Studies on the Nature of Microsome-Bound Substances Involved in the Biosynthesis of Acidic Glycoproteins of Guinea-Pig Serum

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1. In further studies on the biosynthesis of components of fraction I, an acidic glycoprotein-containing fraction from guinea-pig serum, an investigation was made of the substances bound to liver microsomes that had earlier been implicated to participate in the synthesis of components of fraction I present in serum. These substances were normally liberated by ultrasonic vibrations. Antisera to sub-fractions of fraction I were used for characterization. 2. At pH 8.6, most of the microsome-bound substances showed electrophoretic mobilities lower than components of fraction I but similar to components of sialic acid-free fraction I. 3. The sialic acid/protein ratios of immune precipitates formed by microsome extracts were similar to those of precipitates formed by sialic acid-free fraction I. 4. On chromatography on Sephadex G-150, most of the microsomal substances were eluted at an essentially similar volume to the main components of fraction I. 5. It was concluded that most of the microsome-bound substances lack sialic acid residues, and, as appreciable degradation of completed molecules is unlikely, these substances appear to be precursors of serum glycoprotein molecules with incomplete prosthetic groups. 6. Evidence was obtained on the submicrosomal localization of incomplete and complete serum glycoprotein molecules.

This work forms part of a study on the biosynthesis of components of fraction I. Fraction I is an α -globulin fraction containing acidic glycoproteins isolated from guinea-pig serum (Simkin, Skinner & Seshadri, 1964b). In initial investigations on biosynthesis, we have shown that the microsome fraction of guinea-pig liver contains the site or sites of biosynthesis of both the polypeptide and carbohydrate moieties of components of fraction I (Simkin & Jamieson, 1967a). It was demonstrated that substances could be extracted from the microsome fraction of liver by the detergent Lubrol W that react with antisera to fraction I or to sub-fractions of it. These substances became labelled after administration of [^{14}C]leucine or [^{14}C]glucosamine before appreciable label appeared in components of fraction I present in serum. Labelling also took place in certain systems *in vitro*. These substances could also be extracted from microsome material by several other procedures such as treatment with ultrasonic vibrations, and it was suggested that they are associated with membranous structures present in the microsome fraction or are enclosed within vesicles formed by membranes. Several of the substances gave reactions of immunological identity with components present in guinea-pig serum. It was, however, pointed out that the

microsome-bound substances might not be glycoprotein molecules identical with those present in serum, but rather might be precursors possessing incomplete carbohydrate prosthetic groups, because components of fraction I bearing incomplete carbohydrate groups can still react with antisera to fraction I or to its subfractions. The aim of the present work was to investigate the nature of these microsome-bound substances. Studies involving immunoelectrophoresis, chromatography on Sephadex and chemical analysis of material precipitated by antiserum strongly suggest that the substances predominantly represent precursors of serum glycoproteins. Some further evidence on the localization of these substances within components of the microsome fraction is also presented. A preliminary account of this work has been published (Simkin & Jamieson, 1967b).

EXPERIMENTAL

Materials. Pevikon C870 was purchased from Shandon Scientific Co. Ltd., London, N.W. 10. Neuraminidase from *Vibrio comma* (formerly *Vibrio cholerae*) (500 units/ml.) was obtained from Behringwerke A.-G., Marburg/Lahn, Germany. Other materials were as used in previous work (Simkin *et al.* 1964b; Simkin & Jamieson, 1967a).

Animals. Guinea pigs similar to those taken in previous work (Simkin & Jamieson, 1967a) were used after starvation overnight.

Preparation of serum fractions. Fraction I was prepared from guinea-pig or human serum as in previous work (Simkin *et al.* 1964b; Simkin & Jamieson, 1967a). Guinea-pig serum albumin was prepared by the trichloroacetic acid-ethanol procedure of Campbell, Greengard & Kernot (1960), and purified by chromatography with CM-cellulose (Cohen, 1957). A human serum α -globulin-containing fraction was prepared by the method of Weimer, Mehl & Winzler (1950). The final product was not reprecipitated but 150 mg. samples were chromatographed on a 1.1 cm. $\times$ 30 cm. column of DEAE-cellulose. The column was equilibrated with 10 mM-phosphate buffer, pH 8.0, and eluted in a stepwise manner with 65 ml. of 100 mM-phosphate buffer, pH 6.9, 70 ml. of 100 mM-phosphate buffer, pH 5.2, and 35 ml. of 300 mM-NaH₂PO₄, pH 4.3 (cf. Biserte, Havez, Laturaze & Hayem-Levy, 1960). The material eluted by 300 mM-NaH₂PO₄ was further fractionated by electrophoresis on a block of Pevikon with 27 mM-sodium acetate-24 mM-sodium diethylbarbiturate-32 mM-HCl, pH 8.6, as buffer; the protein of faster mobility was taken for use.

Sialic acid-free glycoprotein preparations were prepared by heating with 25 mM-H₂SO₄ at 85° for 60 min. After dialysis against water the non-diffusible material was freeze-dried.

Preparation of liver microsome material and microsome extracts. Except where otherwise stated, the procedures used were similar to those of Simkin & Jamieson (1967a), as were conditions of labelling with radioactive precursors when required before isolation of microsomes. Medium A (0.25 M-sucrose, 50 mM-tris-32 mM-HCl buffer, pH 7.8, 25 mM-KCl, 4 mM-MgCl₂) was used for subcellular fractionations. The method for extraction of microsomes with ultrasonic vibrations was usually modified from that of Simkin & Jamieson (1967a) thus: microsome material (about 90 mg. of protein) suspended in 6 ml. of 0.15 M-NaCl was treated with a Dawe Soniprobe fitted with an 11 mm. $\times$ 13 mm. tip for 2 min. at 20–30 w. An equal volume of 0.15 M-NaCl was added and suspensions were centrifuged at 105 000 *g*_{av} for 120 min. Unless otherwise stated, supernatants were dialysed against water for 24 hr. at 2°, centrifuged and freeze-dried. Residues were dissolved at about 200 mg./ml. for immunoelectrophoresis and normally at about 10 mg./ml. for other purposes; the solutions were centrifuged before use and the supernatants obtained are referred to as ultrasonic extracts.

Immunological methods. Unless otherwise stated, procedures followed those of Simkin & Jamieson (1967a). To compare material present in ultrasonic extracts with substances of different electrophoretic mobility in fraction I or serum, 3 mm.-diameter circles of agar gel were removed after electrophoresis and transferred to wells of similar size for immunological analysis. The precipitin technique used was normally scaled up 1.5–2-fold as compared with that of Simkin & Jamieson (1967a) and precipitation with human fraction I-anti-(human fraction I) carried out only once. For chemical analysis, precipitates were dissolved in 0.12–0.25 ml. of 25 mM-H₂SO₄. Samples were removed for determination of protein by a micro-modification of the method of Lowry, Rosebrough, Farr & Randall (1951); bovine serum albumin was used as standard. The remaining solution was heated at 90° for 60 min. and analysed for

sialic acid by a scaled-down version of the method of Aminoff (1961); *N*-acetylneuraminic acid was used as standard.

Investigation of possible degradation of fraction I on exposure to liver homogenate or subfractions. To test for release of sialic acid, mixtures (total volume 1.0 ml.) containing 0.8 ml. of microsome suspension (about 6 mg. of protein) treated with ultrasonic vibrations (see above), 60 μ g. of fraction I and either 50 mM-tris-42 mM-HCl buffer, pH 7.4, or 109 mM-sodium acetate-13 mM-acetic acid buffer, pH 5.5, were incubated for 90 min. at 37°. Mixtures were then centrifuged for 30 min. at 135 000 *g*_{av} at 2°. The supernatants were fractionated at 2° on 0.8 cm. $\times$ 6 cm. columns of Dowex 2 (formate form). The columns were eluted with 15 ml. volumes of water and 0.3 N-formic acid. The formic acid eluates were evaporated to dryness *in vacuo*, and residues were assayed for sialic acid as described above; spectra were determined with a Unicam SP.800 spectrophotometer. To test for release of sialic acid-containing oligosaccharides, *N*-formic acid was used in place of 0.3 N-formic acid, and the residues of the eluates were heated with 25 mM-H₂SO₄ at 90° for 60 min. before assay.

To test for changes in electrophoretic behaviour of components of fraction I occurring: (a) during preparation of ultrasonic extracts, fraction I (about 1–10 μ g./mg. of microsome protein) or guinea-pig serum (about 0.5 μ l./mg. of microsome protein) was added to microsome suspensions before treatment with ultrasonic vibrations; subsequent procedures in the preparation of ultrasonic extracts were as described above; (b) during early stages of subcellular fractionation, fraction I was added to liver homogenate at about 250 μ g./ml. or to microsome-cell-sap fraction at 230 or 350 μ g./ml.; cell sap was subsequently isolated by procedure (1) of Simkin & Jamieson (1967a) and was concentrated about fourfold by ultrafiltration before examination. Similar procedures were carried out with human α -globulin-containing fraction, which was added at about 1.5 μ g./mg. of protein to a microsome suspension before ultrasonic treatment, or at about 200 μ g./ml. to liver homogenate or microsome-cell-sap fraction.

RESULTS

Except where noted, anti-(fraction I_b) was used in the investigations described.

Immunoelectrophoresis of ultrasonic extracts of liver microsome material. Lubrol extracts of liver microsome material used for double-diffusion analysis in previous work (Simkin & Jamieson, 1967a) gave considerable non-specific precipitation on immunoelectrophoresis. Use was thus made in the present studies of ultrasonic extracts of microsomes, which show this disadvantage much less but react in a qualitatively similar way to Lubrol extracts on double-diffusion analysis. It was shown by means of sequential extraction that ultrasonic vibrations liberate a large proportion of the material that can be extracted by direct addition of Lubrol to microsomes.

On immunoelectrophoresis of ultrasonic extracts in conjunction with anti-(fraction I_b) or anti-(fraction I_{b2}) (Plates 1 and 2), a precipitin line

EXPLANATION OF PLATE 1

Immunoelectrophoresis (diethylbarbiturate-acetate buffer, pH 8.6) in conjunction with anti-(fraction I_b) of: US, ultrasonic extract of microsome material from guinea-pig liver; I, fraction I; US_R and US_S, ultrasonic extracts of rough-membrane and Mg²⁺-free smooth-membrane subfractions respectively of guinea-pig liver microsome material. Photographs were taken towards end of development of precipitin lines.

EXPLANATION OF PLATE 2

Immunoelectrophoresis in conjunction with anti-(fraction I_{b2}) of: US_g and US_a, ultrasonic extracts of guinea-pig liver microsome material isolated after 15 min. labelling *in vivo* with [¹⁴C]glucosamine or [¹⁴C]leucine respectively; I, fraction I. (a) and (c), Gels stained for protein; (b) and (d), corresponding radioautographs.

formed that corresponded to a substance with electrophoretic mobility similar to the principal components of fraction I; it was sometimes accompanied by another, faint, line. Most of the material precipitated consisted, however, of components with electrophoretic mobilities slower than those of components of fraction I. The principal precipitin line formed on the positive side of the origin and was accompanied by one or two minor lines. With anti-(fraction I_b) (Plate 1), a precipitin line was also seen on the negative side of the origin.

The precipitin lines, formed by components of ultrasonic extracts with electrophoretic mobility similar to the main components of fraction I, interacted with certain of the precipitin lines formed by components of slower mobility. This was observed particularly after short periods of electrophoresis or after addition of fraction I or serum to extracts. To investigate more precisely the immunological correspondence between components of ultrasonic extracts and those of fraction I or serum, circles of gel were removed after electrophoresis of ultrasonic extracts, fraction I or serum. Substances of similar and different electrophoretic mobility were compared by double-diffusion analysis. The major precipitin line, formed by a circle removed after electrophoresis of ultrasonic extracts from the region of maximum precipitation determined by conventional immunoelectrophoresis, showed a reaction of immunological identity with a line formed by circles removed from areas of fraction I or serum containing their principal components. Circles taken from fraction I or serum at a corresponding position to this circle from ultrasonic extracts did not, however, form a precipitin line. Though other precipitin lines formed by circles of gel taken from ultrasonic extracts were found to interact with lines formed by circles from fraction I or serum, precise immunological correspondence could not be established in these instances because of faintness of lines and differences in concentration between tissue and serum constituents.

The electrophoretic mobility of components of fraction I or serum was not altered by treatment with ultrasonic vibrations and subsequent procedures in the preparation of ultrasonic extracts.

When ultrasonic extracts were examined, either separately or as a mixture with fraction I from which sialic acid had been removed by hydrolysis with acid, the electrophoretic mobilities of the principal components of the ultrasonic extracts were similar to those of the principal components of sialic acid-free fraction I. Treatment with acid was used for removal of sialic acid because the latter could not be removed completely from fraction I by means of neuraminidase (cf. Simkin *et al.* 1964b).

When radioautography was applied after im-

munoelectrophoresis of ultrasonic extracts of microsome material, isolated after labelling for 15 min. *in vivo* with either L-[U-¹⁴C]leucine or D-[1-¹⁴C]glucosamine (Plate 2) or after incubation of a cell-free microsome-cell-sap system with the former, all but certain minor precipitin lines showed radioactivity. After exposure to amino acid, the precipitin lines of fastest electrophoretic mobility showed lower specific radioactivities than lines formed by slower components. After labelling with glucosamine, however, the specific activities of faster and slower precipitin lines were similar. In control experiments similar to those of Simkin & Jamieson (1967a), the precipitates formed when mixtures of labelled ultrasonic extract and human fraction I were subjected to immunoelectrophoresis in conjunction with anti-(human fraction I) showed little or no radioactivity.

The presence in ultrasonic extracts of liver microsomes of substances that interact immunologically with serum components of faster electrophoretic mobility could be explained by: (1) the microsome-bound substances lack sialic acid residues; the latter occupy terminal non-reducing positions in the prosthetic groups of components of fraction I (Simkin, Seshadri & Skinner, 1964a; Simkin *et al.* 1964b) and a deficiency of them would lower electrophoretic mobility at pH 8.6; (2) the substances form a complex with other material and are thereby altered in electrophoretic mobility.

Analysis of precipitates formed on reaction of microsome extracts with anti-(fraction I_b). If the microsome-bound substances lack sialic acid residues, precipitates formed when microsome extracts are allowed to react with rabbit anti-(fraction I_b) should show a lower sialic acid/protein ratio than precipitates formed by fraction I or guinea-pig serum. As predicted on the basis of published data (e.g. Press & Porter, 1966) on the carbohydrate composition of rabbit γ -globulins, precipitates formed by sialic acid-free fraction I have sialic acid/protein ratios about half of those of precipitates formed by fraction I or serum (Fig. 1). Further, ratios similar to those obtained with sialic acid-free fraction I were found with precipitates formed on reaction of anti-(guinea-pig serum albumin) with guinea-pig serum albumin, which contains little or no carbohydrate. Precipitates formed by either ultrasonic or Lubrol extracts of microsomes, when allowed to react with anti-(fraction I_b), showed sialic acid/protein ratios similar to those of precipitates formed by sialic acid-free fraction I at approximately comparable levels of precipitation (Fig. 1). In some experiments, a wider range of precipitation was investigated than shown in Fig. 1. Sialic acid/protein ratios of precipitates formed by mixtures of ultrasonic extract and fraction I or serum were intermediate

Fig. 1. Sialic acid/protein ratios (*a* and *b*) and protein contents (*c* and *d*) of immune precipitates isolated after reaction between anti-(fraction I_b) and: fraction I (○); sialic acid-free fraction I (△); guinea-pig serum (□); ultrasonic extract of guinea-pig liver microsome material (●); and Lubrol extract of liver microsome material (▲).

between those obtained for either component alone. The sialic acid/protein ratios of precipitates formed by fraction I or serum were not altered by application of the procedures involved in preparation of ultrasonic extracts.

Chromatography of ultrasonic extracts of microsome material on Sephadex G-150. The results described above eliminate the possibility that differences in electrophoretic mobility between microsome-bound and serum constituents are due to complex-formation with material of low molecular weight, as this phenomenon would not alter the sialic acid/protein ratio of immune precipitates. The possibility that complex-formation with material of high molecular weight takes place (cf. Roussel, Sautière, Havez & Biserte, 1965) is not, however, eliminated, but evidence that this is unlikely was provided by the results of chromatography of ultrasonic extracts on Sephadex G-150 (Fig. 2). As only fraction (c) (Fig. 2) contained appreciable material that reacted with antiserum on immunoelectrophoresis, most of the reactive material was eluted at a volume similar to or slightly greater than that required for the principal

Fig. 2. Chromatography at 2° of ultrasonic extract (20 mg. of freeze-dried material) of guinea-pig liver microsome material on a 1.1 cm. × 130 cm. column of Sephadex G-150; the eluent was 100 mM-phosphate buffer, pH 6.2. Fractions were pooled as indicated, dialysed against water, centrifuged and freeze-dried for examination by immunoelectrophoresis. Arrows indicate elution volumes of: I, principal components of fraction I; G, bovine γ-globulin (IgG); A, bovine serum albumin; O, ovalbumin.

components of fraction I. Moreover, the components forming almost all of this material showed the characteristic slow electrophoretic mobilities of components of unfractionated ultrasonic extracts.

Studies on possible degradation of fraction I brought about by exposure to liver homogenate or subfractions. The finding that most of the immunologically reactive material present in ultrasonic extracts lacks sialic acid residues could be explained by the molecules in question either being precursors of completed glycoprotein molecules found in serum, or being present as a result of degradative processes. The latter possibility was investigated in two ways.

(1) Microsome material treated with ultrasonic vibrations was incubated with fraction I at either pH 7.4, the pH value existing during ultrasonic treatment, or pH 5.5, a value approximating to that existing during dialysis of ultrasonic extracts and to the optimum pH of mammalian neuraminidases (Warren & Spearing, 1960; Taha & Carubelli, 1967). The liberation of sialic acid was investigated by the periodate-thiobarbituric acid method of Aminoff (1961), but, because of small amounts of interfering material from microsomes, the spectra of butanol extracts were determined rather than simple measurement of extinction at 549 mμ. No liberation of sialic acid or sialic acid-containing oligosaccharides was found; the liberation of about 20% of the sialic acid of the fraction I added could have been detected. Controls to test for *N*-acetylneuraminatase-pyruvate lyase (EC 4.1.3.3) activity, which is present in guinea-pig liver (Brunetti,

Jourdan & Roseman, 1962), indicated that appreciable sialic acid was not destroyed under the conditions used. Addition of neuraminidase from *Vibrio comma* (at 25 units/ml. in the presence of 2.5 mM-calcium chloride) to microsome suspensions at pH 5.5 caused a very large increase in the value obtained for liberation of sialic acid in the absence of added fraction I. No significant increase in this value was encountered in the absence of neuraminidase.

(2) Fraction I or guinea-pig serum was added at various concentrations to microsome suspensions before treatment with ultrasonic vibrations. Ultrasonic extracts were examined by immunoelectrophoresis for an increase in components of slower electrophoretic mobility than constituents of fraction I. Little evidence for such an increase was obtained. It is difficult, however, to estimate components by this technique and to achieve precise reproduction of ultrasonic treatment. Therefore, in one experiment an α -globulin-containing fraction isolated from human serum, which contained orosomucoid as a prominent constituent, was added to microsomes before ultrasonic treatment. On immunoelectrophoresis, in conjunction with anti-(human fraction I), the ultrasonic extracts showed no precipitin lines with mobilities slower than those of the glycoproteins added. Similar negative results were obtained when degradation at early stages in subcellular fractionation was investigated by addition of fraction I or human α -globulin fraction to liver homogenate or microsome-cell-sap fraction and subsequent examination of cell sap isolated therefrom. Control experiments, in which the fraction I added was replaced by a similar concentration of sialic acid-free fraction I, showed that appreciable conversion into sialic acid-deficient molecules could have been detected.

Examination of subfractions of microsome material. When ultrasonic extracts of Mg^{2+} -free smooth-membrane and rough-membrane subfractions of microsome material, prepared by the method of Dallner (1963), were examined by immunoelectrophoresis (Plate 1), extracts of smooth subfractions showed a marked increase in the proportion of material present with an electrophoretic mobility similar to that of the principal components of fraction I in comparison with unfractionated microsomes, whereas extracts of rough subfractions showed a relative decrease of such material. These findings were not changed by perfusion of a liver with saline and sucrose before subcellular fractionation. The sialic acid/protein ratios of immune precipitates, formed by ultrasonic extracts of subfractions, were determined in one experiment (numbers of values and ranges given in parentheses): the mean value of 3.5 (4, 3.0–3.9) obtained for the Mg^{2+} -free smooth-membrane sub-

fraction was higher than the mean values of 2.5 (3, 2.4–2.6) and 2.3 (3, 2.1–2.5) found for the corresponding unfractionated microsome material (cf. also Fig. 1) and rough-membrane subfraction respectively.

DISCUSSION

Previous work showed that substances associated with the microsome fraction of liver are involved in the biosynthesis of components of fraction I present in serum (Simkin & Jamieson, 1967a). Although the present work confirms that at least most of the microsome-bound components interact immunologically with constituents of serum, most of these components showed electrophoretic mobilities lower than those of the latter but similar to those of components of sialic acid-free fraction I. Chemical analysis of immune precipitates provides strong evidence that the substances of slow mobility lack sialic acid residues. Other evidence consistent with this view was provided by the failure to recover appreciable radioactivity in carrier fraction I after addition to microsomes, labelled *in vivo* with [^{14}C]leucine, and isolation by ion-exchange chromatography after liberation of microsome-bound material with deoxycholate (J. L. Simkin & J. C. Jamieson, unpublished work).

Evidence has been presented that suggests that degradative reactions are not the main factor in the production of sialic acid-deficient components of fraction I. Indirect evidence supporting this view includes the finding that liver microsomes readily released sialic acid on treatment with a bacterial neuraminidase but not in its absence. The occurrence of some degradation cannot, however, be excluded. The concentration of material present in ultrasonic extracts that reacts with antiserum is low (about 1 μg /mg. of microsome protein); therefore, to detect products of degradation, in most experiments the amount of fraction I added was greater (up to tenfold) than the reactive material present in the extracts. It was assumed that relatively large amounts of exogenous fraction I did not inhibit breakdown of endogenous material.

It is postulated that the microsome-bound substances with electrophoretic mobilities similar to those of the principal components of fraction I are completed glycoprotein molecules in the course of secretion. These molecules are present largely in smooth-membrane subfractions, a conclusion supported by the finding that immune precipitates isolated from a smooth-membrane subfraction showed higher sialic acid/protein ratios than precipitates derived from a rough-membrane subfraction.

It is not yet known whether the microsome-bound substances postulated to be precursors of serum glycoproteins lack sugar residues other than sialic

acid. Attempts to isolate material after dissociation of antigen-antibody complexes, or to investigate hexose and hexosamine contents by analysis of immune precipitates, have so far been unsuccessful. Previous studies (Simkin & Jamieson, 1967a) on the degradation of an immune precipitate isolated from microsome extracts after administration of [^{14}C]-glucosamine do, however, suggest that at least an appreciable proportion of the substances that react with antiserum have relatively large prosthetic groups.

As precipitin lines formed by Lubrol extracts of both smooth- and rough-membrane subfractions of microsome material show radioactivity after labelling *in vivo* with glucosamine (Simkin & Jamieson, 1967a) or leucine (J. L. Simkin & J. C. Jamieson, unpublished work), both subfractions contain components with at least partially complete prosthetic groups. A preliminary experiment indicated that sialic acid accounts for 20% or less of the total radioactivity of immune precipitates isolated from microsome extracts after labelling with glucosamine. This would be expected to be preferentially present in smooth-membrane subfractions.

The present work, together with that previously reported (Simkin & Jamieson, 1967a), suggests that the microsome-bound substances are at least predominantly contained within vesicles enclosed by membranous structures, since they can be liberated in very large measure by treatment with ultrasonic vibrations (e.g. Decken & Campbell, 1964; Redman & Sabatini, 1966). The results obtained support the view that precursors of components of serum fraction I that contain at least some *N*-acetylglucosamine residues are present within vesicles enclosed by rough-surfaced endoplasmic reticulum, and that components bearing completed prosthetic groups are found within vesicles present in the smooth-membrane microsome subfraction. The results can be explained by a process in which polypeptide chains, possibly bearing initial *N*-acetylglucosamine residues (Lawford & Schachter, 1966), pass from ribosomes into the cisternae of the rough-surfaced endoplasmic reticulum (Redman & Sabatini, 1966), and while they are present successively in the rough-surfaced and smooth-surfaced endoplasmic reticulum (Peters, 1962) acquire sugar residues (e.g. Molnar, Robinson & Winzler, 1965; Sarcione, Bohne & Leahy, 1964). The terminal sialic acid residues are probably added in association with a component of the smooth-membrane microsome subfraction. Although other interpretations are possible, the differential labelling of complete and incomplete molecules found after administration of amino acid, but not glucosamine, supports such a mechanism of synthesis.

The results described are supported by the findings of other workers, in particular O'Brien Canady, Hall & Neufeld (1966) with respect to the occurrence of sialic acid-deficient glycoproteins in liver microsomes, and Lawford & Schachter (1966) with respect to the subcellular localization of components involved in the biosynthesis of glycoproteins.

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