Glycogen Turnover Studies in Human Hormone-Producing Trophoblastic Cells in Continuous Culture*

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ABSTRACT: The effects of glucose and glycogen concentration on glycogen synthesis and turnover in human hormone-producing cytotrophoblastic cells (BeWo line) were examined. Glycogen synthesis was studied by incubating cultures for 30 min with [6-^{3}H]glucose at various times after replenishment of the daily medium containing either 1 or 3 g of glucose per l. The rate of glycogen synthesis in cells on 1 g of glucose/l. was 30–150 times greater than the respective rate in cells on 3 g of glucose/l. at corresponding times after medium change. In general, the rate of glycogen synthesis was inversely proportional to intracellular glycogen concentration, but did not correlate with glucose concentration in the medium. Thus, when glycogen-rich cells were starved for glucose, the rate of glycogen synthesis began to increase when glycogen concentration dropped below 350 mg/g of protein, and reached a maximum 6.5 hr later when glycogen concentration reached 100 mg/g of protein. In the reverse experiment, when glycogen-depleted cells were fed 3 g of glucose/l., a decrease in rate of glycogen synthesis rapidly occurred when glycogen concentration exceeded 100–200 mg/g of protein (1–2% of wet weight), and was maximal 8 hr later. Glycogen turnover was measured by labeling glycogen-rich or glycogen-depleted cells for 30 or 60 min with [6-^{3}H]glucose, removing the radioactive medium, and incubating with medium containing nonradioactive 1 or 3 g glucose per l. in order to "chase" the label from [^{3}H]glycogen. The half-life of [^{3}H]glycogen was 55 hr in high-glycogen cultures chased with 3 g of glucose/l., 3 hr in high-glycogen cultures chased with 1 g of glucose/l., and 4 hr in low-glycogen cultures chased with 1 g of glucose/l. The kinetics of [^{3}H]-glycogen degradation were compatible with the view that the peripheral nonreducing glucosyl residues of glycogen are metabolically the most reactive in these trophoblastic cells. In glycogen-rich cells pulsed with [^{3}H]glucose, 60% of the radioactivity in the cells was initially present in the trichloroacetic acid precipitate, and 20–40% in glycogen, compared to 5 and 80%, respectively, in glycogen-depleted cells. In glycogen-rich and glycogen-depleted cells, essentially all the radioactivity eventually appeared in the trichloroacetic acid precipitate, concomitant with the loss of radioactivity from glycogen. It was concluded that in cytotrophoblastic BeWo cells the concentration of glycogen plays an important role in regulating metabolic functions in addition to those involving glycogen directly.

The essential role of glycogen metabolism in reproductive physiology has been emphasized by the work of Hughes *et al.* (1969). These investigators found that endometria from infertile patients failed to provide adequate glycogen turnover essential for the nutritional demands of endometrial tissue, such as required in blastocyst implantation. The cytotrophoblast of early pregnancy depends heavily on glycogen as an energy source (Frihandler *et al.*, 1961, 1967; Thomson and Brinster, 1966; Stern and Biggers, 1968; Gregoire and Richardson, 1970). Since a more controlled environment can be obtained with cells in tissue culture than *in vivo*, the BeWo line of hormone-producing cytotrophoblastic cells, derived 5 years ago from human choriocarcinoma (Pattillo *et al.*, 1968), provides an ideal model system with which to study separately hormone and metabolite regulation of glycogen metabolism in trophoblastic cells.

As evidence for hormonal regulation of glycogen metabolism in BeWo, 24-hr incubation with pregnenolone (10 μg/ml), the precursor of progesterone in BeWo cells (Huang *et al.*, 1969), caused depletion of glycogen concomitant with an increase in V_{max}, and decrease in K_M for Glc-1P,[1] of glycogen phosphorylase (Pattillo *et al.*, 1970a; Bernstein *et al.*, 1971). After 3 days of pregnenolone incubation, the secretion of human chorionic gonadotrophin into the medium was inhibited. Similar effects on glycogen metabolism have been noted *in vivo* in rat and mouse uterus (Hall, 1965; Gregoire *et al.*, 1967).

In addition to hormonal control, glycogen metabolism in BeWo cells is regulated by metabolites. Glycogen-depleted cells utilized glucose at twice the rate observed in glycogen-rich cells (Pattillo *et al.*, 1971). Furthermore, the glycogen-depleted cells had a glycogen synthetase activity three times greater than cells rich in glycogen, with a sixfold increase in the *I* form of the enzyme (Pattillo *et al.*, 1970b). It has recently been suggested (Pattillo *et al.*, 1971) that different regulatory mechanisms for glycogen metabolism may exist in trophoblastic cells that produce high levels of glycoprotein hormones, in contrast to other cell types. The present investigation extends previous work on carbohydrate and glycogen metabolism in the BeWo cell line (Knoth *et al.*, 1969; Pattillo *et al.*, 1969–1971; Bernstein *et al.*, 1971). The relationships between the concentration of glucose and glycogen, and the rates of glycogen synthesis and degradation, were examined to provide better insight into nonhormonal regulatory mechanisms of glycogen metabolism in trophoblastic cells. A previous publication in this series has recently appeared (Pattillo *et al.*, 1971).

Materials and Methods

Incubation of BeWo cells. Trophoblastic cells of the BeWo line were cultured in glass Leighton tubes (tube 1962, 11 ×

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[1] Abbreviations used are: Glc-1P, glucose 1-phosphate; UDPG, uridine diphosphate glucose.

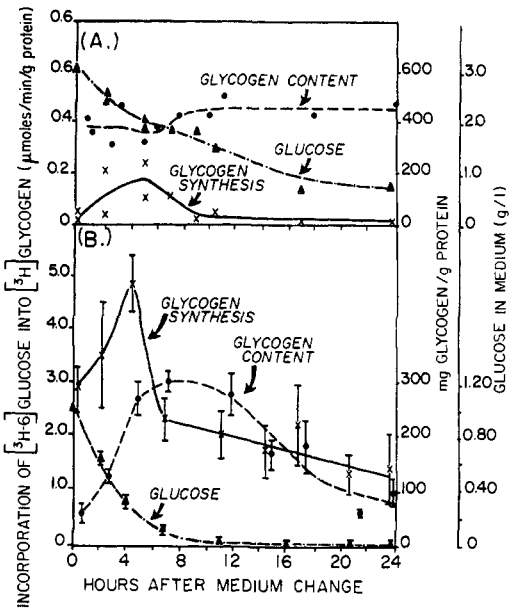

FIGURE 1: Synthesis of [^{3}H]glycogen from [6-^{3}H]glucose (×), intracellular glycogen content (●), and glucose concentration in the medium (▲) at various times following medium change in cultures of human choriocarcinoma cells (BeWo line) maintained daily on 4510 medium containing either 3 (A) or 1 g of glucose per l. (B). At the times designated on the abscissa, each Leighton tube was incubated for 30 min with 2 ml of medium containing 20 μCi of [6-^{3}H]glucose and 2 mg of nonradioactive glucose as described in Materials and Methods. Note the different scales on ordinate in A and B. Each point represents a single Leighton tube in A, and the mean of 5–11 Leighton tubes in B. Vertical bars in B indicate the standard error of the mean.

55 mm window size, Bellco Glass, Inc., Vineland, N. J.), in 2 ml of 4510 medium containing 3 g of glucose/l., as previously described (Pattillo and Gey, 1968; Pattillo *et al.*, 1969). BeWo cells consume more than 1 mg of glucose/hr per 100-mg cells (Pattillo *et al.*, 1971), necessitating the addition of high glucose (3 g/l.) to the daily culture medium to maintain maximum cell proliferation over long periods of time (Pattillo *et al.*, 1969). Some of the Leighton tubes were adapted to low glucose by reducing the glucose concentration in the medium to 1 g/l. (Pattillo *et al.*, 1971). All culture tubes received daily (9–11 AM) 2 ml of fresh medium containing either 1 g of glucose/l. (low glucose) or 3 g of glucose/l. (high glucose), as specified in the text. In the case of cultures on 1 g of glucose/l., labeling experiments were performed on the third day following adaptation of the cells to low glucose, unless stated otherwise.

Labeling with [6-^{3}H]Glucose. At various times after replenishment of nutrients to the cultures, the medium was removed and stored frozen until glucose analyses could be performed. The cells were rinsed for 15 sec at 37° with 4510 medium containing low glucose to insure a constant specific activity of [6-^{3}H]glucose in all experiments; the rinse was discarded. Low-glucose 4510 medium (2 ml) containing 10 μCi of [6-^{3}H]-glucose/ml (International Chemical and Nuclear Corp., Waltham, Mass.) was then added at 37° to the cells to begin the incubation.

The same procedure was routinely employed with cultures that had previously been maintained on either low or high glucose in order to achieve a greater specific activity of [6-^{3}H]-glucose during incubation. However, comparable results were obtained when cells were incubated with [6-^{3}H]glucose in medium containing high glucose instead of low glucose.

Incubation was carried out at 37° for 30 min. The reaction was terminated by the addition of 1 ml of 20% glucose. The medium was removed and discarded. The cells were rinsed twice with water, which was discarded. Water (2 ml) was then added to each tube, which was stored frozen until the glycogen and protein analyses could be performed.

For pulse-chase experiments (*e.g.*, turnover studies) cells on low glucose were incubated as described above, for 30 min with 10 μCi of [6-^{3}H]glucose/ml in 2 ml of low-glucose medium. Cells previously maintained on high glucose were incubated for 60 min with 10 μCi of [6-^{3}H]glucose/ml in 2 ml of high-glucose medium. Following incubation the cells were rinsed briefly with 4510 medium containing either 1 or 3 g of glucose per l., the medium was discarded, and 2 ml of fresh medium containing the appropriate glucose concentration (1 or 3 g per l.) was added to the cells. Incubation was carried out at 37°, with daily medium changes, until time for glycogen analysis.

Chemical Determinations. Glucose was estimated as described by Dubowski (1962). For glycogen and protein analyses, the frozen cells were disrupted by freezing and thawing twice. Glycogen isolation and measurement, and protein estimation were performed exactly as described by Pattillo *et al.* (1971). Radioactivity in glycogen was determined by counting 0.4 ml of the final aqueous solution in 10 ml of a scintillation mixture composed of: 4 g of 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene, 80 g of naphthalene, 400 ml of 2-methoxyethanol, and 600 ml of toluene (Rowland, 1969). The total radioactivity in the lysate was determined with the same scintillation mixture (tritium efficiency, 20%). The trichloroacetic acid precipitable radioactivity in the original suspension of lysed cells was measured by a filter disk method (Hussa *et al.*, 1970) (tritium efficiency, 7.5%). Samples were counted in a Packard TriCarb liquid scintillation spectrometer (Packard Instrument Co., La Grange, Ill.).

Expression of Results. Glycogen content was expressed as milligrams per gram of protein. The disintegrations per minute in glycogen were converted to micromoles glucosyl per minute (1 μmole = 4×10^6 dpm, since 1 ml of medium contained 10 μCi of [6-^{3}H]glucose and 1 mg of glucose), and were expressed on the basis of either glycogen or protein. Results expressed in this manner could readily be compared to activities obtained in enzyme assays. When it was desirable to compare the radioactivity in glycogen with that in the trichloroacetic acid precipitate, both values were determined and expressed relative to the total radioactivity in the lysate.

Results

Glycogen Synthesis from [6-^{3}H]Glucose. BeWo cells maintained daily on high glucose (Figure 1A) contained a constant high glycogen content throughout the day. The glucose concentration in the medium never fell below 0.7 g/l. The low rate of [6-^{3}H]glucose incorporation into glycogen increased to a maximum of about 0.2 μmole/min per g of protein 4–5 hr after medium change, and thereafter decreased tenfold in the next 6 hr, to a constant low rate for the remainder of the day. The early peak of synthetic activity was observed in all cultures on either high or low glucose (Figures 1 and 2), and was a consequence of replenishment with fresh medium (R. O. Hussa, unpublished data).

Cells receiving low glucose in the daily medium (Figure 1B) contained very little glycogen at the time of medium replenishment. Glycogen rapidly accumulated in the cells as the extracellular glucose was consumed. After all the glucose

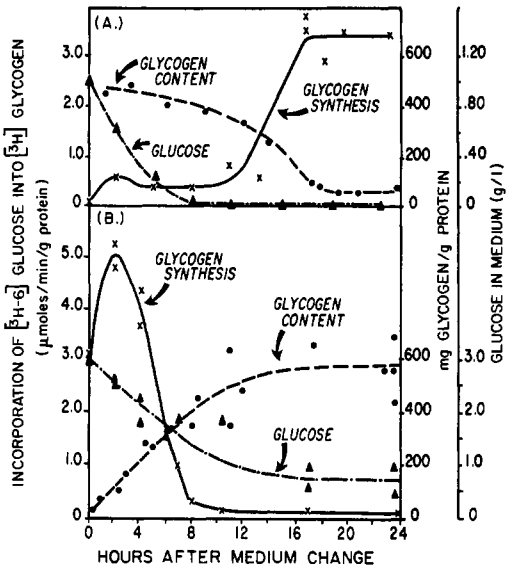

FIGURE 2: Glycogen synthesis, glycogen content, and glucose concentration at various times following medium change in BeWo cells previously receiving 3 (A) or 1 g of glucose per l. (B) in the daily medium, and given either 1 (A) or 3 g of glucose per l. (B) at zero time. Other details were as described in legend to Figure 1. Each point represents a single Leighton tube.

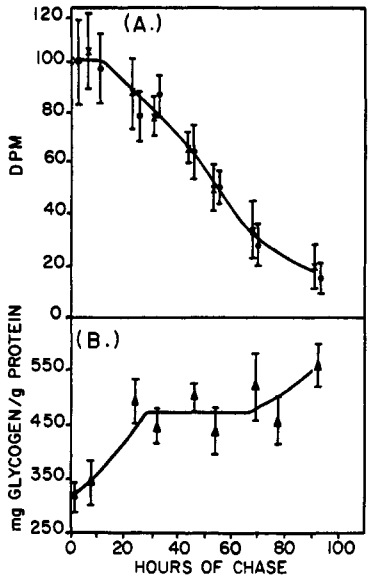

FIGURE 3: Glycogen turnover (A) and glycogen content (B) in BeWo cells maintained daily on 3 g of glucose/l.. Cells (3 days after subculture) were pulsed for 1 hr with 2 ml of 4510 medium containing 20 μCi of [6-^{3}H]glucose and 6 mg of nonradioactive glucose, then chased with 4510 medium containing 3 g of glucose/l., beginning at zero time. At the times designated on the abscissa, samples were analyzed for dpm in glycogen on a protein (×) or glycogen (●) basis, and for glycogen content (▲). Results in (A) are expressed as per cent of value at zero-time chase, which is taken as 100%. Each point represents the mean of three to twelve Leighton tubes, and the vertical bars the standard error of the mean.

was used up from the medium (10 hr after medium change), the intracellular glycogen concentration decreased. These observations confirmed the results of earlier studies (Pattillo *et al.*, 1971). The rate of glycogen synthesis from [6-^{3}H]glucose was 2.9 μmoles/min per g of protein at the time of medium change, increasing to a maximum of 4.9 μmoles/min per g of protein at 4 hr. When the glycogen concentration exceeded 200 mg/g of protein (4 hr), the rate of glycogen synthesis decreased to 2.2 μmoles/min per g of protein in the next 2 hr, after which it remained fairly constant for the remainder of the day. Only the rate of synthesis at 4 and 21 hr differed significantly ($p < 0.05$, Student's t test) from the rate at zero time. The rate of glycogen synthesis from [6-^{3}H]glucose in cells on low glucose was 150, 30, 70, and 100 times greater than the respective rate in cells on high glucose at 0, 4, 10, and 24 hr after medium change (Figure 1).

It was of interest to determine, in glycogen-rich cells, the time required for the rate of glycogen synthesis to increase after lowering the glucose concentration to 1 g/l. in the medium (Figure 2A). Following depletion of glucose from the medium (8 hr), the cytoplasmic glycogen became depleted (8–18 hr). The rate of increase in glycogen synthesis correlated with the depletion of cytoplasmic glycogen, beginning when the glycogen concentration dropped below 350 mg/g of protein, reaching half-maximum activity in about 4 hr (250 mg of glycogen/g of protein), and reaching maximum in another 2.5 hr (100 mg of glycogen/g of protein). The time necessary for the complete increase in glycogen synthesis to occur was 6.5 hr (by 17 hr after medium change). The increase in rate of glycogen synthesis lagged several hours behind the depletion of glucose from the medium.

In the reverse experiment, glycogen-depleted cells were refed medium containing high glucose (Figure 2B). Following the usual initial rise in glycogen synthesis (0–2 hr), the rate rapidly began decreasing (glycogen concentration greater than 100 mg/g of protein), reaching a 50-fold lower activity approximately 10 hr after medium change (420 mg of glycogen/g of protein). The glycogen content and rate of glycogen synthesis did not change during the remainder of the day, and the glucose concentration in the medium did not fall below 0.7 g/l.

Glycogen Turnover. In order to determine the turnover rate of glycogen, glycogen-rich cells were first given a 1-hr pulse with high-glucose medium containing 10 μCi of [6-^{3}H]-glucose/ml, then chased with nonradioactive high-glucose medium. When disintegrations per minute in glycogen on either a protein or glycogen basis were plotted against hr of chase (Figure 3A), both curves fell on the same line. The time necessary for half the radioactivity to disappear from [^{3}H]-glycogen was approximately 55 hr (protein or glycogen basis). The lower glycogen content per gram of protein during the first 32 hr of chase was a consequence of the young age of the cells. After the first 32 hr of chase, the cellular glycogen content per gram of protein did not change significantly (Figure 3B).

Glycogen turnover was next examined in glycogen-rich and glycogen-depleted cultures that were pulsed with [6-^{3}H]-glucose and then chased with low glucose (Figure 4). In BeWo cells fed daily with 1 g of glucose/l. (dashes), half the label disappeared from glycogen about 4 hr after the initial decrease in radioactivity and 8–9 hr after the beginning of glycogen degradation (15 hr of chase, Figure 4A). Similarly, half the glycogen was gone from these cells after 14 hr of chase. In glycogen-rich cells (solid line), half the label had disappeared from glycogen approximately 3 hr (protein basis) or 4.5 hr (glycogen basis) after the initial decrease in radioactivity (7.5 and 8.5 hr of chase, respectively). In this case, the decrease in glycogen content of the cells lagged behind, reaching 50% of original after about 11.5 hr of chase. The events that occurred during the chase were characterized by four distinct phases. (1) 0–4.5 hr: the cells consumed 80% of the extra-

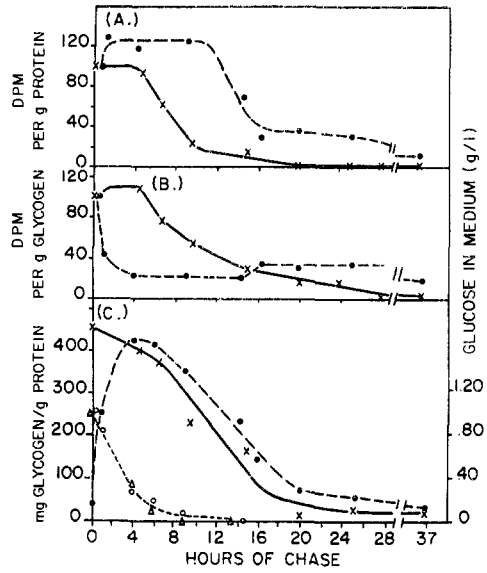

FIGURE 4: Glycogen turnover in BeWo cells chased with 1 g of glucose/l. Cells previously maintained for 3 days on 1 g of glucose/l. (●) or 3 g of glucose/l. (×) in the daily medium were pulsed for 30 min with 2 ml of 4510 medium containing 20 μCi of [6-^{3}H]glucose and 2 mg of nonradioactive glucose, then chased with 4510 medium containing 1 g of glucose/l., beginning at zero time. At the times designated on the abscissa, samples were analyzed for disintegrations per minute in glycogen per gram of protein (A), disintegrations per minute in glycogen per gram of glycogen (B), and milligram of glycogen per gram of protein (C). The glucose concentration in the medium of cells previously maintained on 3 (○) or 1 g of glucose per l. (△) is also shown in part C. In parts A and B, results are expressed as per cent of value at zero-time chase, which is taken as 100%. Each point represents the mean of at least two Leighton tubes.

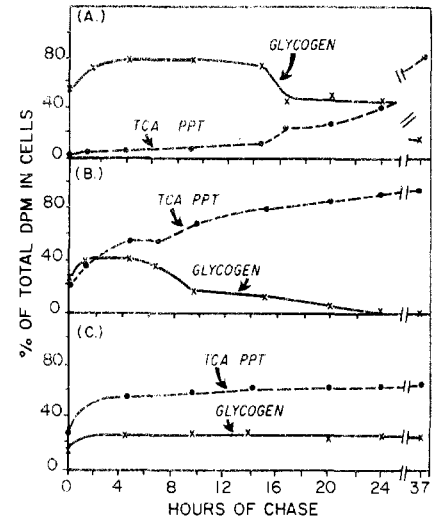

FIGURE 5: Relative radioactivity in glycogen (×) and trichloroacetic acid precipitate (●) in BeWo cells previously grown on 1 g of glucose/l. (A) and 3 g of glucose/l. (B and C), pulsed with [6-^{3}H]-glucose, and chased with 1 g of glucose/l. (A and B), or 3 g of glucose/l. (C). The data in parts A and B were from the Leighton tubes used in Figure 4, and in part C, from those in Figure 3. The cells in part C received a medium change 24 hr after the start of the chase. Other details were as described in the Materials and Methods section, and in legends to Figures 3 and 4.

cellular glucose. Cells previously maintained on 1 g of glucose/l. of deposited glycogen, concomitant with an 80% decrease in glycogen specific activity, and an increase in total disintegrations per minute in glycogen per gram of protein. In cultures previously grown on 3 g of glucose/l., where the initial glycogen content was high, the glycogen specific activity remained essentially unchanged, as did the disintegrations per minute in glycogen per gram of protein. (2) 4.5–11 hr: glucose became exhausted from the medium, and cellular glycogen began to be utilized. In cultures previously grown on low glucose, no change occurred in the radioactivity of the glycogen (protein or glycogen basis). In cells previously maintained on high glucose, a rapid disappearance of radioactivity from glycogen occurred. (3) 11–18 hr: glycogen concentration became low in the cells. In cells previously grown on low glucose, radioactive glycogen was rapidly degraded (protein basis). The glycogen specific activity of the same cells actually *increased* (15–16 hr of chase), rising from 20 to 34% of the original value. In cells previously maintained on high glucose, a gradual decline was observed in the remainder of the radioactive glycogen; after 18 hr of chase, only 5% (protein basis) or 20% (glycogen basis) remained. (4) 18–37 hr: cells became nearly exhausted of glycogen. In cells previously maintained on low glucose, the radioactivity in glycogen slowly decreased to low (but not zero) values. In cells previously grown on high glucose, all radioactivity completely disappeared from glycogen after 24 hr of chase.

The metabolic relationship between glycogen and other (trichloroacetic acid precipitable) macromolecules was investigated by measuring the relative amount of radioactivity in both glycogen and trichloroacetic acid precipitate at various times after glycogen-depleted or glycogen-rich cells received a pulse of [6-^{3}H]glucose, and a chase with nonradioactive low or high glucose (Figure 5). The increase in relative labeling of glycogen in the first 4 hr of chase was observed in all cases (Figure 5A–C), and probably resulted from disappearance of [6-^{3}H]glucose from the cells. In low-glycogen cells after 4 hr of chase with 1 g of glucose/l., 80% of the total radioactivity was present in glycogen, whereas only 5% of the label appeared in the trichloroacetic acid precipitate (Figure 5A). At 14 hr, when glycogen stores were becoming depleted (see Figure 4C), the relative disintegrations per minute in glycogen rapidly began to decrease, with a concomitant increase in radioactivity in the trichloroacetic acid precipitate. From 17 to 37 hr of chase, the relative degree of labeling gradually changed in the same direction until 80% of the total radioactivity was present in the trichloroacetic acid precipitate, and less than 20% in glycogen. The rates of change in this experiment correlated with those in Figure 4A (dashes).

By contrast, in cells previously grown on high glucose, pulsed with [6-^{3}H]glucose and then chased with low glucose, only 40% of the total radioactivity was present in glycogen, and nearly 60% in the trichloroacetic acid precipitate, after 4 hr of chase (Figure 5B). The decrease in radioactive glycogen, and concomitant increase in relative labeling of the trichloroacetic acid precipitate, began earlier (6.5 hr) and was more complete in these cells than in the low-glucose cells in Figure 5A. These results were also in accord with those in Figure 4A (high-glucose cultures). Similarly, in high-glucose cells that were pulsed with [6-^{3}H]glucose and also chased with high glucose, about 26% of the total radioactivity was present in glycogen, and 53% in the trichloroacetic acid precipitate, after 4 hr of chase (Figure 5C). After 37 hr of chase with high glucose, the relative degree of labeling in glycogen had not changed, while that in the trichloroacetic acid precipitate had increased to 66%. The glucose never became exhausted from the medium of the cells of Figure 5C.

Discussion

Glycogen Synthesis. The rate of glycogen synthesis in BeWo cells maintained on low glucose exceeded by more than 30-fold the rate in cells grown on high glucose, at corresponding times after medium change (Figure 1). These results were consistent with observations in liver, where a lower rate of glycogen synthesis was obtained in liver of the intact fed animal than in animals which were fasted and then refed (reviewed by Hers and De Wulf, 1967). BeWo cells possessed a rate of glycogen synthesis from [6-^{3}H]glucose that was inversely proportional to glycogen concentration (Figures 1 and 2) at all times of the day, with a single exception: in cells maintained daily on low glucose (Figure 1B), between 8 and 24 hr after medium change. At least two explanations are possible for the observation that the rate of synthesis did *not* increase, but rather decreased slightly (though not significantly), as glycogen became depleted: (1) the rate of glycogen synthesis is modulated by other factors in addition to glycogen concentration; or (2) the cellular glycogen is compartmentalized, and under the conditions in Figure 1B (8–24 hr) the local concentration of glycogen in the vicinity of synthetase (or synthetase phosphatase) remains high enough to prevent activation of glycogen synthetase. In skeletal muscle, heart (Villar-Palasi and Larner, 1970), and liver (De Wulf and Hers, 1968), glycogen decreases its own synthesis nonhormonally by inhibition of glycogen synthetase (transferase) D phosphatase, the enzyme that converts synthetase D to synthetase I. Our present results suggest that (1) the same mechanism may also be operational in human cytotrophoblastic cells in culture, and (2) the inactivation process is rapid and begins when intracellular glycogen concentration exceeds 100–200 mg/g of protein (1–2% of wet weight).

The rate of glycogen synthesis did not correlate with the glucose concentration in the medium as well as with the intracellular glycogen concentration. Thus, in Figure 1B after 8 hr, the glucose concentration of the medium was zero, but glycogen synthesis remained high; in Figure 2A there was a lag of 3–4 hr between depletion of glucose and increase in glycogen synthesis; and in Figure 2B glycogen synthesis became markedly inhibited at a time (6 hr) when the glucose concentration was greater then 1.5 g/l. in the medium. Furthermore, glycogen-depleted BeWo cells given either 1 or 3 g of glucose per l. displayed comparable rates of glycogen synthesis up to approximately 4 hr following medium change (compare Figures 1B and 2B), in agreement with our previous findings on glycogen deposition (Pattillo *et al.*, 1971). The present experiments do not rule out a possible correlation between intracellular glucose and glycogen synthesis.

Glycogen Turnover. The half-life of [^{3}H]glycogen (55 hr, Figure 3A) in BeWo cells maintained on 3 g of glucose/l. was longer than in liver of fed rats (24–36 hr, Hers and De Wulf, 1967; Stetten and Stetten, 1960), but shorter than in muscle (96 hr, Stetten and Stetten, 1960). The mean rate of [^{3}H]glycogen degradation in glycogen-rich BeWo cells was 0.5–0.6 μmole/min per g of protein, calculated from a glycogen content of 320–470 mg/g of protein, a half-life of 55 hr, and assuming equal distribution of [^{3}H]glucosyl residues in glycogen. The rate of [^{3}H]glycogen degradation in glycogen-rich cells chased with low glucose (instead of high glucose) was 7.0 μmoles/min per g of protein (3 hr half-life, 405 mg of glycogen/g of protein, Figure 4A, solid line), an order of magnitude higher than in comparable cells chased with high glucose. A similar rate of [^{3}H]glycogen degradation, 5.4 μmoles/min per g of protein, was calculated for glycogen-depleted cells that were pulsed with [6-^{3}H]glucose and chased with low glucose (4-hr half-life, 420 mg of glycogen/g of protein, Figure 4A, dashes). In the latter two cases, net glycogen degradation rates calculated from chemical content were 3.0 and 2.2 μmoles per min per g of protein, respectively, for glycogen-rich cells (7-hr half-life) and glycogen-depleted cells (10-hr half-life). The latter value compared favorably with the net degradation rate of 2.8 reported earlier (Pattillo *et al.*, 1971). When the rates were corrected for glycogen synthesis occurring during the same time (about 0.4 μmole/min per g of protein for glycogen-rich cultures between 4.5–11.5 hr of chase, Figure 2A; 2.6 μmole/min per g of protein for glycogen-depleted cultures, averaged between 4 and 14 hr of chase, Figure 1B), the rate of glycogen degradation based on chemical glycogen content was 3.4 (glycogen-rich cells) or 5.2 μmoles per min per g of protein (glycogen-depleted cells). In glycogen-rich cells chased with low glucose, the higher rate of degradation calculated on the basis of radioactivity (7.0 μmoles/min per g of protein), compared to the rate calculated on the basis of glycogen content (3.4 μmoles/min per g of protein), probably reflected uneven distribution of [^{3}H]glucosyl residues in glycogen, with outer chains containing more radioactivity than the inner matrix (see below). In cultures initially depleted of glycogen, both degradation rates were the same.

The half-life and degradation rate of glycogen in BeWo cells, derived from pulse-chase experiments with [6-^{3}H]glucose, should be considered as approximate for several reasons (1) Glycogen content/g of protein increased with time (Figure 3B). (2) Inner branching is known to occur with time (Stetten and Stetten, 1960; Parodi *et al.*, 1970). (3) Glycogen synthetase may enter more deeply into the glycogen matrix than glycogen phosphorylase (Parodi *et al.*, 1970). (4) All the outer chains of glycogen may not be available to glycogen phosphorylase (Parodi *et al.*, 1970). (5) There was probably uneven distribution of [^{3}H]glucosyl residues among (and possibly between) glycogen molecules, since glycogen synthetase adds glucosyl residues only to outer branches of glycogen (Stetten and Stetten, 1960). (6) Some [^{3}H]Glc-1P, formed by phosphorylase action on [^{3}H]glycogen, may have been reutilized for glycogen synthesis.

The different kinetics of glycogen degradation observed when cells initially high in glycogen (Figure 4, solid lines) or depleted of glycogen (Figure 4, dashed lines) were pulsed with [6-^{3}H]glucose and chased with low glucose can be explained completely by the model depicted in Figure 6. Thus, when high-glycogen cells (Figure 6A) are pulsed with [6-^{3}H]glucose, labeled glucosyl residues appear only in peripheral positions, whereas glucosyl residues deep in the inner matrix of glycogen contain radioactivity in low-glycogen cultures (Figure 6B). In the latter, the initial low-glycogen content results in high specific activity of the glycogen, which rapidly decreases early during the chase (see Figure 4B, dashes). During the first 5 hr of chase, prior to glucose depletion, very little addition of unlabeled glucosyl residues takes place in glycogen-rich cells (Figure 6A), whereas large outer regions of unlabeled glucosyl residues accumulate in the growing glycogen deposits of low-glycogen cells (Figure 6B). Upon exhaustion of glucose from the medium of cells that were previously rich in glycogen (Figure 6A), an initial rapid decrease in both glycogen content and radioactivity occurs (5–11 hr) as glycogen is degraded from its nonreducing end, with both rates slowing at longer times of chase as a consequence of the smaller molecular size of the glycogen (11–18 hr); none of the radioactive glucosyl residues remain after 18 hr of chase. In cells initially low in glycogen (Figure 6B), exhaustion of glucose from the medium at 5 hr of chase results in degradation of glycogen *without* any decrease

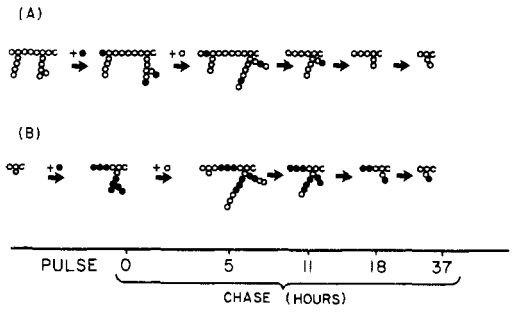

FIGURE 6: Diagrammatic representative glycogen molecule in high-glycogen (A) or low-glycogen (B) BeWo cells, previously maintained on medium containing either 3 or 1 g of glucose per l., respectively, pulsed with [6-^{3}H]glucose (●), then chased with medium containing 1 g of glucose/l. (○), as described in Figure 4. Reducing end of glycogen, C. The number of glucosyl residues indicated between branch points is arbitrary, and meant for illustrative purposes only. Branching activity has also been indicated. The total number of glucosyl residues shown at each time of chase is indicative of the glycogen content in the cells. Other details are described in text.

in radioactivity (5–11 hr) as the unlabeled peripheral glucosyl residues are catabolized. The model also predicts the increase in glycogen specific activity observed after 14 hr of chase (Figure 4B, dashed lines). After 11 hr of chase, the labeled glucosyl residues, now peripheral, are degraded at approximately the same rate as in the cells in Figure 6A, but at a later time (11–18 hr). The very slow rate of decrease in radioactivity between 18 and 37 hr of chase is explained by the inability of glycogen phosphorylase to efficiently degrade low molecular weight glycogen, containing some labeled glucosyl residues as a result of previous branching activity (Stetten and Stetten, 1960; Parodi *et al.*, 1970). The labeled glucosyl residues, now deep within the glycogen matrix, are not removed even after exhaustive periods of chase (37 hr, Figures 4A,B, 6B).

Our present findings are compatible with singly, multi, or multirepetive chain elongation mechanisms (Parodi *et al.*, 1970), and support the view (Stetten and Stetten, 1960; Marshall *et al.*, 1970) that the glycogen molecule (in BeWo cells) is metabolically heterogeneous, and that addition and elimination of glucosyl residues occurs at the peripheral surface of the polysaccharide, accompanied by a branching process. The nonreducing peripheral glucosyl residues are metabolically the most reactive in mammalian systems (reviewed by Stetten and Stetten, 1960) and amoeba (Marshall *et al.*, 1970), but not in yeast (Rothman and Cabib, 1969). Apparently in yeast, each individual molecule of glycogen is completed, possibly in one of the cell organelles, and released prior to initiation of synthesis of a new molecule (Rothman and Cabib, 1969).

Glycogen-rich BeWo cells have the capacity to synthesize other (trichloroacetic acid precipitable) molecules directly from [6-^{3}H]glucose (Figure 5B,C). By contrast, in glycogen-depleted choriocarcinoma cells, the only mechanism by which glucose is converted into macromolecules other than glycogen appears to be by prior conversion to glycogen (Figure 5A). These findings suggest the intriguing possibility that glycogen concentration plays an important role in regulating metabolic functions in addition to those involving glycogen directly, in trophoblastic BeWo cells, and therefore possibly in trophoblastic cells of implanting blastocyst and early placenta.

Current experiments in our laboratory, to be published soon, involve the enzymatic mechanisms regulating glycogen metabolism in cultures derived from both choriocarcinoma and cervical carcinoma and maintained on either low or high glucose.

Acknowledgments

The expert technical assistance of Miss A. C. F. Ruckert is deeply appreciated. The authors also acknowledge the technical assistance of Mr. J. Kurtz, Mrs. J. Martin, Mrs. D. Beeks, and Mr. R. Falsetti, and the valuable discussions with Mr. R. Bernstein. The consent of Dr. Anthony Pisciotta for the use of his scintillation counter is appreciated.

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