The rapid inhibition of host mRNA synthesis by T4 and the concomitant early initiation of T4 mRNA synthesis are apparent from the data in Table 1. *E. coli* B/5 was infected at a multiplicity of 10 in complete K medium ($t = 0$). Aliquots of the infected culture were labeled with H^3-uracil (1 μc/ml) for 1 min at various times after infection. H^3-RNA was isolated as described previously and tested for hybridization with *E. coli* and T4 DNA. During the first minute, the rate of *E. coli* mRNA synthesis decreased from its preinfection value, and T4 mRNA synthesis began. Within 2 min after infection, the rate of host mRNA synthesis was less than 10 per cent of the uninfected control.

The immediate inhibition of host mRNA synthesis cannot be entirely attributed to the activity of some T4 protein, however. As seen from Figures 2 and 3, the rate of T4 mRNA synthesis exceeded that of *E. coli* mRNA even when CAP prevented the synthesis of inhibitor. Such a preferential transcription of T4 DNA by the *E. coli* RNA polymerase could help account for the rapid inhibition of host functions.

Summary.—T4 blocks intracellular λ development by inhibiting the formation of λ mRNA. T4-specific protein synthesis is required for this effect as well as for the inhibition of *E. coli* mRNA. Inhibition of host mRNA occurs immediately after infection and is virtually complete within 4 min.

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[1] Weigle, J. J., and M. Delbrück, *J. Bacteriol.*, **62**, 301 (1951).

[2] Okamoto, K., Y. Sugino, and M. Nomura, *J. Mol. Biol.*, **5**, 527 (1962).

[3] Hall, B. D., and S. Spiegelman, these PROCEEDINGS, **47**, 137 (1961).

[4] Adams, M. H., *Bacteriophages* (New York: Interscience Publishers, Inc., 1959), p. 446.

[5] Green, M. H., *J. Mol. Biol.*, in press; Information Exchange Group No. 7, Memo #51.

[6] Nygaard, A. P., and B. D. Hall, *Biochem. Biophys. Res. Commun.*, **12**, 98 (1963).

[7] Green, M. H., these PROCEEDINGS, **52**, 1388 (1964).

[8] Lieb, M., *Science*, **145**, 175 (1964).

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*THE CELL-FREE BIOSYNTHESIS OF THE GLYCOPROTEIN OF MEMBRANES FROM EHRLICH ASCITES CARCINOMA CELLS**

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Glycoproteins are widely distributed in extracellular fluids and surface membranes. Although the steps of protein synthesis have been illuminated, information on glycoprotein biosynthesis is scarce, and virtually nonexistent on the assembly of membranes from their carbohydrate, protein, and lipid components. Progress on both the biosynthesis and structure of surface membranes rests on their unequivocal isolation. We reported previously[1, 2] on the separation of membranes of Ehrlich ascites cells into two fractions: (1) membranes bearing ribosomes but lacking glycoprotein as shown by the absence of sialic acid and hexosamine, and (2) ribosome-free membranes of which the surface membranes represent one com-

ponent. Thus the glycoprotein, which is an integral part of the surface membranes, is found in the ribosome-free membrane fraction. It was inferred[2] that the membrane glycoprotein is synthesized by a nonsynchronous mechanism in which the protein is first made at the polysome, and later attached to oligosaccharide units (synthesized independently elsewhere) within the complex of the membranes bearing ribosomes.

How and where the oligosaccharide units are synthesized, and how the cell regulates the construction of the final membrane structure is not known. This paper describes an attempt to answer these questions using a cell-free system developed from Ehrlich ascites cells which incorporates monosaccharides and amino acids into soluble and membrane glycoproteins. It is deduced that the enzymes required for synthesis of the oligosaccharide units are located in a postmicrosomal particulate fraction which has been purified by sedimentation at 150,000 *g*. The cell-free system, in conformity with the intact cell data,[2] implicates the membranes bearing ribosomes as a site of union of the oligosaccharide units to a protein receptor to yield membrane and soluble glycoproteins.

Materials and Methods.—Isolation of cellular fractions: A 20,200 *g* supernatant (20*S*) fluid was prepared from Ehrlich ascites cells as described previously,[2] except that homogenization was performed in a medium of 0.20 *M* sucrose, 0.05 *M* Tris buffer (pH 7.5), 0.05 *M* KCl, 0.005 *M* $MgCl_2$, and 0.005 *M* mercaptoethanol. The 20*S* fluid was centrifuged at 150,000 *g* for 1 hr to obtain microsomes, and the supernatant fluid (150*S*) dialyzed 2–4 hr against homogenization media minus sucrose. The dialyzed 150*S* fluid was centrifuged at 150,000 *g* for 5 hr and the contents were divided into 4 fractions: the pellet (150*S* pellet); the lower 10% of the supernatant fluid (which contained a fluffy white material) which was termed the postmicrosomal particulate (PMP) fraction; the middle 30% (supernatant II), and the upper 60% of the supernatant fluid (supernatant I).

Microsomes were purified by sedimentation through two layers of sucrose.[3] Ribosomes were prepared from microsomes by treatment with sodium deoxycholate followed by sedimentation.[2] The 20*S* supernatant fluid was fractionated on a 25–45% linear sucrose gradient to give 4 fractions: the aqueous fraction, located at the top of the tube, which contains the soluble proteins and glycoproteins of the cytoplasm; the ribosome-free membranes; the membranes bearing ribosomes (as a pellet); and the fraction intermediate between the 2 membrane fractions.

Chemicals: ATP, CTP, GTP, UTP, creatine phosphate, creatine phosphokinase, and N-acetylgalactosamine were obtained from Calbiochem; Tris buffer, α-amylase (bacterial type II) and galactose from Sigma Chemical Co.; trypsin (crystallized) from Worthington Biochemical Corp.; N-acetyl-neuraminic acid (synthetic) from General Biochemical Co.; D-glucosamine-C^{14} (180 mc/mM) from Volk Chemical Co.; N-acetylglucosamine-C^{14} (0.2 mc/mM) from New England Nuclear Corp.; galactose-C^{14} (60 mc/mM) and UDP-galactose-C^{14} (40 mc/mM) from International Chemical and Nuclear Corp. Each of the radioactive compounds showed over 98% purity on chromatography in 3 different solvent systems.

Incorporation of labeled compounds: All incubations of subcellular components were terminated by addition of an excess of 10% TCA (trichloroacetic acid). The precipitates were processed and counted as described elsewhere,[2] and the protein content determined by the method of Lowry *et al.*[4] Identification of labeled compounds in glycoproteins of the aqueous (soluble) and ribosome-free membrane fractions of the 20*S* fluid was accomplished by paper chromatography after acid hydrolysis. The fractions were precipitated with 10% TCA and washed[2] to remove glycolipids, hydrolyzed in 3 *N* HCl for 4 hr to liberate glucosamine and galactosamine, and in 1 *N* HCl for 6 hr to liberate galactose. After paper chromatography[2] for 24 hr in pyridine:ethylacetate:acetic acid:water (5:5:3:1), the spots for each sugar were cut out and counted.

Results.—The ability of the 20*S* fluid to incorporate labeled sugars into TCA-precipitable glycoprotein is shown in Table 1. After 0.5 hr, the glucosamine and galactose incorporation per mg of protein is distributed in all fractions but is highest

TABLE 1

CELL-FREE BIOSYNTHESIS OF MEMBRANE AND SOLUBLE GLYCOPROTEINS[5] BY THE 20S FLUID

Fraction	Incorporation (cpm/mg protein) into Glycoprotein of: Glucosamine-C^{14}	Galactose-C^{14}	Incorporation (cpm/mg protein) into Protein of: Leucine-C^{14}	Serine-C^{14}
Aqueous (soluble)	880	112	820	443
Ribosome-free membranes	1240	367	840	616
Intermediate	625	205	1480	764
Membranes bearing ribosomes	930	124	2700	1700

The reaction mixture contained 2.0 ml of the 20S fluid (which had been dialyzed for 2 hr against 0.15 *M* KCl), 0.1 ml ATP solution (20 mg/ml), 0.1 ml creatine phosphate (50 mg/ml), and 10 μc of galactose-C^{14}, glucosamine-C^{14}, serine-C^{14}, or leucine-C^{14}. The fractions were isolated[2] as described in *Materials and Methods* after incubation for 0.5 hr at 37°. The intermediate fraction refers to material located between the ribosome-free membranes and the pellet of membranes bearing ribosomes. Each fraction was precipitated with 10% TCA, washed, and counted.[2] The data represents an average value of three separate experiments for each radioactive compound.

in the ribosome-free membranes. Furthermore, the 20S fluid actively incorporates amino acids into protein; the specific activities of serine and leucine are highest in the rough membrane fraction. Any light precipitate, formed during the incubation, was removed by centrifugation at 5000 *g* for 10 min prior to the sucrose gradient fractionation.

For each experiment shown in Table 1, a control experiment was performed where the labeled compound was added after the incubation. The specific activity of each fraction was corrected for the control value (which did not exceed 20% of the total). The distribution of the glucosamine-C^{14} (9970 cpm per ml 20S fluid total incorporated) was aqueous (soluble) 30 per cent; ribosome-free membrane, 42 per cent; intermediate fraction, 11 per cent; and membranes bearing ribosomes, 17 per cent. For galactose, the distribution of 3550 cpm was, respectively, 13, 52, 3 and 32 per cent, and for leucine (19,300 cpm) the distribution was 14, 14, 17, and 55 per cent.

Paper chromatographic examination of the aqueous (soluble) and ribosome-free membrane fractions revealed over 90 per cent of the glucosamine-C^{14} as hexosamine,

TABLE 2

COMPARISON OF THE CELL-FREE BIOSYNTHESIS OF GLYCOPROTEIN[5] AND PROTEIN BY FRACTIONS FROM EHRLICH ASCITES CELLS

System	Using Glucosamine-C^{14} Cpm	% Change	Using Leucine-C^{14} Cpm	% Change
Complete	2210	—	1330	—
−150S (dialyzed)	990	−55	60	−96
−Microsomes	840	−62	152	−90
−Sugars	1550	−30	1315	0
−ATP	1360	−38	65	−95
+Ribosomes (−Microsomes)	870	−60	1355	+ 2
+Ribosomes	2150	− 2	—	—
+RNase	2235	+ 1	100	−93
+Puromycin	2040	− 8	297	−78
+UTP	1440	−35	—	—
+CTP, GTP	1550	−30	—	—
+UTP, CTP, GTP	950	−57	1380	+ 4
+Trypsin	400	−82	—	—
+α-Amylase	1600	−28	310	−77
+pH 5 Enzymes (−150S)	960	−57	1660	+25

The complete system is used as the reference to compute the % change in each case and contained, in 0.4 ml: 1 μc of D-glucosamine-C^{14} or 0.2 μc L-leucine-C^{14}; 40 μmoles Tris HCl pH 7.5; 20 μmoles KCl; 0.05 ml microsomes (1 mg protein); 0.5 mg sugars (galactose, sialic acid); and 0.5 mg ATP, 4 μmoles $MgCl_2$; 1.6 μmoles mercaptoethanol; and 0.2 ml supernatant fluid. Other components included: 0.05 ml ribosomes (0.95 mg protein); 50 μg RNase; 100 μg puromycin; 100 μg α-amylase; 100 μg trypsin; 0.5 mg UTP; 0.5 mg CTP or 0.5 mg GTP. The pH 5 enzyme preparation was obtained from the 150S fluid as described elsewhere.[6] After incubation at 37° for 40 min, glycoproteins were precipitated with 10 ml of 10% TCA. The precipitate was washed and counted as given in *Materials and Methods*. When N-acetylglucosamine-C^{14} was used in place of glucosamine-C^{14}, similar results were obtained.

with galactosamine comprising 55–60 per cent. About 5 per cent was converted to galactose. Less than 1 per cent of the glucosamine appeared as glucose and mannose. About 85 per cent of the galactose-C^{14} in the smooth membranes was recovered as galactose. Small amounts were recovered as galactosamine, glucose, and mannose. When unlabeled smooth membranes (lipid-extracted) were hydrolyzed, galactosamine, galactose, and mannose were found by paper chromatography.

The fractionation of the 20*S* fluid led to the results shown in Table 2 for a typical experiment. The complete system, used as reference, included the dialyzed 150*S* fluid together with microsomes and the other chemicals shown in Table 2. The regular 150*S* fluid showed a change of −23 and −30 per cent when substituted for the dialyzed 150*S* fluid using glucosamine-C^{14} and leucine-C^{14}, respectively. The use of original 20*S* fluid in place of dialyzed 150*S* fluid showed a per cent change of −62 per cent using glucosamine-C^{14}. Both the 150*S* fluid and the microsomes alone have activity, but their total activity was about 10–30 per cent below that of the complete system. The glucosamine-C^{14} incorporation is stimulated by the presence of galactose and sialic acid. The presence of nucleotide triphosphates CTP, GTP, and UTP is inhibitory, while ATP is stimulatory. The presence of ribosomes, RNase, and puromycin do not influence glucosamine incorporation. The incorporation of either galactose-C^{14} or N-acetylglucosamine-C^{14} was similar to that for glucosamine-C^{14} as shown in Table 2.

The cellular components were further purified, and the results of a typical experiment are shown in Table 3. As in the crude system of Table 2, ATP and monosaccharides stimulate the incorporation, whereas UTP inhibits significantly, especially galactose-C^{14}. In each case nearly all of the activity is present in the postmicrosomal particulate (PMP) fraction. When supernatants I and II or the 150*S* pellet were substituted for the PMP fraction, only the latter showed appreciable ability to incorporate glucosamine-C^{14} or galactose-C^{14}. Although the membranes bearing microsomes alone show only 5–10 per cent of the activity of the complete system (in contrast to the microsomes of Table 2), they stimulate the enzymatic activity of the PMP fraction. However, the membranes bearing ribosomes and the crude microsomes (Table 2) function equally well for leucine-C^{14} incorporation, whereas

TABLE 3

CELL-FREE INCORPORATION OF MONOSACCHARIDES INTO GLYCOPROTEINS

System	Incorporation into Glycoprotein of: Glucosamine-C^{14} Cpm	% Change	Galactose-C^{14} Cpm	% Change	UDP-Galactose-C^{14} Cpm	% Change
Complete	1200	—	840	—	1400	—
−PMP fraction	105	−91	140	−84	86	−94
−Membranes (bearing ribosomes)	460	−60	258	−69	430	−70
+Supernatant I	98	−92	155	−82	110	−92
+Supernatant II	185	−85	178	−79	240	−83
+150*S* Pellet	455	−62	—	—	615	−56
−ATP	700	−38	615	−27	1030	−28
−Sugars	630	−44	545	−35	560	−60

The reaction mixture contained in 0.3 ml: 1 μc of D-glucosamine-C^{14}, D-galactose-C^{14}, or UDP-galactose-C^{14}; 30 μmoles Tris HCl pH 7.5; 13 μmoles KCl; 3 μmoles $MgCl_2$, 1.2 μmoles mercaptoethanol; 0.05 ml of postmicrosomal particulate (PMP) material; 0.1 ml of membranes bearing ribosomes; 0.5 mg of galactose and sialic acid (when glucosamine-C^{14} was used), or 0.5 mg N-acetylgalactosamine and sialic acid otherwise; and 0.5 mg of ATP. As noted, when supernatants I and II (0.15 ml) or the 150*S* pellet (0.05 ml) were added, the PMP fraction was omitted. After incubation at 37° for 40 min, the glycoproteins were precipitated with 10 ml of 10% TCA. The precipitate was washed and counted as given in *Materials and Methods*.

the PMP fraction (alone) is inactive. This illustrates the particulate nature of the PMP fraction and its nonidentity with the membranes bearing ribosomes, especially in view of the full leucine-C^{14} incorporating activity found in supernatants I and II. The specific activity of the PMP fraction was found in 6 different preparations to be near 700 cpm of glucosamine-C^{14} incorporated per mg protein under the conditions of Table 3. This is approximately twice the specific activity of the 150*S* pellet, and 4–5 times the specific activity found in the 20*S* fluid. In a typical case, the total activity in 10 ml of 150*S* dialyzed fluid was distributed 72 per cent (5600 cpm) in the PMP fraction, 19 per cent (1500 cpm) in the 150*S* pellet, and 9 per cent in supernatant II. This distribution was quite variable however, and sometimes as much as 20–30 per cent of the total activity was found in supernatant II.

In Figure 1 the time sequence of glucosamine and galactose incorporation is shown. In both cases incorporation is nearly complete in 60 min. Also in Figure 1 the incorporation of glucosamine as a function of the PMP fraction concentration is plotted. The incorporation increases linearly and finally levels off. In Figure 2, the stimulation of glucosamine incorporation produced by the membranes bearing ribosomes is shown. Regardless of the concentration of PMP fraction present, the membranes bearing ribosomes produce approximately the same increase in incorporation at a given concentration.

Discussion.—The buildup of complex heterogeneous structures such as membranes

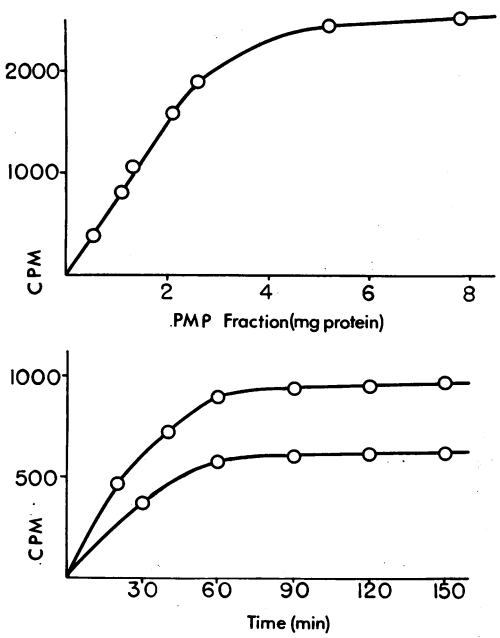

FIG. 1.—*Top:* Cpm (counts per minute) of glucosamine-C^{14} incorporated into TCA-insoluble glycoprotein as a function of postmicrosomal particulate (PMP) fraction during incubation at 37° for 40 min in a complete system as described in Table 3. *Bottom:* The time course of glucosamine-C^{14} (upper curve) and UDP-galactose-C^{14} (lower curve) incorporation into TCA-insoluble glycoprotein in a complete system as described in Table 3.

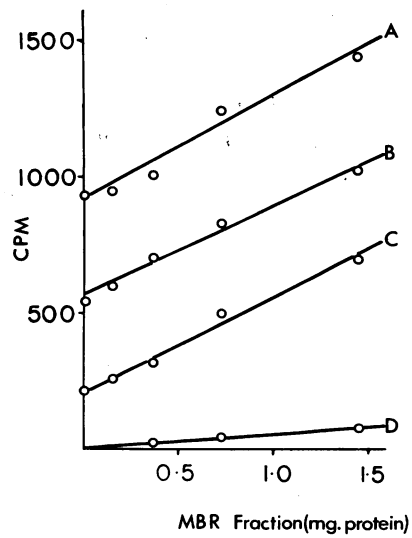

FIG. 2.—Cpm of glucosamine-C^{14} incorporated into TCA-insoluble glycoprotein as a function of amount of membranes bearing ribosomes (mg protein) is illustrated in the presence of various quantities (mg protein) of PMP fraction. For *A*, 2.0 mg.; *B*, 1.0 mg; *C*, 0.5 mg; and *D*, 0.0 mg of PMP fraction was added. The system was incubated for 40 min at 37° as described in Table 3.

poses intriguing biological and chemical questions. Whether the process occurs via spontaneous self-assembly as perhaps with the tobacco mosaic virus components or subunits of macromolecules,[8] or by a mediated template process,[9] can best be answered with an appropriate system. The results in Table 1 clearly establish the cell-free biosynthesis of membrane and soluble glycoproteins[5] by the 20*S* fluid. Neither the glucosamine-C^{14} nor the galactase-C^{14} is converted appreciably to other sugars, although about 60 per cent of the glucosamine is converted to galactosamine. The incorporation per mg of protein was highest in the ribosome-free membranes for both glucosamine-C^{14} and galactose-C^{14} incorporation. This result would be anticipated because the surface membrane,[2] which contains glycoprotein, is probably present[2] in the ribosome-free membrane fraction. After 0.5 hr, the incorporation of amino acids leucine and serine was highest in the membranes bearing ribosomes.

These results demonstrate the presence of the enzymes required for biosynthesis of glycoprotein[5] and protein of the smooth membrane and aqueous (soluble) fractions. Undoubtedly the glucosamine-C^{14} and galactose-C^{14} are incorporated into carbodydrate units which are then joined to protein, because the carbohydrate units alone are soluble in TCA. In fact, some glycoproteins with a high carbohydrate content such as the α_1-glycoprotein[10] from serum are soluble in TCA, and it is possible that some glycoproteins from the Ehrlich ascites cells are not precipitated under the conditions employed.

The incorporation of either galactose from UDP-galactose, or galactosamine and glucosamine into TCA-insoluble glycoprotein shows an absolute requirement for a postmicrosomal particulate (PMP) fraction. The enzymatic activity found in microsomes (obtained by centrifugation at 150,000 *g* for 1 hr) is essentially removed after centrifugation through sucrose as shown by the membranes bearing ribosomes of Table 3. Most of the enzymatic activity is obtained as a fluffy material (at the bottom of the tube) from the dialyzed 150*S* supernatant fluid by centrifugation at 150,000 *g* for 5 hr and termed the PMP fraction. Thus it appears that the enzymes required for biosynthesis of the carbohydrate of the glycoproteins are located in a fluffy particulate complex which sediments less rapidly than the membranes bearing ribosomes, but much more rapidly than the soluble activating enzymes (supernatants I and II) functional in protein synthesis (Table 3). It appears probable that the oligosaccharide-synthesizing enzymes of this PMP fraction are components of (1) the surface membrane[2] itself which is thought to sediment as part of the ribosome-free membrane complex, (2) the Golgi apparatus, or (3) a new undefined structure. It is plausible that the Golgi apparatus contains these enzymes since it has been suggested from cytochemical evidence as a site of surface glycoprotein synthesis.[11]

The results in Table 3 and Figure 2 reveal that an enhanced incorporation from glucosamine, galactose, or UDP-galactose occurs when the membranes bearing ribosomes are added to the PMP fraction. For example, the sum of the activities in Table 3 for the membranes bearing ribosomes and the PMP fraction alone account for only 47 and 37 per cent, respectively, of the cpm of glucosamine-C^{14} and UDP-galactose-C^{14} incorporated in the complete system. The increased incorporation is independent of the amount of enzyme present as represented by the PMP fraction (Fig. 2). The activity in the rough membrane alone was less than 10–20

per cent of the total system. These results suggest the presence of a receptor in the membranes bearing ribosomes—the receptor being part of the membraneous complex since the ribosomes are completely inactive in oligosaccharide synthesis (Table 2). It appears likely that the receptor represents the polypeptide portion of the glycoprotein.

From our results it is deduced that the carbohydrate units are synthesized by enzymes in the PMP fraction probably from their UDP-derivatives. Although the cell-free system was slightly inhibited by UTP, there is indirect evidence that N-acetylglucosamine is activated by conversion to the UDP-derivative before incorporation into liver glycoproteins.[12] In our system, galactose was incorporated into glycoprotein from UDP-galactose which strongly suggests itself as the activated derivative. A mechanism for membrane and soluble glycoprotein biosynthesis can logically be proposed wherein the carbohydrate units, assembled by several enzymes located in the PMP fraction, migrate to the membranes bearing ribosomes where they unite with polypeptide previously synthesized by the polysome complex. The completed glycoprotein would then be integrated into the final membrane structure. This picture is consistent with intact cell data[2] and the 20*S* fluid incorporation data (Table 1), which show that the membranes bearing ribosomes (and not the ribosomes themselves) become labeled with glucosamine-C^{14} and galactose-C^{14}. The PMP fraction is either in close proximity to the membranes bearing ribosomes in the cell or becomes so after cell rupture because considerable enzymatic activity is initially found in the microsomes (Table 2). Most of the activity is lost when the microsome fraction is sedimented through sucrose (Table 3). The close association of the PMP fraction with the membranes bearing ribosomes would explain also the presence of receptor in the PMP fraction where TCA-insoluble glycoprotein is synthesized in the absence of membranes bearing ribosomes (Table 3). The PMP fraction does not contain detectable membranes bearing ribosomes since it will not support protein synthesis unless the latter are added.

It is apparent that the concomitant synthesis of protein, geared to carbohydrate synthesis, is not essential. Intact cell studies[2] demonstrated that the glucosamine incorporation, in contrast to the protein synthesis, was only slightly inhibited by puromycin over a 2-hr period. Similarly, the results of Table 2 show that glucosamine incorporation is not inhibited by puromycin. This conclusion is further emphasized by the destruction of protein synthesis, but not oligosaccharide synthesis, by RNase. The inhibition of protein synthesis by α-amylase, present in excess, suggests the presence of proteolytic enzymes. Thus the inhibition of glucosamine incorporation by 28 per cent can be attributed to proteolytic enzymes, and not to inhibition of possible glycogen synthesis. Both protein and carbohydrate synthesis are inhibited strongly by trypsin.

The synthesis of protein alone markedly differs from the carbohydrate synthesis of Ehrlich ascites cell glycoproteins. Protein synthesis requires ribosomes; carbohydrate synthesis does not. A previous report showed that glucosamine-C^{14}, unlike leucine-C^{14}, did not bind to ribosomes.[2] The soluble enzymes required for protein synthesis (in supernatant I) do not sediment appreciably under conditions where enzymes synthesizing carbohydrate move to the bottom of the tube as part of the PMP fraction (Table 3). In this regard, the pH 5 enzyme preparation,[6] which contains the amino acid-activating enzymes, is inactive in carbohydrate synthesis.

The trinucleotides GTP, CTP, and UTP appear to inhibit considerably the carbohydrate synthesis. However, the carbohydrate synthesis is stimulated by monosaccharides, whereas the protein synthesis is not. Both protein and carbohydrate synthesis are stimulated by ATP, although the latter does not show an absolute requirement at this stage. The role of ATP in the carbohydrate synthesis, as well as the inhibitory effect of UTP, is obscure.

This study renders improbable some of the suggested mechanisms for glycoprotein biosynthesis[13] as applied to the Ehrlich ascites cell. The formation of an sRNA-amino acid-glucosamine intermediate is disallowed, as is the attachment of monosaccharide or carbohydrate units to the growing polypeptide chain attached to the polysome.

Summary.—The incorporation of glucosamine and galactose into soluble and membrane glycoproteins is described. The enzymes which incorporate glucosamine and galactose (from UDP-galactose) into TCA-insoluble glycoproteins were found to reside in a postmicrosomal particulate fraction. It was postulated that the the oligosaccharides, synthesized by this fraction, are attached to a protein receptor at the membranes bearing microsomes as a step in the eventual buildup of the surface membrane. The incorporation of monosaccharides was stimulated by ATP and other monosaccharides, but not by UTP, GTP, CTP, RNase, and puromycin. The incorporation of monosaccharides was contrasted with the incorporation of amino acids into soluble and membrane fractions.

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[1] Cook, G. M. W., M. T. Laico, and E. H. Eylar, *Federation Proc.*, **24**, 230 (1965).

[2] Cook, G. M. W., M. T. Laico, and E. H. Eylar, these PROCEEDINGS, **54**, 247 (1965).

[3] Wettstein, F. O., T. Staehelin, and H. Noll, *Nature*, **197**, 430 (1963).

[4] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[5] Although the incorporation of monosaccharide into glycoprotein is unmistakable because they become insoluble in TCA, it was not demonstrated directly that amino acid-C^{14} was incorporated into the protein moiety of either the soluble or membrane glycoproteins. Preliminary evidence, based on pronase digestion of soluble and ribosome-free membranes, reveals that the isolated glycopeptides contain 20–40% of the total serine-C^{14} in these fractions. Furthermore, since glycoproteins greatly predominate in the aqueous (soluble) fraction, it is highly unlikely, in view of the high incorporation, that the protein moiety would not be labeled with either leucine-C^{14} or serine-C^{14}.

[6] Keller, E., and P. C. Zamecnik, *J. Biol. Chem.*, **221**, 45 (1956).

[7] Fraenkel-Conrat, H., and B. Singer, *Biochim. Biophys. Acta*, **33**, 359 (1959).

[8] Itano, H. A., and S. J. Singer, these PROCEEDINGS, **44**, 522 (1958).

[9] Green, D. E., and O. Hechter, these PROCEEDINGS, **53**, 318 (1965).

[10] Eylar, E. H., and R. W. Jeanloz, *J. Biol. Chem.*, **237**, 622 (1962).

[11] Gasic, G., and T. Gasic, *Proc. Am. Assoc. Cancer Res.*, **45**, 22 (1963).

[12] Molnar, J., G. B. Robinson, and R. J. Winzler, *J. Biol. Chem.*, **239**, 3157 (1964).

[13] Eylar, E. H., *J. Theor. Biol.*, in press.