.

Statistics

Statistical evaluation was performed in paired analysis using Student's *t*-test.

*$N^{6,2'}$O-dibutyryl adenosine 3′-5′: cyclic monophosphate.

†Gestational age was determined from the crown-rump length nomogram of Tanimura et al.[20] The investigators did not participate in the decision to interrupt pregnancy.

RESULTS

As seen in figure 1, the medium glucose concentration remained constant at 10 mM throughout the forty-five-hour culture period. Radioactivity in the media glucose was detectable by the sampling at seven hours and steadily increased throughout the culture period. Radioactivity determined in tissue glycogen after ethanol precipitation at the end of the culture period was found to be of the order of 3 per cent of that incorporated into glucose (figure 1A). Thus, in any regard; the amount of radioactivity in tissue glycogen was indeed a very small fraction of the total glucose radioactivity.

When explants in the same experiment as that described above were incubated with dibutyryl cyclic AMP and theophylline at concentrations that were maximal for stimulation of amino acid uptake and glycogenolysis, i.e. 0.1 mM and 0.5 mM, respectively, the total medium glucose did not change during incubation. However, there was significantly more radioactivity in the medium glucose than in the control experiment (figure 1A). Furthermore, radioactivity in tissue glycogen was significantly decreased to one-fourth the control value, i.e., less than 0.3 per cent of the total glucose radioactivity. It is of interest to note that between the twelfth and eighteenth hour in culture the rate of incorporation increased in both the control and dibutyryl cyclic AMP-incubated dishes. This is not surprising, as the initial twelve to eighteen hours in culture is the period of maximal glycogen depletion. A similar experiment to that described in figure 1A was performed; however, the hormonal agents and the alanine-U-^{14}C were not added until after a twenty-four-hour preincubation. As seen in figure 1B, in either the presence or absence of dibutyryl cyclic AMP plus theophylline, the medium glucose remained constant during the forty-five hours after the addition of the agents. The radioactivity detected in the media glucose was greater in all samples incubated with dibutyryl cyclic AMP plus theophylline. Similarly, the radioactivity in glycogen in control samples was greater than from the samples incubated with the agents. In this experiment, however, there was not the apparent lag period immediately after the addition of the alanine-U-^{14}C, perhaps since a twenty-four-hour preincubation had been performed. From the experiments depicted in figures 1A and 1B, it was seen that radioactivity in tissue glycogen was negligible compared with the total radioactivity in the media glucose; therefore, the following experiments report only the media glucose radioactivity. Furthermore, the constant media glucose concentrations seen during the culture periods (sixty-five hours) were observed in all of the following experiments: with all data pooled, dibutyryl cyclic AMP was found to stimulate the ^{14}C-alanine incorporation by 276 per cent at all times examined (figures 1, 2, 4, 5). These data demonstrate a statistically

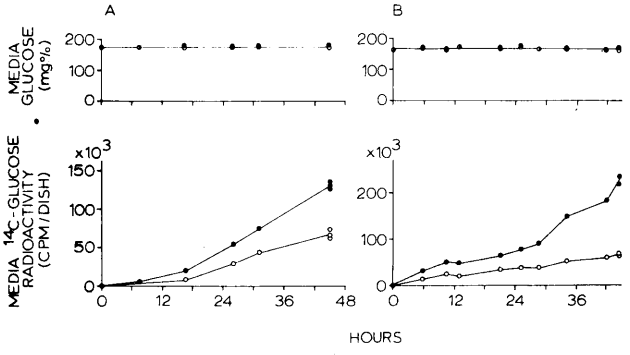

FIG. 1. Effect of dibutyryl cyclic AMP plus theophylline on incorporation into glucose from alanine-U-^{14}C in human fetal liver explants. Explants were prepared from specimens of 82 mm. (A) and 155 mm. (B) crown-rump length. After zero hours' (A) or twenty-four hours' (B) preincubation, alanine-U-^{14}C (5 μCi) was added to each culture dish (final conc. = 2.3 μM). Some dishes also received dibutyryl cyclic AMP (0.1 mM) plus theophylline (0.5 mM) (solid circles). Media samples were collected for the determination of glucose-specific activity. At the end of the culture period, the explants were pooled and frozen for glycogen radioactivity determinations. Total glycogen radioactivity as cpm./mg. protein was 2,200 and 500 for the control and dBcAMP in A, and 3,800 and 1,400 for the control and dBcAMP in B.

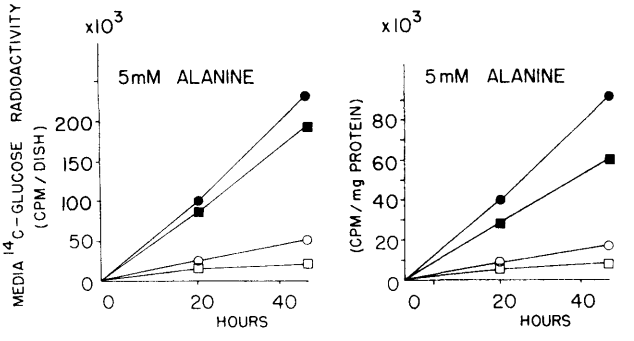

FIG. 2. Effect of insulin and dibutyryl cyclic AMP on incorporation into glucose from alanine-U-^{14}C in human fetal liver explants. Explants were prepared from a specimen of 110-mm. crown-rump length and preincubated for twenty-four hours, after which alanine-U-^{14}C (5 μCi; 5 mM) was added. The following additions were also made simultaneously: o—none; ●—dibutyryl cyclic AMP (0.1 mM) plus theophylline (0.5 mM); □—insulin (1U./ml.); ■—dibutyryl cyclic AMP plus theophylline plus insulin. Twenty and forty-five hours later, media samples were taken for analysis of glucose-specific radioactivity. Total protein concentrations were determined at the end of the culture period for the figure at the right.

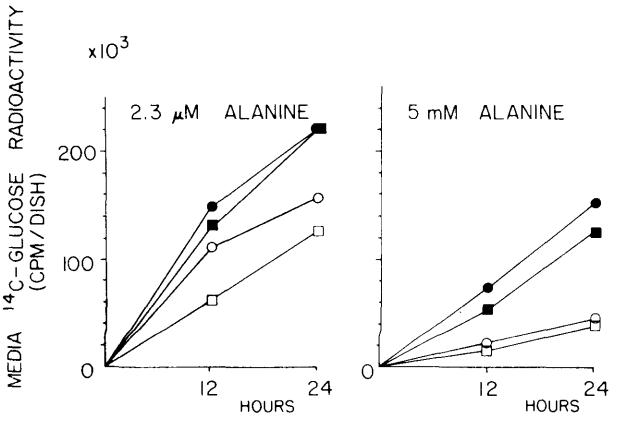

FIG. 3. Effect of insulin on glucagon-stimulated incorporation into glucose from alanine-U-^{14}C in human fetal liver explants. Explants were prepared from a specimen of 120-mm. crown-rump length and preincubated for twenty-four hours with the following additions: o—none; •—glucagon (7.5 μg./ml.) plus theophylline (0.5 mM); □—insulin (1 U./ml.); ■—glucagon plus theophylline plus insulin. After this period, alanine-U-^{14}C (5 μCi; either 2.3 μM or 5 mM) was added and media sampled at twelve and twenty-four hours thereafter. Total glycogen radioactivity as cpm per dish after twenty-four hours with the hormones present was 910 (none), 710 (glucagon), 960 (insulin), 910 (glucagon + insulin) in experiments with 5 mM alanine.

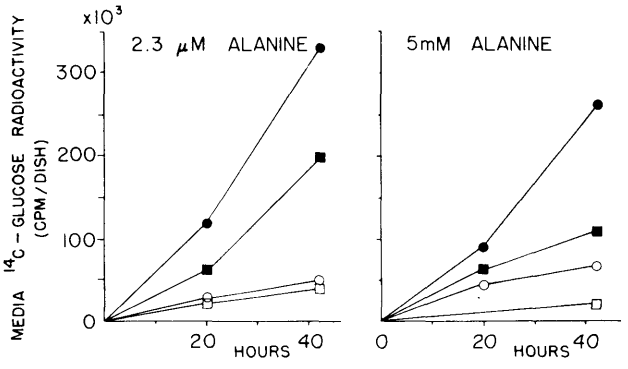

FIG. 4. Effect of dibutyryl cyclic AMP and insulin on incorporation into glucose from alanine-U-^{14}C at low glucose concentration in human fetal liver explants. Explants were prepared from a specimen of 110-mm. crown-rump length. Other details are similar to those in the legend to figure 2, except that this entire experiment was performed with media containing 2.8 mM glucose: o—no additions; •—dBcAMP plus theophylline; □—insulin; ■—dBcAMP plus theophylline plus insulin.

significant effect of the cyclic nucleotide at the $P < 0.01$ level (n = 30 pairs). Similarly, glucagon, which stimulates the accumulation of cyclic AMP in human fetal liver,[14] was found to stimulate incorporation from alanine-U-^{14}C by 195 per cent ($P < 0.01$, n = 6 pairs; figure 3, table 1).

TABLE 1

Effect of glucagon plus theophylline and insulin on incorporation into glucose from alanine-U-^{14}C in human fetal liver explants

		media ^{14}C-glucose radioactivity (10^3 cpm./mg. protein)	
		media glucose:	
addition	final conc.	10 mM	2.8 mM
none	—	7.8	9.7
glucagon + theophylline	7.5 μg./ml. 0.5 mM	27.1	46.5
insulin	1 U./ml.	1.4	8.2
glucagon + theophylline + insulin	7.5 μg./ml. 0.5 mM 1 U./ml.	14.3	26.8

Explants were prepared from a specimen of 150-mm. crown-rump length. Without prior incubation, the appropriate additions were made. Some of the culture dishes contained 2.8 mM glucose, while others contained 10 mM. Alanine-U-^{14}C (5 μCi; 2.3 μM) was added to all dishes at time zero. After forty-eight hours, samples were taken for glucose determinations.

Incubations of explants with only insulin at 1 U./ml., which was maximal for amino acid uptake and glycogen accumulation, consistently decreased incorporation by 35 per cent ($P < 0.01$, n = 11 pairs; figures 2-4, table 1). This effect of insulin was always in the same direction, although the magnitude of the insulin effect varied in different experiments. In addition to this effect on basal ^{14}C-alanine incorporation, insulin reduced the stimulated incorporation that resulted from dibutyryl cyclic AMP and theophylline incubation. When all data are pooled, insulin decreased by 41 per cent the incorporation of ^{14}C-alanine in the presence of dibutyryl cyclic AMP ($P < 0.01$; n = 6 pairs; figures 2, 4) and decreased by 23 per cent the incorporation in the presence of glucagon ($P < 0.01$; n = 6 pairs; figure 3, table 1).‡

These effects of insulin alone and in combination with the other hormonal agents are clearly seen regardless of whether the results are expressed per culture dish or per milligram protein, as seen in figure 2. Furthermore, these hormonal effects are observed at various medium glucose concentrations. As seen in figure 4 and table 1, alterations in medium glucose from 1 mM to 2.8 mM did not alter the pattern of results. In any given experiment the glucose radioac-

‡Furthermore, radioactivity in tissue glycogen was slightly increased in tissues incubated with insulin, but this did not significantly alter the overall effect of insulin on ^{14}C-alanine incorporation into glucose (figure 3). In addition, since radioactivity in tissue glycogen was not assayed at times earlier than forty-eight hours, it is possible that a larger percentage of ^{14}C-alanine radioactivity was present in this fraction at early times.

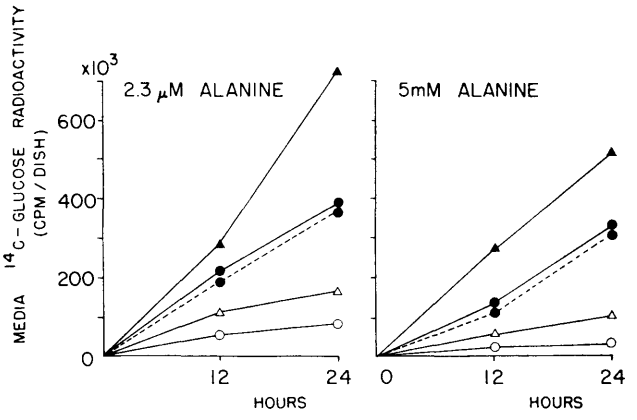

FIG. 5. Effect of triamcinolone on dibutyryl cyclic AMP-stimulated incorporation into glucose from alanine-U-^{14}C in human fetal liver explants. Explants were prepared from a specimen of 120-mm crown-rump length and preincubated for twenty-four hours with the following additions: o—none; •—dBcAMP plus theophylline; Δ—triamcinolone (20 μg./ml.); ▲—dBcAMP plus theophylline plus triamcinolone. After this preincubation, these agents were readded to their respective dishes and alanine-U-^{14}C (5 μCi; 2.3 μM or 5 mM) was added. At twelve hours and twenty-four hours after the alanine addition, media samples were taken for glucose-specific radioactivity determination. One set of dishes had no addition during the initial twenty-four hour preincubation but had dBcAMP plus theophylline added with the alanine (-----).

tivity was greater in dishes containing 2.8 mM glucose than the corresponding control at 10 mM glucose (table 1).

The hormonal effects observed at both 2.8 mM and 10 mM glucose were also observed at different medium alanine concentrations. Incubation in the presence of 5 mM alanine-U-^{14}C reduced total radioactivity in medium glucose from those values observed at 2.3 μM alanine-U-^{14}C (figures 3-5).

Incubation of tissues for twenty-four hours with dibutyryl cyclic AMP before the addition of the radioactive alanine did not alter the rate of incorporation from those of tissues that had the dibutyryl cyclic AMP and alanine-U-^{14}C added simultaneously (figure 5). This suggests that no significant lag period occurs in the cyclic-AMP stimulation of gluconeogenesis. Furthermore, the near linearity of this response as seen in figure 5 and other figures is consistent with this notion. Incubation with corticosteroid increased gluconeogenesis, but not as markedly as dibutyryl cyclic AMP plus theophylline (figure 5). Since a dose response test was not performed, this may be a consequence of the concentration of steroid used, even though the dose selected was maximal for enzyme induction in fetal liver explants.[18] The presence of both stimulatory agents produced greater stimulation than either agent alone and was greater than the sum of the individual effects (figure 5).

The response of the liver explants to steroid incubation is consistent with the well-known effects of corticosteroids on gluconeogenesis.[24] Experiments in adrenalectomized rats demonstrated that corticosteroids are not necessary for basal gluconeogenesis but are required to observe the cyclic AMP-stimulated acceleration.[24] The mechanism of this corticosteroid action is not understood at present. The results depicted in figures 1-5 and table 1 demonstrate that glucagon and dibutyryl cyclic AMP are capable of stimulating incorporation of alanine-U-^{14}C into glucose in fetal liver explants in the absence of corticosteroids. However, the stimulation elicited by glucagon/cyclic AMP in the absence of steroids could be present, but of such small magnitude compared

TABLE 2

Rates of glucose production from 5 mM alanine-U-^{14}C by human fetal liver explants:
Rate of glucose production (nmole/gm. min)

CR length (mm.)	none	dBcAMP*/ glucagon†	insulin	dBcAMP* + insulin/ glucagon† + insulin	triamcinolone	triamcinolone + dBcAMP
150	3.99	14.09*	1.13	6.77*	—	—
110‡	2.63	5.27*	1.54	4.58*	—	—
120	2.47	27.13*	—	—	10.36	51.30
120	4.44	15.29†	3.79	12.43†	—	—

The values in this table are calculated from the data in figures 2-5, assuming 25 mg. of liver tissue per dish and using a counting efficiency of 80 per cent for the ^{14}C determinations:

$$\frac{\text{(radioactivity per sample) (0.5 = moles glucose/moles alanine)}}{\text{(alanine specific radioactivity) (time of sample) (amount of tissue)}}$$

‡This experiment was performed with low glucose (2.8 mM).

with basal gluconeogenesis in the perfused adult rat liver as to be invisible under their experimental conditions. Further investigations are necessary before one can draw firm conclusions regarding the mechanism of steroid action under these circumstances.

Table 2 summarizes the rates of ^{14}C-alanine incorporation calculated from the data in figures 3-5, studies in which alanine was present at 5 mM. For these calculations, it was assumed that each culture dish contained 25 mg. tissue. Since the rate from each fetal specimen is different, this assumption would only slightly alter the absolute rate but not alter the relative results. As seen in table 2, the rate of incorporation into glucose in control experiments is of the order of 3-5 nmoles/gm./min. These rates were increased two- to elevenfold by either dibutyryl cyclic AMP or glucagon and were decreased 1.2-3.5-fold by insulin. Furthermore, insulin decreased the glucagon/cyclic AMP-stimulated rate by 1.2-2.1-fold.

The results of the present study confirm the earlier studies of Villee[13] and Adam et al.,[14] which demonstrated that human fetal liver was capable of performing gluconeogenesis. Upon incubation of human fetal liver slices with 10 mM pyruvate-2-^{14}C in the presence of 11 mM glucose, Villee[13] observed gluconeogenic rates of approximately 16 nmoles/gm./min. More recently, Adam et al. have examined incorporation of radioactivity into glucose from alanine-U-^{14}C (10 mM) or lactate-3-^{14}C (10 mM) in the absence or presence of 2.8 mM glucose in the isolated perfused human fetal liver. Their results demonstrated a de novo production rate of approximately 20 nmoles/gm./min. The basal rates observed in the present study from 5 mM alanine were about 4 nmoles/gm./min. and are similar to those observed in other in vitro systems.

DISCUSSION

Incubation with glucagon or dibutyryl cyclic AMP consistently stimulated the gluconeogenic rate in the present study with fetal liver explants, although an earlier attempt to demonstrate hormonal responsiveness in the isolated perfused human fetal liver was unsuccessful.[14] The inability to observe a stimulated rate in the perfused liver may be explained in part by comparing (1) the concentrations of agent examined, (2) the time of observations, and, most importantly, (3) the use of the perfused liver at a period in which the tissue was undergoing biochemical alterations before reaching a new steady state (e.g. changes in glycogen metabolism, cell intermediates, etc.).

Insulin was capable of decreasing basal ^{14}C-alanine incorporation and, in addition, diminished glucagon- or dibutyryl cyclic AMP-stimulated incorporation. These effects were seen with either 10 mM or 2.8 mM glucose present in the media.

The glucagon/cyclic AMP-stimulated rates were linear. As demonstrated in figure 5, preincubation with dibutyryl cyclic AMP prior to addition of the alanine-U-^{14}C yielded the same rate as that seen when both were added simultaneously, indicating a linear rate of incorporation. In addition, the identity in these rates argues against the dibutyryl cyclic AMP-stimulation resulting from a stimulation of alanine uptake by the liver cells, since this process displays a four-to-seven hour lag period in these same tissues.[17] In addition, the basal rate of amino acid uptake (i.e. α-aminoisobutyrate) in these tissues (approximately 28 mmoles/gm./min.)[17] was stimulated only 40 per cent by dibutyryl cyclic AMP or glucagon, which would provide only an additional 11 nmoles substrate/gm./min., or the equivalent of 6 nmoles glucose/gm./min. This is insufficient to account for the increment of stimulation seen with these agents in most cases (table 2).

Furthermore, any other mechanism for cyclic AMP action that includes enzyme induction is unlikely to be the major control point, since the rate of incorporation was unaltered by preincubation with dibutyryl cyclic AMP. These include PEP-carboxykinase, which was reportedly increased 30 per cent by five hours' incubation with dibutyryl cyclic AMP in human fetal liver explants[25] and glucose-6-phosphatase, which is markedly increased by dibutyryl cyclic AMP in human fetal liver explants.[16] Other key enzyme activities, e.g. pyruvate carboxylase in human fetal liver, have been determined,[26] but their role in the control of this process in unclear. Another possible control point is that of substrate translocation and mitochondrial CO_2 fixation.[27] This has not been examined in human fetal liver but has recently been determined in neonatal dog liver. Chlebowski et al.[28] were able to correlate the development of gluconeogenesis with the appearance of mitochondrial CO_2 fixation in the newborn dog. In addition, it appears that pyruvate carboxylase may be more rate-limiting than PEP-carboxykinase in the developing dog.[28,29] This pattern of metabolic maturity is different from that seen in the neonatal rat, where the development of gluconeogenesis parallels the appearance of PEP-carboxykinase activity that follows birth[30] or hormonal administration[3,4,31]

An additional difference in these models for gluconeogenesis is the appearance of PEP-carboxykinase. This enzyme activity is not detectable in the rat until just prior to birth,[4] whereas it is relatively active in fetal dog liver.[29]

The available information on fetal liver suggests that human liver is more similar to the dog's as a model. (1) PEP carboxykinase is clearly detectable in early human fetal liver,[26] (2) Stimulation by cyclic AMP of ^{14}C-alanine incorporation into glucose does not seem to be associated with an increase in this enzyme activity, (3) Rates of glucose output and gluconeogenesis in human fetal liver[32] (table 2) are similar to those in the fetal dog.[28,29]

The rates of alanine incorporation into glucose detected in this study (4 nmoles glucose/gm./min. ~ 0.2 μmoles glucose/kg. fetus/min.) are not sufficient to account for the physiologic glucose requirements in the newborn.[33] The ability of this tissue to increase its gluconeogenic rate two- to tenfold provides a potential mechanism for the increased production of hepatic glucose, should further fetal development markedly elevate the basal gluconeogenic rate. Such a response to hormonal alterations in the neonatal period may become more important in our understanding of neonatal hypoglycemia.[34]

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