Role of Cortisol on the Glycogenolytic Effect of Glucagon and on the Glycogenic Response to Insulin in Fetal Hepatocyte Culture*

CHRISTIANE PLAS AND JACQUES NUNEZ

From the Unité de Recherche sur la Glande Thyroid et la Régulation Hormonale I.N.S.E.R.M. and the Équipe de Recherche Associée n° 449 Centre National de la Recherche Scientifique, Hôpital de Bicêtre, 94270 Bicêtre, France

The effects of insulin and glucagon on glycogen metabolism were studied in cultured fetal hepatocytes transplanted from 15-day-old fetuses. The effects of these hormones were examined just after transplantation, when the cells contained only minute amounts of glycogen, and during the 3 to 4 day culture period, when the hepatocytes were exposed to 10 µM cortisol and actively accumulated glycogen. At all stages of the culture, glucagon addition (10 nM) was followed by a rapid depletion of labeled glycogen, previously synthesized during a pulse labeling with [14C]glucose: this effect was mimicked by N6,02'-dibutyryl adenosine 3':5'-monophosphate (dibutyryl cyclic AMP) (0.3 to 1 mM). Such a glycogenolytic effect of glucagon was observed even 6 hours after transplantation, i.e. at a time when cortisol was not present. In addition, glucagon clearly induced cyclic adenosine 3':5'-monophosphate (cyclic AMP) accumulation in cells grown for 18 hours in the absence of cortisol. With cells grown for 3 days in the presence of cortisol, glucagon-dependent glycogenolysis was also obtained when cortisol was removed from the medium 20 hours before hormone addition. Thus the presence of cortisol is not necessary either to maintain a response to glucagon or for the onset of the glycogenolytic effect of glucagon. Insulin addition (10 nM) stimulated [14C]glucose incorporation into glycogen at all stages of the culture when grown in the presence of cortisol; no glycogenic response to insulin was observed 6 hours after transplantation where cortisol was not previously introduced. In addition, if the hepatocytes were grown in the presence of insulin alone (i.e. in the absence of cortisol) no significant storage of glycogen occurred. Maximal storage (or labeling) of glycogen was observed when hepatocytes were grown in the presence of both cortisol and insulin. The presence of cortisol was therefore necessary for the expression of the glycogenic effect of insulin. These data show that marked differences exist between the onset of developmental responses towards glucagon and insulin. The glucagon-dependent regulatory pathway should be present very early in fetal development and should not depend on cortisol. On the contrary, the onset of the insulin-dependent regulatory pathway seems to be induced during culture, and it is likely that this is caused by cortisol.

It has been demonstrated that the presence of glucocorticoids in the plasma of the rat fetus is necessary for the accumulation of hepatic glycogen in late gestation (1-6). The presence of high levels of insulin in the plasma of the rat fetus during this period is well established (6-11) and the implication of insulin in the glycogen storage process has been suggested (12). Glucagon has been considered as an hormonal factor contributing to the mobilization of glycogen stores at birth (4, 13, 14). In vitro studies performed with perfused rat liver have clearly shown both a glycogenolytic effect of glucagon (15) which is mediated by cyclic AMP1 (16) and a glycogenic effect of insulin (17).

1 The abbreviations used are: cyclic AMP, cAMP, adenosine 3':5'-monophosphate; dibutyryl cAMP, Rp,cAMP, N6,02'-dibutyryl adenosine 3':5'-monophosphate.
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glycogenolytic response to glucagon was also present before any glycogen storage and in the absence of cortisol. On the contrary, a marked glycogenic effect of insulin was observed only in hepatocytes that have been previously exposed to cortisol.

**EXPERIMENTAL PROCEDURE**

Methods—Primary cultures of hepatocytes were obtained from 15-day-old rat fetuses (Sprague Dawley), using a culture procedure which already has been described (18, 20). This procedure involves dissociation of liver cells by treatment with trypsin, plating of a mixed cell suspension (on a collagen substrate to which only the hepatocytes adhered), and subsequent removal (after 6 hours) of the nonadhering hematopoietic cells (20). In some experiments, in order to study the effects of glucagon and insulin at earlier stages of the culture, the separation step was performed after 2 hours. The hepatocytes were grown for up to 4 days, the culture medium being replaced every 24 hours. Cortisol (10 μm) was introduced in the culture medium at the time of separation of the cells and after each 24-hour period together with the fresh medium. In other words, cortisol was always absent during the first 6 hours of plating. Several experiments were also performed without the presence of cortisol during the culture period chosen (2 to 6 hours to 4 days). The composition of the culture medium was as follows: NCTC 109, 90 parts/fetal calf serum, 10 parts. Fetal calf serum was carefully selected from several batches. The glucose concentration of the fresh medium was 1.06 mg/ml. The medium was replaced every 24 hours in order to minimize the variations in glucose concentration throughout the 4-day culture period. In short labeling experiments (4 to 6 hours of labeling), [14C]glucose was added 16 hours after replacement of the medium; at this time the glucose in the medium was measured and found to vary from 0.90 mg/ml at Day 1 to 0.78 mg/ml at Day 4. Thus the variation in medium glucose concentration during the culture period, where the effects of insulin and glucagon were measured, varied only by about 10%. In some experiments the specific radioactivity of the glucosyl residues of glycogen was determined as previously described (18).

Measurements of glycogen and protein contents (18), of glycogen labeling in the cultures grown in the presence of [3-14C]glucose, of glucose concentration in the medium (18), and of cyclic AMP levels in the cells (19) were performed as previously reported. The results were expressed per million hepatocytes (18, counting of the cells being performed on parallel cultures. The number of cells relative to wet weight was evaluated. For instance, after 3 days of culture in the presence of cortisol, one million hepatocytes corresponds to about 2.7 mg wet weight. Pig glucagon (containing 10 ppm of insulin) and pig insulin (containing less than 0.2 ppm of glucagon) were kindly supplied by Dr. Schlichkrull (Novo Laboratories). [3-14C]Glucose was purchased from the Commissariat à l'Energie Atomique (Saclay). Trypsin (TRSF) was obtained from Worthington, medium NCTC 109 from Diico, dibutyryl cAMP from Roehringer, hydrocortisone-21-sodium succinate from Sigma, cyclic AMP antiserum, and [3H]-succinyl cyclic AMP tyrosine methyl ester used in the radioimmunoassay of cyclic AMP from Institut Pasteur Production Materials—Fetal calf serum was obtained from Sorga (batch 60).

RESULTS

Development of Glucagon-dependent Glycogenolytic Response and Effect of Cortisol—Glucagon clearly produced a breakdown of glycogen stored in fetal hepatocytes taken from 15-day-old fetuses and grown for 4 days in the presence of cortisol (19). This glycogenolytic effect of glucagon was reproduced by cyclic AMP and the addition of glucagon was followed by cyclic AMP accumulation in the cells (19). However, it was not clear from these experiments whether the glycogenolytic response to glucagon was induced by cortisol or whether 15-day-old hepatocytes were already competent to respond to glycogenolytic agonists. To answer these questions the effect of glucagon or dibutyryl cAMP upon [3H]glucose incorporation into glycogen was studied at different times of the culture. Glucagon (10 nM) or dibutyryl cAMP (1 mM) were introduced together with [14C]glucose in 15-day-old hepatocytes for 1, 2, 3, or 4 days in the presence of 10 μM cortisol (Table I). Glycogen radioactivity was measured 4 hours after addition of the label. Table I shows that glucagon produced a clear inhibition of net [14C]glucose incorporation into glycogen not only at Day 4 of culture, when the rate of glycogen synthesis was high but also at Days 3 and 2, and even at Day 1, where no effect of cortisol on glycogen storage function was observed (18). To examine further the effects of glucagon in the absence of cortisol glucagon was added to cultures of 15-day-old hepatocytes 2 to 24 hours after plating in a medium lacking cortisol. Glucagon markedly inhibited the accumulation of [14C] label in glycogen in cells cultured without cortisol for either 6, 18, or 24 hours. Negative results were obtained only when glucagon was added 2 hours after plating the cells (Table II). Dibutyryl cAMP (1 mM) clearly inhibited net [14C]glucose incorpo-

<table>
<thead>
<tr>
<th>Addition</th>
<th>Glycogen radioactivity after glucagon or dibutyryl cAMP</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
</tr>
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<tr>
<td>None</td>
<td>2,200* 3,200* 4,740* 11,430*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10 nM glucagon</td>
<td>1,500* 1,590* 2,330* 2,620*</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
<td>1 mM Bt,cAMP</td>
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<td>None</td>
<td>3,640* 4,950* 13,980* 13,650*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 nM glucagon</td>
<td>2,040* 1,420* 6,000* 4,410*</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 mM Bt,cAMP</td>
<td>720* 796* 2,300* 2,810*</td>
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</tr>
</tbody>
</table>

**TABLE I**

Effect of glucagon and dibutyryl cAMP on [14C]glucose incorporation into glycogen of hepatocytes grown in presence of cortisol

The cultures were performed in the presence of 10 μM cortisol, cortisol being introduced in the medium at 6 hours, the time when the hepatocytes were freed from the hematopoietic cells. At times indicated in the table, [14C]glucose (1.25 μCi/mg) was introduced together with or without glucagon or dibutyryl cAMP (Bt,cAMP). The radioactivity (in counts per min per million hepatocytes) present in the newly synthesized glycogen was measured 4 hours later. Each value is the mean of two cultures.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Glycogen radioactivity after glucagon or dibutyryl cAMP</th>
<th>2 hr</th>
<th>6 hr</th>
<th>18 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3,370* 1,900* 2,880* 5,770*</td>
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<tr>
<td>10 nM glucagon</td>
<td>3,090* 1,160* 1,850* 3,780*</td>
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<td></td>
<td></td>
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<tr>
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<td>3,360* 1,900* 3,120*</td>
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<tr>
<td>1 mM Bt,cAMP</td>
<td>1,000* 675* 1,340*</td>
<td></td>
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</tbody>
</table>

**TABLE II**

Effect of glucagon and dibutyryl cAMP on [14C]glucose incorporation into glycogen of hepatocytes grown in absence of cortisol

The cultures were performed in the absence of cortisol. The hepatocytes were freed from hematopoietic cells 6 hours after plating, or 2 hours (indicated by asterisk) after plating. At times of the cultures indicated in the table, [14C]glucose (1.25 μCi/mg) was introduced together with or without glucagon or dibutyryl cAMP (Bt,cAMP). The radioactivity (in counts per min per million hepatocytes) present in the newly synthesized glycogen was measured 4 hours later. Each value is the mean of two cultures.
corporation into glycogen 2 hours after plating the cells (Table II). Throughout the culture period, dibutyryl cAMP produced similar (but larger) effects than 10 nM glucagon (Tables I and II). The finding that dibutyryl cAMP was more effective than glucagon when employed at a saturating dose was not reproduced with all the dibutyryl cAMP batches (19); thus, this observation cannot be considered as significant.

A second group of experiments was performed under the same conditions except that glucagon (or dibutyryl cAMP) was added only after labeling of the cells during 4 hours with [14C]glucose. Radioactivity incorporated into glycogen was measured after 4 and 6 hours of labeling, i.e., before, and 2 hours after glucagon or dibutyryl cAMP addition. The glycogenolytic effect of these agonists was estimated by the decrease in glycogen radioactivity. Glucagon (Fig. 1) and dibutyryl cAMP (Table III) stimulated throughout the culture period the degradation of the glycogen, newly synthesized in the presence of cortisol. A clear glycogenolytic effect of glucagon and dibutyryl cAMP was observed also 6 hours after plating, i.e., in the absence of cortisol (Fig. 1 and Table III). Therefore the hepatocytes responded to glucagon after 6 to 24 hours of culture in the absence of cortisol (Fig. 1 and Table III). This response was accompanied by an accumulation of cyclic AMP in these young cells. For instance, after 18 hours of culture in the absence of cortisol (Fig. 2), glucagon increased cyclic AMP levels, the peak occurring within minutes. The cyclic AMP level remained elevated for the 4 hours of glucagon exposure. Similar results have been previously described with hepatocytes grown during 4 days in the presence of cortisol (19).

Cyclic AMP accumulation was also observed when glucagon was added 6 hours after plating the cells in the absence of cortisol. A second group of experiments was performed under the same conditions except that glucagon (or dibutyryl cAMP) was added only after labeling of the cells during 4 hours with [14C]glucose. Radioactivity incorporated into glycogen was measured after 4 and 6 hours of labeling, i.e., before, and 2 hours after glucagon or dibutyryl cAMP addition. The glycogenolytic effect of these agonists was estimated by the decrease in glycogen radioactivity. Glucagon (Fig. 1) and dibutyryl cAMP (Table III) stimulated throughout the culture period the degradation of the glycogen, newly synthesized in the presence of cortisol. A clear glycogenolytic effect of glucagon and dibutyryl cAMP was observed also 6 hours after plating, i.e., in the absence of cortisol (Fig. 1 and Table III). Therefore the hepatocytes responded to glucagon after 6 to 24 hours of culture in the absence of cortisol (Fig. 1 and Table III). This response was accompanied by an accumulation of cyclic AMP in these young cells. For instance, after 18 hours of culture in the absence of cortisol (Fig. 2), glucagon increased cyclic AMP levels, the peak occurring within minutes. The cyclic AMP level remained elevated for the 4 hours of glucagon exposure. Similar results have been previously described with hepatocytes grown during 4 days in the presence of cortisol (19).

Cyclic AMP accumulation was also observed when glucagon was added 6 hours after plating the cells in the absence of cortisol. A typical experiment is presented, each value represents the mean of two cultures.

**Table III**

<table>
<thead>
<tr>
<th>Duration of pulse</th>
<th>Addition</th>
<th>Glycogen radioactivity after Bt,cAMP addition:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Glycogen radioactivity after Bt,cAMP addition:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No cortisol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 hr</td>
</tr>
<tr>
<td>4 hr</td>
<td>None</td>
<td>3,260</td>
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<tr>
<td>6 hr</td>
<td>None</td>
<td>2,960</td>
</tr>
<tr>
<td>6 hr</td>
<td>0.3 mM Bt,cAMP</td>
<td>1,310</td>
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</table>

![Fig. 1. Glycogenolytic effect of glucagon at different times of the culture.](http://www.jbc.org/)  
![Fig. 2. Cyclic AMP levels of hepatocytes grown in the absence of cortisol after exposure to glucagon.](http://www.jbc.org/)
glucose. Table IV shows that in the absence of cortisol, no presence of cortisol alone, specific activity was 1.0 (Day 2), 0.95 significantly modified by the presence of insulin: in the specific activity of glucose units in the stored glycogen was not be uniformally labeled. Moreover it was verified that the of transplantation in 15-day-old hepatocytes, glycogen should these conditions, since the glycogen pool is very low at the time of cortisol the glycogen content remained low, whether of the cells in the absence of cortisol (Fig. 5). Further experiments were performed to test if the insulin glycogenolytic response to glucagon does not seem to depend on cortisol.

Development of Insulin-dependent Glycogenic Response: Effect of Cortisol—The glycogenic effect of insulin was first tested by [14C]glucose labeling experiments, hepatocytes being grown in the presence of cortisol from the beginning of the culture. [14C]Glucose was introduced at Days 1, 2, 3, and 4 of culture, with or without 10 nM insulin, and the radioactivity of glycogen was measured 4 hours after addition of the label. Fig. 5 shows that at Days 2, 3, and 4, when cortisol-dependent glycogen storage was present, insulin markedly stimulated glycogen labeling. Insulin produced little glycogen labeling at earlier times when cortisol was ineffective with regard to glycogen metabolism. On the other hand very little, if any increase of glycogen labeling by insulin could be observed 2 hours after plating of the cells in the absence of cortisol (Fig. 5).

Glycogen content (B) and radioactivity present in glycogen (A) were determined on the same cultures.

10 nM glucagon was added only 20 hours after cortisol removal. Fig. 4 shows that glucagon still induced a clear fall in the level of labeled glycogen. Thus maintenance of a glycogenolytic response to glucagon does not seem to depend on cortisol.

In late gestation glycogen synthesis becomes important, whereas at earlier stages only minute amounts of glycogen are synthesized. If 15-day-old hepatocytes are grown for 4 days in the presence of cortisol, both glycogen accumulation (18) and a stimulaton by insulin was detected at any time of the culture.

stimulation by insulin was detected at any time of the culture. In the presence of cortisol, stimulation was present at Days 2, 3, and 4 of the culture as indicated by direct measurement of glycogen content (Fig. 6).

DISCUSSION

In late gestation glycogen synthesis becomes important, whereas at earlier stages only minute amounts of glycogen are synthesized. If 15-day-old hepatocytes are grown for 4 days in the presence of cortisol, both glycogen accumulation (18) and a clear glycogenolytic response to glucagon (19) have been previously demonstrated. It has been shown that this effect is mimicked by cyclic AMP and potentiated by theophylline (19); glucagon addition (10 nM) was also followed by an increase of the tissue levels of cyclic AMP which was maximal after 2 to 5 min (19). Therefore the plasma membranes of 15-day-old hepatocytes grown in the presence of cortisol very likely contain a specific receptor for glucagon and a hormone-

Fig. 3. Glycogenolytic effect of glucagon, added just after cortisol removal. Hepatocytes were grown throughout the culture period in the presence of [14C]glucose, the medium being replaced every 24 hours (specific radioactivity of glucose in fresh medium: 0.80 μCi/mg). After 3 days of culture in the presence of 10 μM cortisol, at the time of replacement of medium, cortisol was either withdrawn (O, Δ), or not (●, △) from the medium and 10 nM glucagon was added. Both glycogen content (B) and radioactivity present in glycogen (A) were determined on the same cultures.

Fig. 4. Glycogenolytic effect of glucagon, added 20 hours after cortisol removal. The experimental protocol was similar to that described under Fig. 3 except that the specific radioactivity of glucose in fresh medium was 0.20 μCi/mg and that the effect of glucagon addition was tested only 20 hours after replacement of the medium. On Day 3, at the time of medium replacement, cortisol either was removed (O, Δ), or not (●, △) from the medium. The glycogen radioactivity was measured 20, 21, 22, 23, and 24 hours after medium replacement. Δ, △, 10 nM glucagon added at time 20 hours. O, ●, control experiment with no glucagon addition.

Fig. 5. Glycogenic effect of insulin at different times of the culture. At times indicated on the abscissa, the effect of 10 nM insulin on the rate of incorporation of [14C]glucose into glycogen was measured. Time 0 hours: hepatocytes freed from hematopoietic cells 2 hours after plating and then grown in the absence of cortisol. Time 24, 48, 72, and 96 hours: hepatocytes freed from hematopoietic cells 6 hours after plating and then grown in the presence of 10 μM cortisol. [14C]Glucose (1.25 μCi/mg) was introduced together with or without 10 nM insulin, and the glycogen radioactivity was measured 4 hours later. Pointed bars, 10 nM insulin was added together with [14C]glucose. Empty bars, control experiments with no insulin addition. Two cultures were used for each point, the individual values being represented by a horizontal line on each bar.

* M. C. Fulchignoni and C. Plas, unpublished results.
resulting either from increased synthesis or from decreased synthesis or enhanced breakdown of the glycogen; conversely, glycogen labeling with [Y]glucose. The use of such isotope incorporation studies requires two comments. First, a decrease in glycogen labeling could be the result of either decreased accumulation in the absence of cortisol is minimal during the early stages of development of the hepatocyte, a direct measurement of cold glycogen was difficult to obtain. Therefore, these data did not provide information concerning the early stages of development of the hepatocyte. A typical experiment is presented, each value represents one culture.

### Table IV

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Glycogen labeling after a culture period:</th>
<th>cpm/10^6 hepatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td>10 μM cortisol</td>
<td>12,300</td>
<td>26,200</td>
</tr>
<tr>
<td>10 μM cortisol + 10 nM insulin</td>
<td>12,700</td>
<td>39,500</td>
</tr>
<tr>
<td>None</td>
<td>7,300</td>
<td>9,250</td>
</tr>
<tr>
<td>10 nM insulin</td>
<td>7,300</td>
<td>9,410</td>
</tr>
</tbody>
</table>

sensitive adenylate cyclase as demonstrated in the adult liver (21). Both activation and inactivation of the cyclic AMP-dependent degradative and synthetic glycogen pathways were observed (19). In addition, a fully active cyclic AMP-dependent protein kinase was found present in these hepatocytes. However these data did not provide information concerning the role of cortisol on the effects of glucagon. Since glycogen accumulation in the absence of cortisol is minimal during the early stages of development of the hepatocyte, a direct measurement of cold glycogen was difficult to obtain. Therefore, the experiments were performed by determining the changes in glycogen labeling with [14C]glucose. The use of such isotope incorporation studies requires two comments. First, a decrease in glycogen labeling could be the result of either decreased synthesis or enhanced breakdown of the glycogen; conversely, an increase in glycogen labeling might be interpreted as resulting either from increased synthesis or from decreased breakdown of the glycogen. The term "glycogenolytic effect" (or "glycogenic effect") used here does not mean that the absolute rate of glycogenolysis (or glycogenesis) is stimulated, but indicates a final effect at the level of glycogen. However in the prelabeling experiments, glucagon induced the loss of 14C from glycogen, indicating that the hormone had triggered the net breakdown of glycogen. It is likely that glucagon produced this effect at least by stimulating the absolute rate of glycogenolysis. In a previous work (19), it was shown that the glycogen half-life was 50 min just after glucagon addition whereas, in the absence of glucagon, the glycogen half-life was 12 to 14 hours; in these experiments the effect of glucagon was measured after 4 days of culture in the presence of cortisol. Second, the amount of labeled glycogen depends on the specific activity of the intracellular glycogen precursor pool which is different from that of the specific activity of the extracellular labeled glucose (18). Changes in extracellular glucose concentration could alter the specific activity of the glucose precursor pool. The experimental procedure we have used permits us to maintain the extracellular glucose concentration throughout the culture period without large variations; most of the experiments were performed with a glucose concentration varying from 0.90 to 0.78 mg/ml. This excluded the possibility of variations of glucose concentration in the effect of the hormones in glycogen metabolism, particularly that for insulin. For instance, the specific activity of glucose units in glycogen stores was not significantly modified by the presence of insulin. These observations allowed us to study the role of cortisol on the response to glucagon and insulin at the earliest stages of the culture.

The experiments reported in this work show that a glycogenolytic response to glucagon was maximally expressed before significant amounts of glycogen were stored (Fig. 1). In addition, this glycogenolytic response was not found to be induced by cortisol (Table II and Fig. 1). Removal of cortisol for 20 hours did not suppress the glycogenolytic effect of glucagon (Fig. 4). Consequently, the presence of cortisol in the medium is not required either for the onset of the glycogenolytic response or for the maintenance of the response to this hormone for at least a period of 20 hours. The absence of response to glucagon (and insulin) after 2 hours of plating may be explained by assuming that the membrane receptor for glucagon (or insulin) has been inactivated by trypsinization (22), the method which is used to isolate the cells. This assumption is supported by the finding (Table II) that dibutyryl cAMP was inhibitory to 14C labeling in glycogen 2 hours after plating. On the contrary, 6 hours after plating, a glycogenolytic response to glucagon was obtained, thus suggesting that the glucagon receptor was either repaired or synthesized de novo. It is not clear from the literature whether in 15-day-old liver fetuses there are glucagon receptors and an active form of adenylate cyclase. Adenylate cyclase activity has been shown to be present (23, 24) but the ability to respond to glucagon at this stage is controversial (23, 24). In addition, the adenylate cyclase thus detected may belong, at least partly, to the hematopoietic cells, which represent at this stage 60% of the liver population (25, 26). In late gestation (18-day-old fetuses), glucagon stimulated both adenylate cyclase activity (23, 27, 28) and glycogenolysis (4, 13, 14). In the system used in this work, cultured 15-day-old hepatocytes, cyclic AMP accumulation was induced by glucagon 6 and 18 hours (Fig. 2) after plating, in the absence of cortisol. Therefore these young hepatocytes plated in the absence of cortisol are competent, or
rapidly become competent, within a 6-hour period, to respond to glucagon. However in the adult perfused liver, Exton et al. (29) have shown some effects of adrenalectomy on the glycogenolytic response to glucagon.

On the other hand, a glycogenic effect of insulin was clearly shown, when cortisol-dependent glycogen storage was present (Fig. 5). From the results obtained when a combination of cortisol and insulin was used during a 4-day period (Fig. 6 and Table IV), it might be assumed that these two hormones produce additive glycogenic effects. In the absence of cortisol, insulin alone might stimulate glycogen synthesis. Fig. 6 and Table IV show that no glycogen storage was obtained in the presence of insulin alone. A similar result was obtained by Eisen et al. (30) with 16-day-old liver in explant culture. However, these authors found that cortisol alone is also ineffective in stimulating glycogen synthesis, but that previous exposure to the steroid hormone for 40 hours, permits the expression of the glycogenic effect of insulin. They assumed that when cortisol by itself stimulates glycogen synthesis in our culture system (18), some insulin could be introduced with the fetal calf serum present in the serum medium (38). Careful examination of the immunoreactive insulin content of the fetal calf serum (batch 60, Sorga) used in the experiments reported above, revealed that the immunoreactive insulin content was only 5 microunits/ml; this activity was not detectable in the low (fat pad assay: lower than 25 microunits/ml, i.e. a value below the sensitivity of the assay; binding assay: 7.9 microunits/ml). However, even if the cortisol effect upon glycogen accumulation should depend on the presence of some unknown factor present in the serum, it remains clear that insulin (10 nM) is still able to induce a stimulation (Figs. 5 and 6, Table IV). In addition, stimulatory effects of cortisol were obtained in cultured liver explants (31) in the absence of both fetal serum (i.e. in absence of nonsuppressible insulin-like activity) and insulin. The present data strongly suggest, therefore, that cortisol is responsible for the onset of glycogen storage and that insulin only potentiates this effect. No true additivity could be shown but, rather, permissive effect of cortisol towards insulin for the development of the glycogen synthetic pathway. Cortisol might induce some step necessary for insulin action. If this step were to occur later in gestation, it would explain why insulin alone is effective in stimulating glycogen synthesis in liver explants from term fetuses (32) and in perfused adult liver (17). This step might also be the rate-limiting enzyme, glycogen synthetase, as it has been suggested that cortisol might be responsible for the synthesis of this enzyme during development (5, 18, 30, 33). It may be also concluded from in vivo (34, 35) and in vitro (18) studies that in the presence of cortisol, glycogen synthetase is mostly in the active form. Insulin might produce a further increase of the activity of the glycogen synthetase, as was suggested by the results obtained by studies performed with adult perfused liver (17) and fetal liver explants (30, 36).

In conclusion, a parallel study between glucagon and insulin response in cultured young fetal hepatocytes has been performed in this work. Marked differences have been found between them. Glycogen storage potential depends on cortisol and develops during the culture. The glucagon-dependent regulatory pathway is present very early in development, before any net storage of glycogen occurs and does not require cortisol. The onset of an insulin-dependent, regulatory pathway parallels that of the glycogen synthetic pathway and seems to depend on a permissive effect of cortisol. In the fetal hepatocyte in culture, cortisol appears as a hormone required both for the development of glycogen synthesis and for its regulation by insulin, but not for its regulation by glucagon.

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Role of cortisol on the glycogenolytic effect of glucagon and on the glycogenic response to insulin in fetal hepatocyte culture.
C Plas and J Nunez


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