The Regulation of Gluconeogenesis in Isolated Rat Liver Cells by Glucagon, Insulin, Dibutyryl Cyclic Adenosine Monophosphate, and Fatty Acids*

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SUMMARY

Suspensions of rat liver cells from starved rats converted three carbon precursors to glucose and responded to hormones such as glucagon and insulin. Cells were isolated by perfusion of rat livers with calcium-free Hanks' solution containing 0.01 g% of collagenase and 0.08 g% of hyaluronidase in the presence of 4% defatted albumin. Cells from 18- to 24-hour starved rats synthesized glucose de novo from L-alanine at rates comparable to those observed in the perfused liver. Glucagon increased cyclic adenosine 3',5'-monophosphate accumulation in liver cells with the maximal effect being observed some 2 min after its addition. In cells from fed rats there was an immediate increase due to glucagon of glucose release which could be accounted for by glycogenolysis. In cells from starved rats there was almost no glycogen (0.5% for starved rats as compared to 7.5% for fed rats), and there was a 20-min lag period before glucose output was increased by glucagon. Glucagon stimulated gluconeogenesis in the presence of alanine, lactate, or pyruvate whereas added fatty acids inhibited gluconeogenesis from alanine and stimulated that from lactate or pyruvate. Insulin increased glycogen deposition in cells from starved rats, and this accounted for the decrease in glucose output seen with insulin. Dibutyryl cyclic AMP also stimulated gluconeogenesis in isolated liver cells. The effects of insulin and glucagon on liver cells were abolished by short term treatment of cell suspensions with trypsin. In contrast, the response to dibutyryl cyclic AMP was not altered in the trypsin-treated cells.

The perfused rat liver has been used extensively and successfully in studies of gluconeogenesis (1-10). A major disadvantage of the system is that it is not exclusively a population of parenchymal cells. Secondly, the technique is rather cumbersome and limits the number of variables that can be studied simultaneously.

Liver slices, at first glance, would appear to be a simple system which would offer an ideal method for studying cellular phenomena. They provide many samples from the same animal, offering the possibility of studying many variables and simultaneously providing internal control samples. However, slices are actually nonuniform and complex systems in which oxygen diffusion to cells on the "inside" of the slice is limited, and the cells on the outer surface are mechanically damaged.

Homogeneous preparations of intact parenchymal cells isolated from one animal would allow the study of many variables at one time and would overcome many of the disadvantages mentioned in the other systems. There have been several procedures published for the isolation of rat liver cells (11-15), but we have found only the procedure of Berry and Friend (15) to be satisfactory for studies on the hormonal regulation of gluconeogenesis.

MATERIALS AND METHODS

All experiments were done with 130- to 160-g female Sprague-Dawley rats (Charles River, CD strain) fed laboratory chow ad libitum. The rats were deprived of food for 18 to 24 hours prior to the start of each experiment except where indicated. Each rat was anesthetized by intraperitoneal injection of 10 mg of sodium pentobarbital, the abdomen opened, the portal vein cannulated, approximately 500 units of heparin injected to prevent clotting, and the liver placed in a perfusion-aeration apparatus at 37°. The complete procedure should not take more than 4 min from the time the liver is cannulated until the liver is connected in the perfusion-aeration apparatus (Metaloglass Co.). The gas phase was 95% oxygen-5% carbon dioxide. The liver was next perfused with 50 ml of calcium-free Hanks' solution containing 4% defatted albumin which was not recirculated but discarded. Following this, the blanched liver was perfused for 40 min with 50 ml of calcium free Hanks' solution containing 4% defatted albumin, 0.08% hyaluronidase, and 0.01% collagenase which was recirculated. The liver was removed at the end of the 40 min of perfusion with enzymes, placed in a plastic Petri dish, and gently pressed with a spatula to disperse the cells. The dispersed tissue was incubated with shaking for another 15 min in plastic flasks containing the...
enzyrne solution previously used for perfusion in an atmosphere of 95% oxygen-5% carbon dioxide. At the end of this period, the cells were filtered through nylon chiffon, sedimented by centrifugation at 50 × g for 2 min, and the supernatant removed. The cells were washed three times and resuspended in Krebs-Ringer bicarbonate buffer.

All incubations were done at 37° in an atmosphere of 5% carbon dioxide-95% oxygen. The Hanks’ solution contained NaCl (137 mM), KCl (5.4 mM), NaHPO₄ (0.2 mM), KH₂PO₄ (0.4 mM), MgSO₄ (0.4 mM), glucose (6.6 mM), MgCl₂ (0.5 mM), and NaHCO₃ (4.2 mM). The Krebs-Ringer bicarbonate buffer contained NaCl (120 mM), KCl (4.8 mM), CaCl₂ (1.3 mM), KH₂PO₄ (1.2 mM), MgSO₄ (1.2 mM), and NaHCO₃ (24 mM). The bovine fraction V albumin powder (pH 7.0) was defatted prior to use by the procedure of Guilloy and Racker (16). The pH of the Hanks’ solution containing 4% albumin was approximately 7.1 at the start of the experiment and approximdately 7.0 at the end of the enzymatic perfusion of the liver. The procedure for isolation of liver cells was that of Berry and Friend (15) modified by the use of 0.01% collagenase instead of 0.05% and 0.08% hyaluronidase instead of 0.10%. Viability of the cell preparations was assessed by the exclusion of vital dyes. Preparations used in these experiments contained 90 to 98% viable cells as indicated by their exclusion of trypan blue.

Aliquots of the cell suspension containing 5 to 8 × 10⁷ cells in 0.25 ml were added to plastic incubation tubes (17 × 100 mm) containing 1.25 ml of the bicarbonate buffer, which contained 4% albumin and the indicated substrate. The drugs and hormones were added prior to the addition of the cells unless otherwise indicated. Tubes were gassed with 95% oxygen-5% carbon dioxide, stoppered, and incubated with vigorous shaking at 37°. At the end of the incubation, the samples were chilled in an ice bath, centrifuged, and 25-µl aliquots of medium were taken for glucose assay by the glucose oxidase method (17).

The cell pellet remaining in each tube was analyzed for glycogen by the anthrone procedure after digestion in 30% (w/v) KOH (18).

Cyclic AMP was assayed by an adaptation of the binding assay described by Gilman (19). The assay was conducted at pH 6.0 in a total volume of 35 µl using a binding protein which was purified up to the diethylaminoethyl cellulose step (19). Instead of collecting the bound cyclic AMP on Millipore filters, we separated the bound from the unbound cyclic AMP by adding 0.6 ml of Norit A charcoal suspended (60 mg per 100 ml) in 20 mM phosphate buffer (pH 6.0) containing 0.5% bovine serum albumin. After a maximum of 10 and a minimum of 5 min, the tubes were centrifuged to pellet the charcoal. Aliquots (0.5 ml) of the supernatant were taken to determine the bound radioactive cyclic AMP. The use of charcoal is similar to the method of Brown et al. (20).

For the figure and table where cyclic AMP measurements are given, tubes were set up in parallel to the tubes used for glucose and glycogen measurements. Trichloroacetic acid was added to the cells plus the medium to a final concentration of 5% in those tubes in which cyclic AMP was measured.

Lactate dehydrogenase activity was assayed in both the medium and cell homogenates by the method of Bergmeyer et al. (21). Sources of chemicals were: crude bacterial collagenase from Clostridium histolyticum, twice crystallized, dialyzed salt-free and lyophilized trypsin and chicken egg yolk which inactivated approximately 0.8 mg of trypsin per mg of inhibitor (Worthington), glucagon-free bovine insulin (a gift of Eli Lilly Co.); bovine albumin fraction V powder (Penetex Division of Miles Products); NAD⁺-Alanine dehydrogenase 3',5'-cyclic phosphate (Schwarz BioResearch); oleic acid (99% pure), octanoic acid, sodium pyruvate, glycogen, and bovine testicular hyaluronidase (300 NF units per mg, type 1) (Sigma); heparin (Organon, Inc.); sodium pentobarbital (Abbott Laboratories); f-alanine (Eastman Organic Chemicals); and lactate (Mann).

RESULTS

The procedure of Berry and Friend (15) for the isolation of liver cells resulted in parenchymal cells which responded to hormones, were morphologically intact, and could be isolated in high yield. Electron micrographs of cells isolated by the modified Berry and Friend procedure indicated that the structure of the cells, including nuclei, mitochondria, rough surfaced endoplasmic reticulum, and other cytoplasmic organelles were well preserved.²

Berry and Friend (15) did not use buffers containing albumin, but we found that the yield of cells and hormonal sensitivity was greater for cells isolated and incubated in the presence of 4% defatted bovine albumin.³ We also found that the additional step of perfusing the liver with calcium- and magnesium-free Hanks’ solution containing 2 mM EDTA recommended by Berry and Friend (18) was unnecessary for a large yield of viable cells. Occasionally we had had problems with impurities in the albumin, collagenase, or hyaluronidase. These problems were overcome by the use of defatted albumin and a reduction in the concentration of hyaluronidase and collagenase used in the perfusion medium.

A high yield of cells was obtained using our modification of the procedure of Berry and Friend (Table I). The leakage of lactate dehydrogenase was less than in cells prepared by the procedure of Howard et al. (13) in which liver slices were digested with collagenase and hyaluronidase (Table I).

The accumulation of cyclic AMP was enhanced by the addi-

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³ E. H. Leduc, personal communication.
² M. E. Johnson and N. Das, unpublished results.
tion of glucagon to liver cells from fed rats, with the largest increase being noted after 1 or 2 min of incubation (Table II). Upon further incubation (for up to 2 hours) the cyclic AMP accumulation in the presence of glucagon decreased but was still higher than in controls. There was an appreciable amount of glycogen in the cells from fed rats, and glucagon increased glycogen breakdown and the appearance of glucose in the medium at all times periods tested (Table II). The increase in glucose production in cells from fed rats occurred immediately and could be accounted for solely by enhanced glycogen breakdown.

Since we were interested in examining effects of agents on gluconeogenesis, all further experiments were conducted with cells from rats starved for 24 hours whose glycogen content was very low (approximately 10 μg per 10^6 cells or less).

A test of metabolic integrity in liver preparations is the ability to synthesize glucose from 3-carbon precursors. Krebs et al. (9) have reported that 0.66 μmole of glucose per min per g of tissue was formed, with 10 mM L-alanine as substrate, in perfused livers from 48-hour starved rats. The rate of glucose production obtained by Haynes (22) from liver slices of fasted rats using 10 mM alanine as the substrate was 0.14 μmole per min per g of tissue. The rate of glucose production, 0.5 to 0.7 μmole per min per g of tissue (assuming that each liver cell weighs 2 × 10^-5 g) (23), which we observed in isolated liver cells preparations in the presence of alanine, compares favorably with that of the perfused liver and is higher than the values obtained in liver slices (Table III). The addition of glucagon to cells from starved rats incubated with 10 mM pyruvate increased glucose accumulation in the medium after a lag period of more than 20 min (Fig. 1). The increase in glucose output was due to stimulation of gluconeogenesis rather than glycogenolysis, since the glycogen content in control cells was 0.5% (11 μg per 10^6 cells in glucose equivalents) and the decrease due to glucagon was less than 1 μg per 10^6 cells for the experiment shown in Fig. 1.

Cyclic AMP accumulation was increased by glucagon with the maximal effect being seen at 2 min; by 20 min, the values had dropped almost to the level of controls (Fig. 1). At the later time period (60 to 120 min) when gluconeogenesis was stimulated, the cyclic AMP values were indistinguishable from those for controls. Effects similar to those shown in Fig. 1 have also been seen when liver cells from starved rats were incubated with 10 mM L-alanine.

Gluconeogenesis was stimulated by glucagon in cells from starved rats incubated in the presence of 10 mM pyruvate, lactate, or alanine, as well as 5 mM alanine plus either 5 mM pyruvate or lactate (Table III). Oleate at a concentration of 0.5 or 1 mM decreased gluconeogenesis from alanine but increased gluconeogenesis from lactate or pyruvate (Table III). There was no inhibition of gluconeogenesis due to 1 mM oleate in the presence of glucagon (2.7 μM).

In an experiment conducted on aliquots of the same cells used for Experiment 1 in Table III, it was found that epinephrine bitartrate (50 μM) stimulated glucose release by 40% after 2 hours of incubation and 97% after 3 hours of incubation in the presence of 10 mM L-lactate. Cyclic AMP levels in control cells were 3.8 ± 0.2 pmoles per 10^6 cells at 2 min and 4 ± 0.1 pmoles per 10^6 cells at 5 min. In the presence of epinephrine, cyclic AMP increased to 5.5 ± 0.4 pmoles per 10^6 cells at 2 min and 4.5 ± 0.5 at 5 min.

### Table II

| Time | Medium glucose | Total cyclic AMP | Glycogen content |
|------|----------------|------------------|------------------|
|      | Basal | Δ due to glucagon | Basal | Δ due to glucagon | Basal | Δ due to glucagon |
| min  | μg/10^6 cells | pmoles/10^6 cells | μg glucose/10^6 cells | μg glucose/10^6 cells |
| 1    | 27    | + 6              | 3.0             | + 4.0             | 156   | - 12              |
| 2    | 28    | + 6              | 2.8             | + 5.1             | 142   | - 15              |
| 5    | 27    | + 8              | 2.8             | + 1.7             | 153   | - 40              |
| 20   | 33    | + 9              | 2.7             | + 0.8             | 93    | - 47              |
| 60   | 40    | + 10             | 3.0             | + 0.3             | 81    | - 48              |
| 120  | 47    | + 16             | 2.4             | + 0.6             | 44    | - 23              |

### Table III

| Percentage of change due to fatty acids or glucagon |
|----------------------------------------------------|
| Substrate                                          |
|         | 0.5 mM Oleate | 1.0 mM Oleate | 2.7 μM Glucagon | 1.0 mM oleate |
| 10 mM Alanine                                     | 29 | - 15 | - 33 | + 37 | + 22 |
| 10 mM Alanine                                     | 33 | - 2 | - 14 | + 36 | + 2 |
| 10 mM L-Lactate                                   | 29 | + 10 | + 24 | + 52 | + 77 |
| 10 mM L-Lactate                                   | 22 | + 21 | + 41 | + 63 | + 116 |
| 10 mM Pyruvate                                    | 46 | + 16 | + 14 | + 21 | + 21 |
| 10 mM Pyruvate                                    | 42 | - 16 | - 34 | - 28 | - 56 |
| 5 mM Alanine + 5 mM pyruvate                       | 29 | + 3 | - 10 | + 14 | + 2 |
| 5 mM Alanine + 5 mM pyruvate                       | 41 | + 33 | + 36 | + 34 | + 56 |
| 5 mM L-Lactate + 5 mM L-lactate                   | 50 | + 14 | + 7 | + 18 | + 16 |
| 5 mM L-Lactate + 5 mM L-lactate                   | 47 | + 37 | + 33 | + 24 | + 47 |

The remainder of the studies were done with 10 mM L-alanine as the substrate. The data in Fig. 2 indicate that there was no gluconeogenesis in the absence of added substrate and that maximal stimulation of gluconeogenesis was seen with 5 mM...
GLUCOSE PRODUCTION

FIG. 1. Stimulation of cyclic AMP accumulation and glucose production by glucagon. Liver cells (7.4 x 10⁶ cells per tube) obtained from a starved rat were incubated in buffer containing 4% albumin and 10 mM pyruvate for 5 min prior to addition of glucagon (2.7 μM). The glucose content in the medium at this time was equivalent to a glucose production of 4 μg per 10⁶ cells, and the increments over that value during the incubation are shown. The glycogen content at the start of the experiment was approximately 11 μg in glucose equivalents per 10⁶ cells, and the values ranged from 10 to 11 μg in the tubes incubated with glucagon.

GLUCOSE PRODUCTION

FIG. 2. The effect of alanine concentration. Liver cells were incubated for 2 hours in Krebs-Ringer bicarbonate buffer containing 4% albumin and various concentrations (0 mM, 2.5 mM, 5 mM, 10 mM) of L-alanine. The values are the means ± standard errors of six paired experiments. Alanine, a concentration of 2.5 mM alanine gave about half-maximal stimulation of gluconeogenesis.

GLUCOSE PRODUCTION

FIG. 3. The effects of octanoate and dibutyryl cyclic AMP (DCA/M) on glucose production. The liver cells were incubated for 2 hours in Krebs-Ringer bicarbonate buffer containing 4% albumin and 10 mM L-alanine. The concentration of dibutyryl cyclic AMP was 0.04 mM; the concentration of octanoate ranged from 0.5 mM to 5 mM. The values as percentage of change from basal are shown as the mean ± standard errors of six paired experiments. Basal glucose formation was 25.2 ± 3.4 μg of glucose per 10⁶ cells over 2 hours. The initial glycogen content in glucose equivalents was 8.3 ± 1.5 μg per 10⁶ cells, whereas that at the end of the incubation period was 5.5 ± 0.5 μg per 10⁶ cells for control cells and was not significantly decreased by any of the added agents.

Insulin has been shown to reduce the amount of glucose released by the fasting liver (7, 8), and this was also found to be the case with liver cells (Table IV and Fig. 4). Insulin increased glycogen deposition in liver cells as compared to controls (Fig. 4). The decrease in glucose release to the medium seen with insulin was probably not the result of inhibition of gluconeogenesis, since within the limits of experimental error the decrease in glucose release could be accounted for by diversion of glucose into glycogen.

In the studies shown in Fig. 4, cells were incubated with dibutyryl cyclic AMP in the presence and absence of insulin. Basal glucose production was reduced with insulin and elevated by all three concentrations of dibutyryl cyclic AMP. In the presence of insulin, the stimulation of glucose production due to the higher concentrations of dibutyryl cyclic AMP was unaffected, whereas glucose production due to the lowest concentration of dibutyryl cyclic AMP was reduced by insulin. Dibutyryl cyclic AMP also markedly reduced the stimulation of glycogen deposition due to insulin. In fact, glycogen con-
Inhibition of the hormonal changes in glucose production by treatment with trypsin

Liver cells from 18-hour fasted rats were isolated by perfusion and divided into two lots; one served as the control and the other was treated with trypsin. In Experiment 1, cells were exposed to 1 mg per ml of trypsin for 5 min. In Experiments 2 and 3, the cells were incubated with only 33 μg per ml of trypsin for 30 s. After trypsin treatment, the cells were washed with buffer containing trypsin inhibitor. Cells were then incubated for 2 hours in Krebs-Ringer bicarbonate buffer containing 4% albumin and 10 mM L-alanine.

The method of cell isolation using tetraphenylborate (14) resulted in high yields of cells, the majority of which did not divide among many tubes and are responsive to hormones. Experience with this procedure indicated great variability in the preparation. The procedure of Berry and Friend (15), and poor response to hormones all of which led to our abandonment of this procedure. The procedure of Berry and Friend (15) to be the most satisfactory due to the very low yields of cells and the distorted structure seen in electron micrographs.

Table IV

| Glucose production | Experiment 1 | Experiment 2 | Experiment 3 | Average |
|--------------------|--------------|--------------|--------------|---------|
| **Basal**           | 8.8          | 45.2         | 15.9         | 23.3    |
| **DCAMP** (0.18 mM) | + 9.6        | + 13.0       | + 7.5        | + 10.0  |
| **Glucagon** (2.7 μM) | + 5.3       | + 4.5        | + 2.3        | + 4.0   |
| **Insulin** (12 milliunits/ml) | - 2.3       | - 1.7        | - 2.7        | - 2.2   |
| **Insulin and glucagon** | + 0.6       | - 0.6        | - 10.6       | - 3.5   |
| **Trypsin treated** | 1.6          | 29.4         | 13.5         | 14.8    |
| **Basal**           | + 4.9        | + 12.3       | + 9.6        | + 8.9   |
| **DCAMP**           | + 1.2        | + 1.0        | - 1.3        | + 0.3   |
| **Glucagon**        | + 1.8        | - 1.2        | + 1.5        | + 0.7   |
| **Insulin and glucagon** | + 3.4       | + 0.3        | - 4.5        | - 0.3   |

* DCAMP, dibutyryl cyclic AMP.

Discussion

We have tested several methods for the isolation of liver cells and found that of Berry and Friend (15) to be the most satisfactory. The procedure of Jacob and Bhargava (11), which involved perfusion of the liver with calcium chelators followed by mechanical separation of the cells, proved unsatisfactory due to the very low yields of cells and the distorted structure seen in electron micrographs. Cells prepared by homogenization of liver were able to incorporate amino acids into proteins at very low rates (26) and synthesized very little lipid from acetate (26-28).

The method of cell isolation using tetraphenylborate (14) resulted in high yields of cells, the majority of which did not exclude the vital dyes. From ultrastructural studies in this laboratory (not shown) and those by Harris and Leone (29), it was apparent that these cells were grossly distorted.

The procedure of Howard et al. (13) employed digestion of liver slices with collagenase and hyaluronidase. Experience with this procedure indicated great variability in the preparations, low yields of cells, high rates of enzyme leakage (Table I), and poor response to hormones of all of which led to our abandonment of this procedure. The procedure of Berry and Friend results in large numbers of parenchymal cells which can be divided among many tubes and are responsive to hormones.

It has been shown that glucose production from L-alanine is stimulated in the perfused livers from fasted rats by the addition of glucagon (30). Friedmann et al. observed that the increase in glucose production was nearly 4-fold over basal conditions after the addition of glucagon to the perfusate (31).

Gluconolactone-induced activation of gluconeogenesis has been attributed to its lipolytic action (32), and many investigators have tried to correlate fatty acid oxidation with increased gluconeogenesis. Brendel and Bressler (33) observed that long chain fatty acids stimulated gluconeogenesis (in the presence of pyruvate) in pigeon liver homogenates and postulated that the key step linking the two processes was the production of acetyl-CoA. Acetyl-CoA is the end product of fatty acid

* N. Das, unpublished studies.
oxidation and has been shown to activate pyruvic carboxylase, one of the key regulatory sites of gluconeogenesis (33).

Inhibition of gluconeogenesis by long chain fatty acids in perfused liver has been observed in the presence of alanine (9). However, Struck et al. (32) and Krebs et al. (9) reported an enhancement of glucose formation from lactate in the presence of oleic acid and glucagon. The reports by Krebs (9) and Struck (32) suggest that inhibition of gluconeogenesis by fatty acids or their metabolic products occurs at the conversion of alanine to pyruvate. Our data are in agreement with the observation that the inhibitory effect of fatty acids is unique for alanine since opposite effects are seen with other substrates. Also the greatest increase in glucose production was observed from lactate in the presence of oleate plus glucagon (Table I11).

Exton et al. (4) observed a rapid increase in glucose output due to glycolysis with perfused livers from fed rats in the presence of glucagon (lactate as substrate). We obtained similar results with isolated fed rat liver cells (Table II).

Several investigators have shown that free fatty acids can affect gluconeogenesis in the perfused rat liver (3, 34–36). However, Exton et al. (3) were unable to observe any significant effects of albumin-bound oleate on gluconeogenesis from lactate under conditions where ketogenesis was increased (3). Fröhlich and Wieland (37) observed that glucagon in the presence of oleate did not stimulate ketogenesis when glycolysis was present. However, they observed an additional effect of glucagon on gluconeogenesis which exceeded that observed in the absence of fatty acid. These findings indicate that fatty acid oxidation may supply additional factors which are needed for optimal glucagon stimulation of gluconeogenesis and basal stimulation.

Exton et al. (3) found that the ketogenic and gluconeogenic actions of glucagon in the liver were separable and that glucagon had a primary and direct effect on gluconeogenesis. Therefore, it seems unlikely that fatty acid oxidation can be considered as the physiological regulator of gluconeogenesis (3, 38).

The action of insulin in regulating glucose release is particularly interesting. Under basal conditions in the normal fed rat, insulin has no effect on glycolysis or gluconeogenesis (10). According to Exton et al. (4, 5), insulin can block the production of glucose in livers from starved rats by lowering the tissue concentration of cyclic AMP. Recently, Hepp (39) proposed that insulin inhibits glucagon-stimulated adenylate cyclase activity in partitcular fractions of mouse liver. House (40) claims that the effect is not on cyclase but on phosphodiesterase. Our results indicate that insulin opposes the stimulatory effect of glucagon on glucose release (Table IV). Whether this effect of insulin is on the accumulation of cyclic AMP, the action of cyclic AMP, or some other process remains to be demonstrated. Glinsmann and Mortimore (10) were the first to report that insulin antagonizes the stimulation of glucose release by cyclic AMP in the perfused liver, a finding which was recently confirmed by Exton et al. (4). If the sole action of insulin was to inhibit adenylate cyclase, it should not affect the stimulation of glucose release by added cyclic AMP.

The major effect of insulin was not on gluconeogenesis in our experiments but rather to divert glucose into glycogen. This suggests that our effects may be similar to those of Bishop and Larner (41) and Gerschenson and Casanello (42), who found a rapid increase in glycogen synthesis in liver after infusion of insulin (which could not be accounted for by changes in cyclic AMP content)

Kono (25) and Fain and Loken (24) found that the response of both adipose tissue and isolated white fat cells to insulin or glucagon was abolished by preliminary treatment with trypsin. Similar results were seen in the present studies with liver cells. The failure of trypsin treatment to affect the response to dibutyl cyclic AMP suggests that trypsin impairs some early step in hormone action. Possibly trypsin blocks the binding of insulin and glucagon to their receptors in hepatic cells since trypsin abolished the ability of fat cells to bind insulin (43).

The present results indicate that it is possible to obtain suspensions of liver cells by a modification of the procedure of Berry and Friend (15) which are responsive to those agents which affect gluconeogenesis in the intact liver. The suitability of these cells for studies on the induction of enzymes, regulation of cyclic AMP accumulation, and other processes affected by hormones is currently under investigation.

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