Primary cultures of rat hepatocytes respond to hormones or amino acid deprivation by increasing System A-mediated neutral amino acid transport. Previous reports have shown this stimulation to be dependent on RNA and protein synthesis, whereas the present report describes the inhibition of System A by tunicamycin (TM), an inhibitor of asparagine-linked glycoprotein biosynthesis. The basal System A activity, as monitored by Na+-dependent 2-aminoisobutyric acid uptake, was decreased by TM when hepatocytes were cultured for 24 h in the presence of the antibiotic. System Gly activity was also sensitive to TM, whereas the activities of Systems L1, L2, and N were relatively resistant and that of System ASC was only moderately affected. The increase in System A-mediated uptake after incubation of hepatocytes in the absence of amino acids (i.e. adaptive control) was almost completely abolished by including TM. Likewise, stimulation of hepatic 2-aminoisobutyric acid transport by glucagon, dexamethasone, insulin, or vasopressin was also blocked by the inhibitor. When glucagon alone or glucagon plus dexamethasone was added, the inhibition by TM was transient such that the degree of inhibition decreased with incubation time after the initial 2 h. Addition of TM to cells which had been treated previously for 2 to 4 h with glucagon and dexamethasone blocked any further increase in transport indicating that the glycoprotein component of System A must be continually synthesized to sustain the increase in activity. Treatment of hepatocytes with various lectins did not inhibit 2-aminoisobutyric acid transport.

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MATERIALS AND METHODS

Hepatocyte Isolation and Culture—Male Sprague-Dawley rats weighing 100 to 200 g were fasted overnight prior to surgery between 7 and 9 a.m. on the day of the experiment. Hepatocytes were isolated and placed in primary culture as described previously (13, 14). Except where indicated, freshly isolated cells were plated in multiwell (24-well) trays (300,000 cells per well) in an amino acid-enriched (14-well) medium; AAF, amino acid free (medium); TM, tunicamycin.

Transport Assays—Amino acid transport was measured by a modification of the method of Gazzola et al. (15). Generally, the cells were washed twice for 15 min each with CholKRP* (37°C, pH 7.4) prior to the uptake assays to remove the residual culture medium and to minimize possible trans-effects by allowing depletion of the intracellular amino acid pools. After the transport assays were completed, the protein content of each well was determined by a modified Lowry method (14). The data were calculated with the aid of computer programs which incorporated standard statistical analyses. The results are reported as picomoles of AIB transport per mg of protein per unit time or as per cent of control uptake rates. The data shown are from replicate determinations for a single experiment. Each experiment was performed on 2 to 4 different batches of cells.

Materials—The rats were obtained from a colony maintained by the Division of Animal Resources, University of Florida. The AIB was purchased from ICN Pharmaceuticals, the L-[4,5-3H]leucine was from Schwarz/Mann, and the L-[4,5-3H]leucine, L-[G-3H]histidine, and [G-3H]glycine were from Amersham Corp. Highly purified insulin

* The abbreviations used are: CholKRP, Na+ -free (choline-containing) Krebs-Ringer phosphate buffer; AIB, 2-aminoisobutyric acid; NaKRP, Na+-containing Krebs-Ringer phosphate buffer; NaKRB, Na+-containing Krebs-Ringer bicarbonate buffer; AAR, amino acid rich (medium); AAF, amino acid free (medium); TM, tunicamycin.

1 The abbreviations used are: CholKRP, Na+-free (choline-containing) Krebs-Ringer phosphate buffer; AIB, 2-aminoisobutyric acid; NaKRP, Na+-containing Krebs-Ringer phosphate buffer; NaKRB, Na+-containing Krebs-Ringer bicarbonate buffer; AAR, amino acid rich (medium); AAF, amino acid free (medium); TM, tunicamycin.
and glucagon were generous gifts from Dr. Mary Root of Lilly. The unlabeled amino acids, Type I collagenase, tunicamycin, dexamethasone, and lectins were from Sigma. Flow Laboratories was the source for the Waymouth’s medium and the fetal bovine serum was purchased from K.C. Biologicals.

RESULTS

Inhibition of Specific Transport Systems by Tunicamycin—
Six systems which are known to mediate hepatic neutral amino acid transport were tested for their sensitivity to TM by establishing primary cultures of rat hepatocytes in the presence of 0.05 μg/ml of the inhibitor. After 24 h, the Na+-independent neutral amino acid transport, mediated by Systems L1 and L2 (19), and the Na+-dependent uptake by Systems ASC and N were only marginally inhibited by the TM treatment (Table I). In contrast, Systems A and Gly were significantly depressed (90 and 77%, respectively) when the cells had been maintained for 24 h in the presence of TM. These results indicate that the individual amino acid transport systems vary in their sensitivity to TM.

Effect of Tunicamycin on Adaptive Control of System A Transport—The activity of System A is enhanced when hepatocytes are incubated in the absence of all extracellular amino acids. This process, referred to as adaptive control, is dependent on de novo synthesis of both RNA and protein (2–4). To test the concentration dependence of TM on System A transport, we transferred cultured hepatocytes to AAF or AAR medium for up to 18 h. The results were the same whether Waymouth’s medium or NaKRB containing 10 mM AIB was used as the AAR medium. In all cases, the AAF medium was NaKRB lacking amino acid supplement. Incubation of hepatocytes in the AAF medium resulted in a 25-fold stimulation of activity after 8 h and including TM at concentrations between 0.05 and 1 μg/ml in the AAF medium inhibited this stimulation in a concentration-dependent manner (Fig. 1). The effect of 1 μg/ml of tunicamycin on the incorporation of either L-[3H]leucine or D-[3H]mannose into trichloroacetic acid-insoluble material was tested in cells maintained in AAF or AAR medium for 6 h. The inhibition of protein synthesis by tunicamycin was not significant (269 ± 38 versus 239 ± 38 pmol·mg−1 protein·30 min−1), whereas the incorporation of mannose was decreased by 44% (43.4 ± 2.1 versus 24.3 ± 2.1 pmol·mg−1 protein·30 min−1). It is not known whether the TM-insensitive mannose incorporation is the result of glycoprotein synthesis by a TM-resistant pathway or the result of incorporation into products other than asparagine-linked oligosaccharides.

To determine the effects of TM on the ability of hepatocytes to undergo adaptive control for a longer period of time, hepatocytes which had been cultured overnight in Waymouth’s medium containing 10% fetal bovine serum were transferred to either AAF or AAR medium and the transport of AIB was monitored during the next 18 h. As shown in Fig. 2, the addition of TM to the cells maintained in the AAF medium blocked most of the increase in amino acid transport. Similar results are observed if cycloheximide or actinomycin is used instead of TM.

Inhibition of Hormone-stimulated System A Activity by Tunicamycin—A variety of hormones also stimulate hepatic System A transport by a protein synthesis-dependent process (for a review see Ref. 2). To determine if this induction also required asparagine-linked glycoprotein biosynthesis, the effect of TM on the hormonal control of AIB uptake was measured. Freshly isolated hepatocytes were placed in culture with Waymouth’s medium for 2 h and then transferred to NaKRB plus 10 mM AIB containing 10−7 M glucagon in the presence or absence of 1 μg/ml of TM. The purpose of the 10 nm AIB in the incubation is to prevent stimulation of System A activity by adaptive control. Despite this precaution, the basal rate of uptake was increased after 4 to 6 h (Table II). Glucagon caused a 30% stimulation in System A activity within 15 min after the addition of the hormone (Table II). This small degree of stimulation was protein synthesis-independent as measured by cycloheximide sensitivity (data not shown).

### Table I

| System tested      | Tunicamycin | Transport activity measured | % inhibition |
|--------------------|-------------|-----------------------------|-------------|
|                    | NaKRP       | CholKRP                     | Na+-dependent |
| System A           | −           | 32.5 ± 2.4                  | 22.5 ± 1.6  | 10.0 ± 2.9  | 90\* |
|                    | +           | 27.1 ± 1.6                  | 26.0 ± 1.5  | 11.1 ± 2.2  | 29   |
| System ASC         | −           | 59.3 ± 4.8                  | 42.2 ± 2.8  | 17.1 ± 5.6  | 27   |
|                    | +           | 49.7 ± 2.9                  | 37.5 ± 3.5  | 12.2 ± 4.5  | 29   |
| System Gly         | −           | 26.9 ± 0.4                  | 5.2 ± 0.1   | 21.7 ± 4.5  | 29   |
|                    | +           | 10.9 ± 1.0                  | 6.0 ± 0.7   | 4.9 ± 1.2   | 77\* |
| System N           | −           | 166 ± 6.0                   | 20.0 ± 1.0  | 146 ± 6.0   | 20   |
|                    | +           | 133 ± 7.0                   | 10.0 ± 1.0  | 117 ± 7.0   | 20   |
| System L1          | −           | 87.8 ± 7.4                  | 72.5 ± 6.9  | 17   |
|                    | +           | 541 ± 47                    | 444 ± 74    | 18   |

\* Significant at \( p < 0.01 \).

\* Significant at \( p < 0.025 \).

**Fig. 1.** Effect of concentration on the tunicamycin-dependent inhibition of adaptive control. Hepatocytes were placed in primary culture for 24 h in Waymouth’s medium containing 10% fetal bovine serum. The culture medium was then changed to NaKRB containing 10 mM AIB and NaKRB containing 10 mM AIB (AAR medium). Tunicamycin was present at concentrations between 0.05 and 1.0 μg/ml as shown. The Na+-dependent uptake of 50 μM AIB was measured for 1 min at 37 °C at the times indicated. The data are reported as Na+-dependent AIB transport and were calculated by subtracting the uptake rate in the absence of Na+ (NaKRP, pH 7.4) from that observed in the presence of Na+ (NaKRP, pH 7.4). The rate of transport for cells maintained in the presence of amino acid. The results are the averages ± S.D. of four determinations.
FIG. 2. Time course of the inhibition by tunicamycin of starvation-induced System A transport. Hepatocytes were cultured for 24 h in Waymouth's medium plus 10% fetal bovine serum and then transferred to NaKRB buffer (pH 7.4) with (−) or without (×) 10 mM AIB. Control conditions were as described in Fig. 1. The results are given as the averages ± S.D. of at least three determinations. Where not shown, the S.D. bars are contained within the symbol.

TABLE II

Inhibition of glucagon-stimulated System A activity by tunicamycin

Hepatocytes were placed in primary culture with Waymouth's medium containing 10% fetal bovine serum. After 2 h, the medium was changed to NaKRB plus 10 mM AIB with or without glucagon (16 μg/ml) and tunicamycin (1 μg/ml) as indicated. For each experiment, the cells were assayed at 4 and 6 h; the medium was replaced with fresh medium of the same composition after the first 2 h. After a 15-min depletion in CholKRP at 37 °C, transport was measured as described in Fig. 1. The results are the averages ± S.D. of 3 or 4 individual determinations.

| Incubation conditions | Time of incubation with hormone and inhibitor | 2 h | 4 h | 6 h |
|-----------------------|-------------------------------------------|-----|-----|-----|
| Control               |                                          |     |     |     |
| NaKRP                 | 37.0 ± 3.0                                | 38.6 ± 1.1 | 60.3 ± 4.5 | 58.0 ± 8.6 |
| ChoKRP                | 26.7 ± 3.0                                | 23.6 ± 1.0 | 19.9 ± 2.4 | 27.7 ± 1.3 |
| Na+-dependent         | 10.3 ± 0.5                                | 5.7 ± 0.3 | 10.4 ± 0.4 | 30.2 ± 4.0 |
| Glucagon              |                                           |     |     |     |
| NaKRP                 | 28.7 ± 3.0                                | 48.8 ± 4.1 | 111 ± 22  | 128 ± 18  |
| ChoKRP                | 26.7 ± 3.0                                | 23.6 ± 1.0 | 19.9 ± 2.4 | 27.7 ± 1.3 |
| Na+-dependent         | 10.3 ± 0.5                                | 5.7 ± 0.3 | 10.4 ± 0.4 | 30.2 ± 4.0 |
| Glucagon plus cAMP    |                                           |     |     |     |
| NaKRP                 | 40.7 ± 3.3                                | 41.1 ± 1.4 | 63.1 ± 7.3 | 93.5 ± 5.4 |
| ChoKRP                | 26.3 ± 3.0                                | 25.8 ± 4.5 | 29.3 ± 3.9 | 37.1 ± 2.5 |
| Na+-dependent         | 14.4 ± 3.3                                | 15.3 ± 3.3 | 33.8 ± 5.6 | 56.4 ± 4.0 |

*a Significant at p < 0.01 when compared to the values obtained with the hormone-treated cells in the absence of tunicamycin.

showed) and, as shown in Table II, was TM-insensitive as well.

These results confirm the data of Edmondson et al. (16) who have reported that glucagon stimulation of System A transport in freshly isolated cell suspensions is composed of an initial period which is protein synthesis-independent followed by a second period which is protein synthesis-dependent. We find that the rapid protein synthesis-independent portion of the glucagon stimulation is not detectable after the cells have been maintained in culture for 24 h or more (data not shown).

The results depicted in Table II show that the stimulation of transport by glucagon is blocked by TM. The glucagon stimulation of System A transport could be completely abolished by the TM for approximately 2 h, but the effectiveness of the inhibitor was decreased significantly if the exposure time was extended to 4 h without supplying fresh TM. This phenomenon can be observed in the data of Table II by comparing the degree of inhibition of transport at 2 or 4 h (fresh TM had been added 2 h prior to the transport assays in each case) with the degree of inhibition at 6 h which was 4 h after the last addition of fresh TM.

Stimulation of System A activity in cultured hepatocytes by insulin, dexamethasone, or vasopressin was also tested for a sensitivity to TM. Cells were placed in primary culture with medium containing the hormone to be tested in the presence or absence of TM. As previously shown (14, 17), insulin and dexamethasone stimulated System A activity and their effects were additive (Table III). TM blocked the stimulation of transport by these two hormones whether they were added alone or in combination. Vasopressin, a calcium-dependent hormone, produced only a 40% stimulation of System A activity after 4 h of incubation, but this increase was also abolished by TM (Table III).

Duration of Tunicamycin Inhibition of System A Activity Induced by Glucagon and Dexamethasone—As mentioned above, during the course of these studies we noted that the glucagon induction of System A was completely abolished for the initial 2 h after the addition of the hormone and the inhibitor, but if fresh inhibitor was not added the degree of inhibition decreased with time. The data shown in Fig. 3 illustrate the time-dependent nature of the TM effect on System A activity when the cells are exposed to the combination of glucagon and dexamethasone. Dexamethasone has been shown to potentiate the glucagon-mediated stimulation of System A (18) and so the combination of these two hormones results in a greater induction than glucagon alone.

In this series of tests, the TM was added with the hormones at the beginning of the experiment (time = 0) and in some instances fresh tunicamycin was added to the cells at 2 or 4 h after initiation of the hormone treatment. After the first 2 h, the uptake in cells incubated in presence of the hormones and TM was not significantly increased above the control values (Fig. 3). For the hormone-treated cells, the rate at
which the System A activity increased was similar between 2 and 4 h of culture whether the TM was present with the glucagon and dexamethasone or not, suggesting that after the initial 2 h exposure to the inhibitor the synthesis of the necessary System A protein was no longer inhibited by the antibiotic.

After 6 h of culture, we measured AIB uptake in glucagon-treated cells which: 1) had been maintained in the presence of TM for the entire 6-h period with no medium changes; 2) had fresh TM-containing medium added after the first 2 h of culture; or 3) had been placed in new TM-containing medium after 4 h of culture (Fig. 3). The later the medium change, that is, the closer the medium change was to the assay time of 6 h, the greater the degree of inhibition of the hormone induction. This phenomenon is best illustrated by the inset of Fig. 3 which depicts the relation between the transport rate observed after 6 h of culture and the time of the last addition of fresh TM to the culture. One finds a linear inverse correlation between the length of time that the cells have been exposed to fresh TM-containing medium and the degree of inhibition of System A activity. If the TM-containing medium which has been exposed to cells for 6 h is removed and placed on a new hepatocyte population, full inhibitory activity can be demonstrated. It is not clear at the present time why this time-dependent relation does not appear to hold for the inhibition of System A activity induced by adaptive control (Figs. 1 and 2).

Effect of Tunicamycin Addition after Glucagon Stimulation of System A Activity—As already discussed, most of the glucagon-stimulated transport activity has been shown to be sensitive to cycloheximide and other protein synthesis inhibitors. Furthermore, Potter and his coworkers (18) have reported that these inhibitors can block further increases in transport activity even if added after the initial stimulation of uptake has occurred. However, inhibitors of protein synthesis cannot distinguish between the production of a plasma membrane-bound component of System A or of a cytoplasmic protein which may serve to regulate or modify the activity of existing transport carriers within the plasma membrane. To test the possibility that hormone treatment causes continued synthesis of a cell surface component, we measured the ability of TM to block further increases in AIB uptake by cells which had been exposed to glucagon and dexamethasone prior to the addition of the inhibitor. The results indicate that even after the transport activity has begun to increase the addition of TM blocks any further stimulation within 1 h (Fig. 4). We interpret these results as evidence for a glycoprotein component of System A, probably located in the plasma membrane, for which continued de novo synthesis is necessary to sustain the hormone-induced stimulation of activity.

Effect of Lectins on System A Transport Activity—In an effort to determine the accessibility of the putative oligosaccharide-transport activity of glucagon-stimulated transport activity to lectins, we measured the transport activity towards six lecithins, which are shown in Table IV. The data presented in this table show that lectins are effective inhibitors of glucagon-stimulated transport activity. For example, the lectin, Concanavalin A (Con A), was the most effective inhibitor, reducing the transport activity by 90%.

TABLE IV

| Lectin added     | NaKRP | CholKRP | Na* dependent |
|------------------|-------|---------|---------------|
| Adaptive control | 67.0±3.0 | 21.0±1.2 | 47.2          |
| None             | 75.9±6.9 | 22.7±1.5 | 53.2          |
| Wheat germ agglutinin | 63.2±1.2 | 19.8±1.6 | 43.4          |
| Lentil           | 71.3±1.3 | 22.2±0.9 | 49.1          |
| Phytohaemagglutinin | 66.8±2.4 | 20.6±1.4 | 46.2          |
| Glucagon-treated | 86.4±4.0 | 27.9±2.8 | 58.5          |
| Ricin            | 82.8±3.6 | 29.0±3.3 | 53.8          |
| Soybean          | 72.7±6.5 | 26.2±3.9 | 46.5          |

2 Although we refer to the protein composition of System A in singular terms for discussion purposes, we recognize that it is quite possible that the activity is the result of a multimeric protein complex. We know of no compelling evidence for either conclusion, but for the present we wish to use the more simplistic model.  

FIG. 3. Time-dependent suppression by tunicamycin of the System A induction by glucagon. Freshly isolated hepatocytes were placed in culture in NaKRP buffer containing 10 mM AIB with (□, △, ∗, ○) or without (○, ●, ●) the combination of 10−7 M glucagon and 10−7 M dexamethasone. In some cases, the initial incubation medium also contained 1 μg/ml of TM (□, △, ○, ●), whereas in others it did not (□, ○). The Na+-dependent uptake of 50 μM AIB was measured as described in the legend to Fig. 1. For some of the cells (○, ●, △, □), the initial incubation medium was not changed during the entire 6 h, but other cells were given fresh buffer still containing the hormones and TM at 2 h (△) or 4 h (○), respectively. ---, serve to show the time of the last addition of fresh TM. The inset shows a replott of the data obtained after 6 h; the abscissa reflects the time at which fresh buffer was added. The results are the averages ± S.D. of 3 or 4 determinations. Where not shown the S.D. bars are enclosed within the symbol.

FIG. 4. Effect of adding tunicamycin after glucagon induction of System A in hepatocytes. Cells in primary culture were transferred to NaKRP buffer (pH 7.4) containing 10 mM AIB in the presence (□, △, ○, ●) or absence (○) of the combination of 10−7 M glucagon and 10−7 M dexamethasone. TM at 1 μg/ml was added 2 h (△), 3 h (○), or 4 h (●) after the exposure of the cells to the hormones. After a 30-min depletion, the Na+-dependent uptake was measured for 1 min at 37°C. The results are the averages of at least three determinations and the standard deviations, omitted for clarity, were typically less than 10%.

Effect of various lecithins on System A transport activity

Cultured hepatocytes were incubated in the AAF medium (adaptive control) or treated with glucagon (10−7 M) for 6 h to produce a stimulus of System A activity. The cells were then incubated for 15 min at 37°C in the presence of 10 μg/ml of the lectins prior to assaying transport. The uptake of 50 μM AIB was measured for 1 min at 37°C. As discussed in the text, similar results were obtained whether the incubation with lectins was done at 4 or 37°C. The results are the averages ± S.D. of 3 or 4 individual determinations.

Transport activity measured

| Lectin added     | NaKRP | CholKRP | Na* dependent |
|------------------|-------|---------|---------------|
| Adaptive control | 67.0±3.0 | 21.0±1.2 | 47.2          |
| None             | 75.9±6.9 | 22.7±1.5 | 53.2          |
| Wheat germ agglutinin | 63.2±1.2 | 19.8±1.6 | 43.4          |
| Lentil           | 71.3±1.3 | 22.2±0.9 | 49.1          |
| Phytohaemagglutinin | 66.8±2.4 | 20.6±1.4 | 46.2          |
| Glucagon-treated | 86.4±4.0 | 27.9±2.8 | 58.5          |
| Ricin            | 82.8±3.6 | 29.0±3.3 | 53.8          |
| Soybean          | 72.7±6.5 | 26.2±3.9 | 46.5          |
chiridic moiety of System A, we tested the effect of a variety of lectins on AIB uptake by either hormone-treated or amino acid-deprived hepatocytes. The transport activity of System A in either the basal or the stimulated condition was not inhibited significantly by the lectins tested (Table IV). Although the data shown in Table IV were obtained after incubation of the hepatocytes with the lectins for 15 min at 37°C, incubation with the lectins at 37 or 4°C for 5, 15, 20, or 60 min yielded similar results. These data are in agreement with the observations of Cecchini et al. (19) and Todderud and Kletzien (20) who have reported that solubilized proteins associated with System A activity do not bind to ConA-Sepharose. These negative results should not be taken as evidence against the presence of a glycoprotein component for System A, because Cummings and Kornfeld have shown that 35 to 40% of all asparagine-linked complex-type glycopeptides do not bind to ConA-Sepharose (21). It is also possible that lectin binding does not inhibit the transport process.

DISCUSSION

The present report demonstrates the effect of TM on the biosynthesis of the neutral amino acid transport systems in rat hepatocytes. System A, a highly regulated transport system (1–4), was decreased in activity to a greater extent than any of the others with the possible exception of System Gly. It is possible that those systems which are less sensitive to the inhibitor: 1) do not contain asparagine-linked carbohydrate chains; 2) are not inhibited by the absence of asparagine-linked carbohydrate chains due to TM action; or 3) the associated glycoproteins are not being synthesized as rapidly as those for Systems A or Gly. With respect to the last point, the high degree of inhibition for System A may reflect a short half-life of a necessary glycoprotein component. Studies to be published elsewhere indicate that for cultured hepatocytes the glucagon-stimulated System A activity decays, after removal of the hormone, by a first order process with a half-life of about 2 h or less. Whether the sensitivity to TM, in general, reflects the turnover of the other systems tested is not certain because we do not have quantitative estimates for the half-life of each. It is worth noting that System Gly, the only other system which was inhibited to a large degree, has been suggested to be a variant of System A.

The transient nature of TM-mediated inhibition of the glucagon induction of System A is not easily explained. The data argue against several possibilities. An effect on energization by the Na+ gradient appears unlikely because two Na+-dependent agencies (Systems ASC and N) were not inhibited to the same extent as System A, although one cannot eliminate the possibility of different degrees of energetic coupling. It is also conceivable that glucagon induces two components of System A, only one of which is sensitive to tunicamycin, yet extensive kinetic analysis and competitive inhibition studies by several laboratories have produced no evidence for such heterogeneity (14). One may also question the effects of TM on glucagon binding or glucagon receptor number. It has been demonstrated that much of the induction of System A still occurs if the glucagon is removed from the medium after only a 15-min incubation with the cells (18, 22). The data of Fig. 4 indicate that TM inhibits further stimulation of transport when added 2 or 4 h after the addition of glucagon. If a significant amount of the glucagon-induced intracellular signal has already been generated after 15 min, any effect of TM on glucagon binding or glucagon receptor number would not play a role when the inhibitor is added after 2 or 4 h. Further studies will be required to explain the transient nature of the inhibition of glucagon action by TM.

Although most of our knowledge about the regulation of neutral amino acid transport has come from descriptive studies at the cellular level, a few of the molecular processes involved in hormonal control of System A, especially by glucagon, are known. As mentioned above the increase in System A transport will continue after the hormone has been removed (18, 22), suggesting that there is a committed step in the pathway beyond which dissociably bound hormone is no longer necessary. Furthermore, de novo synthesis of the protein component must continue for nearly as long as the activity is increasing; the addition of cycloheximide at any time during the stimulation blocks further increases in activity (18). Our results support these findings and extend them by demonstrating that the protein which must be continually synthesized during glucagon induction is a glycoprotein. Our interest in this observation arises from the implication that the newly synthesized protein is a plasma membrane-bound molecule rather than a cytoplasmic one. The data of Samson and Fehlmann (23) and Quinlan et al. (24) are consistent with our interpretation of the present results. Those laboratories have shown that plasma membrane vesicles isolated from hepatocytes containing elevated rates of System A transport retain the stimulated activity. Further work will be necessary to determine if this glycoprotein represents the translocation mediator itself or a regulatory subunit.

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