EXPERIENTIA

Vol. 32 – Fasc. 2 Pag. 135 – 268 15. 2. 1976

## GENERALIA

# The Role of Sialic Acid in Determining the Life-Span of Circulating Cells and Glycoproteins

by V. Bocci*

*Istituto di Fisiologia Generale, Università di Siena, I–53100 Siena (Italy).*

*Summary* The role of sialic acid in determining the life of circulating cells and glycoproteins is reviewed and an attempt is made to assess its real importance and to interpret the desialylation pathway. An overall view of factors, including desialylated glycoprotein fragments, that may regulate hepatic synthesis of glycoproteins is presented

Recently much attention[1–3] has been focused on the role played by sialic acid present as a terminal sugar in the carbohydrate chain of free or membrane-bound glycoproteins. The aim of this review is to weigh the available experimental evidence for the effective role of sialic acid, to evaluate probable sites of desialylation and to speculate whether asialoglycoproteins or their fragments, after being selectively bound by hepatocytes[4], exert a regulatory activity on glycoprotein synthesis.

Some years ago the possibility was considered[5] that the decaying cell metabolism, as well as external factors, could be responsible for deterioration of the cell membrane and for the removal of erythrocytes from the circulation. It was envisaged that aged erythrocytes have a reduced surface charge mostly due to the action of plasma or tissue sialidase. The hypothesis has been further substantiated[6] accelerating the loss of sialic acid from the membrane of erythrocytes by means of a brief period of stagnation of the splenic and renal circulation in vivo and by showing a much shorter half-life of extensively desialylated erythrocytes. Lower sialic acid and N-acetylgalactosamine content has also been found in the old human red cells[7]. Moreover earlier[8,9] and more recent results confirm that the survival of influenza virus neuraminidase-treated[10,11] and natural sialic acid deficient[12] erythrocytes is considerably shortened.

The loss of sialic acid is unlikely to be the only cause by which these anucleated cells are removed from the circulation; progressive desialylation is not restricted to erythrocytes and may represent a general mechanism to ease, at least in part, elimination of senescent platelets[13,14], erythroid nuclei[15] and perhaps granulocytes and spermatozoa. Moreover it has the effect of altering the fate and distribution of desialylated thymocytes[16] and lymphocytes[17] although once these nucleated cells have regenerated their membrane, they may leave the liver again to follow their normal circulatory pathways. The enhanced hepatotropism of these neuraminidase-treated cells indicates that their membrane glycoproteins, like plasma asialoglycoproteins, have high affinity for a hepatic binding protein[18,19].

* Acknowledgments. The author wishes to thank Drs. A. Viti and G. P. Pessina for helpful discussion and comments.

1 S. Roseman, Chem. Phys. Lipids, *5*, 270 (1970).
2 P. J. Winterburn and C. F. Phelps, Nature, Lond. *236*, 147 (1972).
3 G. Ashwell and A. G. Morell, in *Advances in Enzymology* (Ed. A. Meister; J. Wiley New York 1974), vol. 41, p. 99.
4 W. E. Pricer jr. and G. Ashwell, J. biol. Chem. *246*, 4825 (1971).
5 V. Bocci, Experientia *24*, 626 (1968).
6 V. Bocci, G. P. Pessina and T. Conti, *Protides of the Biological Fluids*, 21st Colloq. (Ed. H. Peeters; Pergamon Press Oxford 1973), p. 49.
7 C. Balduini, C. L. Balduini, A. A. Castellani and E. Storti, Boll. Soc. ital. Biol. sper. *48*, Abstract 65 (1972).
8 W. B. Stewart, C. W. Petenyi and H. M. Rose, Blood *10*, 228 (1955).
9 E. Gardner jr., C.-S. Wright and B. Z. Williams, J. Lab. clin. Med. *58*, 743 (1961).
10 J. Jancik and R. Schauer, Hoppe-Seyler's Z. physiol. Chem. *355*, 395 (1974).
11 J. R. Durocher, R. C. Payne and M. E. Conrad, Blood *45*, 11 (1975).
12 P. Lalezari and H. Mendhiry, Br. J. Haemat. *25*, 399 (1973).
13 S.-I. Choi, J. V. Simone and L. J. Journey, Br. J. Haemat. *22*, 93 (1972).
14 K. A. Grottum and M. Jeremic, Thromb. Diath. haemorrh. *29*, 461 (1973).
15 E. Skutelsky and D. Danon, J. Cell Biol. *43*, 8 (1969).
16 S. N. Berney and B. M. Gesner, Immunology *18*, 681 (1970).
17 J. J. Woodruff and B. M. Gesner, J. exp. Med. *129*, 551 (1969).
18 R. L. Hudgin, W. E. Pricer jr., G. Ashwell, R. J. Stockert and A. G. Morell, J. biol. Chem. *249*, 5536 (1974).
19 R. J. Stockert, A. G. Morell and I. H. Scheinberg, Science *186*, 365 (1974).

The hypothesis may be applied also to tissue cells: stationary cells are probably undergoing continuous desialylation and regeneration of the membrane (or resialylation). For perennial cells this situation may remain in a steady state condition for years, while for shorter lived cells the rate of resialylation eventually slows down to a point when cells have lost a great part of their membrane charge and become susceptible to be phagocytized by surrounding fixed or mobile reticuloendothelial cells.

As far as plasma glycoproteins are concerned, MORELL et al.[20] revealed that enzymatic removal of sialic acid from ceruloplasmin resulted in a protein that was rapidly disappearing from the circulation while accumulating at the same time within the hepatocytes, and being catabolized in the lysosomes[21]. It was further shown[22] that removal of any two sialic residues from ceruloplasmin (or exposure of no more than two galactosyl residues) was sufficient for the liver to recognize and bind the defective protein; removal or alteration of galactose resulted in a serum survival time almost identical with that of native protein[20].

These observations have been extended[23] to the asialoderivatives of orosomucoid, fetuin, haptoglobin, $\alpha_2$-macroglobulin, human chorionic gonadotropin, follicle-stimulating hormone[24], prothrombin[25], thyroxine-binding globulin[26], erythropoietin[27] and transcortin[28]. This list is likely to increase with time since, with very few notable exceptions, most circulating proteins have carbohydrates including sialic acid.

It has been known for some time that glycoproteins, after treatment with neuraminidase, have their isoelectric point raised, their electrophoretic mobility reduced, while their function, at least in vitro, remains unaltered. It appears that one role[2] of sialic acid is to mask the adjacent galactosyl residues thus preventing recognition by the liver. It also implies that catabolism of glycoproteins is a two-step process, the first one being removal of sialic acid and the second being hepatic uptake.

The question is whether catabolism of all plasma glycoproteins can be interpreted by this biphasic process. Experimental control of this new concept is not easy, mainly because the injected asialoglycoproteins have a very short half-life and probably most of them are cleared by a single passage through the liver. On this basis and because the liver is probably a secondary desialylating organ, available data on catabolism of some plasma protein by using this isolated organ need to be freshly evaluated, and it appears useless to test the clearance of a native (desialyzable in vivo) protein using the isolated and perfused liver.

There are some examples worth mentioning that seem to exclude desialylation as a main and generalized first step in glycoprotein catabolism.

The fractional catabolic rate of asialotransferrin is only slightly higher than that of normal transferrin[20,23,29]; further work by WONG et al.[30] has shown that little, if any, desialylation of transferrin occurs in vivo, although human asialotransferrin which has exposed terminal galactose residues, is rapidly eliminated from the circulation of heterologous hosts[29,31]. The only hint of desialylated-like transferrin[32] is in the cerebrospinal fluid (CSF), where a slow iron-binding globulin (or $\tau$ fraction[33]) can be demonstrated, composing about 15% of the whole CSF transferrin[34]. As yet the exact chemical composition and origin of the slow transferrin remain undecided: it could be synthesized by the choroid plexus as an incomplete transferrin and it could be derived from the faster transferrin of hepatic origin after desialylation or partial proteolytic degradation[35] by a neuraminidase or cathepsin-like enzyme. This problem is being investigated in our laboratory[36].

As discussed at length in previous papers[37,38], intravascular hemolysis in mammals yields an amount of hemoglobin similar to that of haptoglobin consumed daily, so that most of the haptoglobin breakdown can be accounted for by formation of a complex with hemoglobin that is taken up and digested by the Kupffer cells[39–41]. I like to recall that the late FREE-

[20] A. G. MORELL, R. A. IRVINE, I. STERNLIEB, I. H. SCHEINBERG and G. ASHWELL, J. biol. Chem. *243*, 155 (1968).
[21] G. GREGORIADIS, A. G. MORELL, I. STERNLIEB and I. H. SCHEINBERG, J. biol. Chem. *245*, 5833 (1970).
[22] C. J. A. VAN DEN HAMER, A. G. MORELL, I. H. SCHEINBERG, T. HICKMAN and G. ASHWELL, J. biol. Chem. *245*, 4397 (1970).
[23] A. G. MORELL, G. GREGORIADIS, I. H. SCHEINBERG, J. HICKMAN and G. ASHWELL, J. biol. Chem. *246*, 1461 (1971).
[24] W. H. YANG and H. PAPKOFF, Fert. Steril. *24*, 633 (1973).
[25] G. L. NELSESTUEN and J. W. SUTTIE, Biochem. biophys. Res. Commun. *45*, 198 (1971).
[26] J. S. MARSHALL, A. M. GREEN, J. PENSKY, S. WILLIAMS, A. ZINN and D. M. CARLSON, J. clin. Invest. *54*, 555 (1974).
[27] D. W. BRIGGS, J. W. FISHER and W. J. GEORGE, Am. J. Physiol. *227*, 1385 (1974).
[28] H. VAN BAELEN and G. MANNAERTS, Arch. Biochem. Biophys. *163*, 53 (1974).
[29] E. REGOECZI, M. W. C. HATTON and K.-L. WONG, Can. J. Biochem. *52*, 155 (1974).
[30] K.-L. WONG, P. A. CHARLWOOD, M. W. C. HATTON and E. REGOECZI, Clin. Sc. molec. Med. *46*, 763 (1974).
[31] M. W. C. HATTON, E. REGOECZI and K.-L. WONG, Can. J. Biochem. *52*, 845 (1974).
[32] W. C. PARKER and A. G. BEARN, J. exp. Med. *115*, 83 (1962).
[33] T. BÜCHER, D. MATZELT and D. PETTE, Klin. Wschr. *30*, 325 (1952).
[34] E. C. LATERRE, Ricerca Clin. Lab. *3*, 736 (1973).
[35] J. CLAUSEN, T. MUNKNER, Nature, Lond. *189*, 60 (1961).
[36] G. P. PESSINA, L. PAULESU, P. PESSINA and V. BOCCI, Boll. Soc. ital. Biol. sper. *51*, 75 (1975).
[37] V. BOCCI, Archo Fisiol. *67*, 314 (1970).
[38] R. J. WINZLER and V. BOCCI, *Glycoproteins*, Part B (Ed. A. GOTTSCHALK (Elsevier, Amsterdam 1972), p. 1228.
[39] R. K. MURRAY, G. E. CONNELL and J. H. PERT, Blood *17*, 45 (1961).
[40] R. ENGLER, J. MORETTI and M. F. JAYLE, Bull. Soc. Chim. biol. *49*, 263 (1967).
[41] T. WADA, H. OHARA, K. WATANABE, H. KINOSHITA and H. NISHIO, J. reticuloendoth. Soc. *8*, 185 (1970).

MANN[42], very imaginatively, classified haptoglobin as a suicidal protein and there is therefore little ground to think that desialylation of haptoglobin could be a significant preliminary step in its catabolism.

The fibrinolytic and fibrinogenolytic pathways[43–45] appear to be independent of the desialylation process, at least down to fragments D and E. Rabbit fragments D appear to contain sialic acid as their electrophoretic mobility is reduced after neuraminidase treatment[46]. Desialylation of fragments as a prerequisite of liver uptake could be an important step if, according to the assumption discussed later on, they have a role in the regulation of fibrinogen synthesis.

Desialylated immunoglobulin G, like transferrin and fibrinogen, do not display a shorter half-life than native protein, and for this reason have been indicated[3] as examples of 'refractory' glycoproteins. MARSHALL et al.[26] have suggested that the removal of sialic acid from immunoglobulin may unmask an antigenic site (recalling the hypothesis of BEZKOROVAINY[47]), to which autoantibodies may be produced. In this sense, the hepatic uptake of desialylated glycoproteins is viewed as a significant protective mechanism.

Ceruloplasmin has oxidase activity and plays a role in transporting copper, and perhaps in regulating intestinal absorption of the metal. Conversion to apoceruloplasmin may represent a first significant step towards catabolism as, in contrast to apotransferrin, circulating apoceruloplasmin cannot bind copper again and is eliminated more rapidly than the holoprotein from the circulation[48–50]. It is in fact well-known[51,52] that metal-proteins are more resistant to enzymatic proteolysis than corresponding apoproteins. An interesting possibility suggested by MORELL et al.[20] is that in Wilson's disease, a defective or absent sialyltransferase, rather than desialylation, may be responsible for the synthesis of asialoceruloplasmin incapable of surviving in the circulation.

$\alpha_2$-Macroglobulin is known to bind and hence to diminish the proteolytic activity of a number of enzymes, and its physiological importance[53] may reside in regulating hemostatic and inflammatory reactions. It could also bind and inactivate sialidase and the same possibility applies to an even more effective enzyme inhibitor such as $\alpha_1$-antitrypsin.

Orosomucoid, which has a sialic acid content of 12.1%[54], may be the most susceptible protein to desialylation, but the quantitative importance of this process for it and other glycoproteins remains uncertain. At present the evidence that traces of asialoglycoproteins may exist in vivo is indirect and based upon the ability of serum to compete with the binding of a purified labelled asialoglycoprotein by the liver plasma membrane preparation (inhibition assay system of VAN LENTEN and ASHWELL[55]). The finding[26] that sera from cirrhotic patients have increased levels of asialoglycoproteins does not necessarily mean that the desialylation process is really occurring. About 10 years ago, SCHMID et al.[56] showed that, in various chronic diseases, human orosomucoid lacks sialic acid up to 50% of the normal value. Whether this microheterogeneity is due to a defective sialyltransferase, or to non-availability of sugar nucleotides, or to partial failure in adding up the terminal sugar residues in case of increased synthesis (as it happens in chronic diseases) is not known. Therefore several possibilities have to be taken into account: 1. decreased hepatic uptake caused by alteration of the plasma membrane in liver diseases; 2. increased peripheral desialylation; 3. altered hepatic synthesis with release of normal and uncompleted glycoproteins and 4. a combination of the former conditions. With some limitations these possibilities can be tested experimentally.

Assuming that some circulating glycoproteins are continuously desialylated, what are the probable sites and mechanisms of desialylation?

Sialidase is an ubiquitous enzyme that has been located in mammary gland[57], liver and kidney[58], brain[59], intestinal mucosa[60], spleen[57,61], bone marrow[62], leucocytes[63], testis[57], sperm[64], corpus luteum[65] and

[42] T. FREEMAN, *Protides of the Biological Fluids*, 15 Colloq. (Ed. H. PEETERS; Elsevier, Amsterdam 1968), p. 1.
[43] L. MESTER, in *Fibrinogen* (Ed. K. LAKI; Dekker, New York 1968), p. 165.
[44] M. W. MOSESSON, Thromb. Res. *2*, 185 (1973).
[45] E. REGOECZI and K.-L. WONG, *Protein Turnover*, Ciba Foundation Symposium 9 (ASP, Amsterdam 1973), p. 181.
[46] G. P. PESSINA, T. CONTI, A. PACINI and V. BOCCI, Ital. J. Biochem. *21*, 155 (1972).
[47] A. BEZKOROVAINY, Physiol. Chem. Phys. *4*, 492 (1972).
[48] R. J. CARRICO, H. F. DEUTSCH, H. BEINERT and W. H. ORME-JOHNSON, J. biol. Chem. *244*, 4141 (1969).
[49] N. A. HOLTZMAN and B. M. GAUMNITZ, J. biol. Chem. *245*, 2350 (1970).
[50] N. A. HOLTZMAN and B. M. GAUMNITZ, J. biol Chem. *245*, 2354 (1970).
[51] P. R. AZARI and R. E. FEENEY, J. biol. Chem. *232*, 293 (1958).
[52] J. W. DRYSDALE and H. N. MUNRO, J. biol. Chem. *241*, 3630 (1966).
[53] H. RINDERKNECHT and M. C. GEOKAS, Biochim. biophys. Acta *295*, 233 (1973).
[54] N. HEIMBURGER, K. HEIDE, H. HAUPT and H. E. SCHULTZE, Clin. chim. Acta *10*, 293 (1964).
[55] L. VAN LENTEN and G. ASHWELL, J. biol. Chem. *247*, 4633 (1972).
[56] K. SCHMID, J. F. BURKE, M. DEBRAY-SACHS and K. TOKITA, Nature, Lond. *204*, 75 (1964).
[57] R. CARUBELLI, R. E. TRUCCO and R. CAPUTTO, Biochim. biophys. Acta *60*, 196 (1962).
[58] S. MAHADEVAN, J. C. NDUAGUBA and A. L. TAPPEL, J. biol. Chem. *242*, 4409 (1967).
[59] R. OHMAN, A. ROSENBERG and L. SVENNERHOLM, Biochemistry *9*, 3774 (1970).
[60] N. K. GHOSH, L. KOTOWITZ and W. H. FISHMAN, Biochim. biophys. Acta *167*, 201 (1968).
[61] G. P. PESSINA and V. BOCCI, Boll. Soc. ital. Biol. sper. *50*, 1201 (1974).
[62] G. P. PESSINA and V. BOCCI, Boll. Soc. ital. Biol. sper. *50*, 1194 (1974).
[63] W. GIELEN and R. SCHAPER, Hoppe-Seyler's Z. physiol. Chem. *355*, 660 (1974).
[64] P. N. SRIVASTAVA, L. J. D. ZANEVELD and W. L. WILLIAMS, Biochem. biophys. Res. Commun. *39*, 575 (1970).
[65] V. UNBEHAUN, Hoppe Seyler's Z. physiol. Chem. *351*, 705 (1970).

choroidal plexus[36]; in most cases it is present as a 'particulate' enzyme with a low pH optimum (4.0–4.5) and as a 'soluble' enzyme in the cytosol that exhibits pH optima that most frequently range from pH 5.5 to pH 6, but displays activity up to neutral pH. As a contrast, in fresh serum there is no sialidase activity[26], although the enzyme has been found in Cohn Fraction VI[66], thus suggesting that even in normal plasma, there might be some trace activity although undetectable with current assay. It is probable that plasma sialidase, having a pH optimum at 5.5 and being very diluted, will be practically ineffective at physiological pH.

An explanation of the process and sites of desialylation may be found in considering that the internal environment may not be absolutely constant everywhere because, in certain areas, the circulation is at times either sluggish, or at a standstill. There is evidence that cells and plasma can remain transitorily trapped or slowed down inside the vascular system in organs of which the spleen is the most representative[67–69], and in others such as bone marrow[67,68,70,71], and perhaps liver[68], kidney and skin.

When blood is trapped in the venous sinuses of the spleen and some of the plasma is filtered off (plasma skimming of WEISS[72]), the hematocrit value tends to rise and oxygen tension to lower. Hypoxia acts as a lysosomal labilizer and could provoke a sudden release of lysosomal enzymes. In other organs with high lysosomal activity, independently of variation of oxigen tension, sialidase might also be slowly freed by exocytosis into the surrounding fluid. By one or both mechanisms, the concentration of sialidase could rise significantly in certain areas of the vascular system, whereas it could become considerably diluted and ineffective in the general circulation because of: a) mixing in a large pool, b) fast catabolism, c) a hypothetic sialidase binding protein and d) higher pH value. A diagrammatic representation of the hypothetically uneven body-distribution of sialidase is attempted in Figure 1. GREGORIADIS et al.[73] have indeed demonstrated that most of the injected enzyme is removed from the circulation within 5 h. It appears pertinent to note that also the CSF circulation has been found sluggish and uneven[74,75]; a fact to take into account when investigating the origin of the slow transferrin in the CSF.

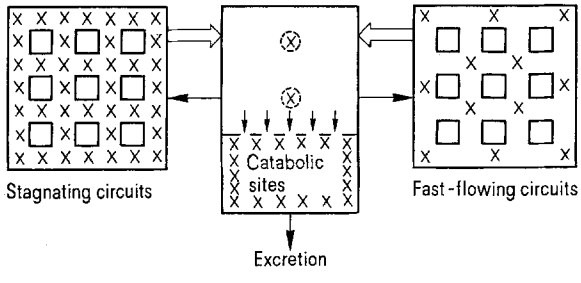


Fig. 1. The left and right compartments include capillary and interstitial circulation while the central one represents the volume of plasma (not in scale) contained in larger vessels. Cells are indicated by the squares. It is presumed that the concentration of sialidase (×) is high in the stagnating circuits, low in the fast-flowing circuits and negligible in the plasma of the large vessels. Catabolic sites (including excretion) are imagined to be in rapid equilibrium with the intravascular pool.

In hypoxic conditions, a local increase of lactic acid can provoke a slight decrease of pH that will favour enzymatic activity. Either for cells or plasma glycoproteins, the desialylation process is thus envisaged to occur in a microenvironment characterized by flow stagnation, high sialidase activity and slightly lower pH; these 'unphysiological' conditions may be very short in some parts of the vascular system (sinusoids of the bone marrow)[70] and longer in the spleen[69] so that, at any one time, the enzymatic activity on the substrates may be limited. However, as sialidase remains bound at the cell surface[76,77], the enzymatic action might continue to go on at a lower rate. By recalling[78] that the number of circulations of erythrocytes in mammals is close to $1.7 \times 10^5$ and by assuming a random circulation, a cell or a glycoprotein have fewer chances to pass through 'stagnating' circuits. Probably not all glycoproteins have the same reactivity towards sialidases: there could be chemical reasons in the sense of different chain terminants, sialic acid content, steric hindrance and biophysical reasons in the sense of different intra-extravascular space distribution and transit time within the extravascular compartment. Moreover endothelial or circulating cells are competing with glycoproteins in binding sialidase.

There are also other possibilities such as the action of membrane-bound sialidase onto the endogenous substrate located on the plasma membrane itself, or onto either circulating cells or glycoproteins transiently attached to the membrane. Glycoproteins could be desialylated also during transcapillary movement or at any time when they are taken up by a cell. It is however difficult to admit that lysosomal digestion of glycoproteins will not proceed beyond the stage of

[66] L. WARREN and C. W. SPEARING, Biochem. biophys. Res. Commun. *3*, 489 (1960).
[67] C. A. OWEN JR., A. L. ORVIS and J. M. KIELY, Am. J. Physiol. *211*, 273 (1966).
[68] J. SORBIE and L. S. VALBERG, Am. J. Physiol. *218*, 647 (1970).
[69] S. H. SONG and A. C. GROOM, Am. J. Physiol. *220*, 779 (1971).
[70] P. F. MARTON, Scand. J. Haemat. *7*, 177 (1970).
[71] P. F. MARTON, Scand. J. Haemat. *10*, 81 (1973).
[72] L. WEISS, Am. J. Anat. *111*, 131 (1962).
[73] G. GREGORIADIS, D. PUTMAN, L. LOUIS and D. NEERUNJUN, Biochem. J. *140*, 323 (1974).
[74] H. F. GRUNDY, J. Physiol. *163*, 457 (1962).
[75] L. E. MCCARTHY and H. L. BORISON, Expl. Neurol. *17*, 57 (1967).
[76] P. MCQUIDDY and J. E. LILIEN, Biochim. biophys. Acat *291*, 774 (1973).
[77] P. SACHTLEBEN, R. GSELL and J. N. MEHRISHI, Vox sang. *25*, 519 (1973).
[78] A. C. ALLISON, Nature, Lond. *188*, 37 (1960).

desialylation. The kidney offers a unique situation for studying this problem but as yet has not permitted a definite conclusion to be drawn[79] as to whether protein molecules below 60,000 MW can cross renal tubular cells and return intact into the circulation.

Ageing of the circulating cells and of some glycoproteins (or of a percentage of it) can be visualized as due at least in part to the progressive loss of some sialic acid residues. Possibly for a period of time, either cells, or glycoproteins do not show any impairment because, as has been established[3,6], the loss of sialic acid is very slow and, up to a certain degree, is not critical for survival.

Desialylated cells and glycoproteins appear to have well defined fates: most of the cells are taken up by reticuloendothelial cells[80,81] concentrated in the bone marrow, spleen and liver and the process of erythrophagocytosis needs no further comment after the authoritative work by KNISELY[82] and BESSIS[83]. Nucleated cells and perhaps also erythrocytes may get stuck to hepatocytes until the former cells have regenerated their membrane or are taken up by Kupffer cells. These and reticuloendothelial cells in general have an undisputed fundamental role[84] in taking up old erythrocytes and denatured proteins. The hepatocytes appear to be the main graveyard for asialoglycoproteins as, after being bound by the hepatic plasma membrane, they are catabolized intracellularly. It is also clear that the hepatocytes, while being unable to take up cells or fragments, play a key role in the uptake and catabolism of plasma hemoglobin[85,86].

The final aspect to consider is desialylation as a process able to feed back components and perhaps instructions to sites of synthesis. At least in man where the bone marrow is the main erythroclastic organ[70], this seems true for old erythrocytes which, while flowing in the slow blood current of the bone marrow sinusoids, are recognized and engulfed by macrophages thus providing materials for local reutilization. Less apparent is the usefulness of blocking desialylated platelets and nucleated cells by the spleen or liver unless the uptake of these cells promotes the origin of marrow-stimulant factors from these organs.

Regulation of synthesis of glycoproteins is poorly understood: the fragmentary experimental evidence barely allows the proposal, as a working hypothesis, of the scheme reported in Figure 2. It is well known that the liver is practically the only site of synthesis of glycoproteins, except immunoglobulins. It appears that desialylation of glycoproteins as a continuous physiological process may occur to a different extent and it is possible that also glycoprotein fragments undergo desialylation and are selectively bound by the hepatic plasma membrane. The fate and usefulness of the desialylated glycoproteins and of the fragments may be quite different: while the former may be extensively broken down in a phagolysosome, thus serving to replenish the intracellular amino acid pool, the latter may be better suited to work as a chemical messenger. It seems that some glycoproteins and fragments are taken up by Kupffer cells[41,87], and a group of authors[88–90] have proposed for fibrinogen that its fragments could influence its own rate of synthesis. Whether this effect, still controversial[91], is mediated by a smaller fragments released after partial digestion

[79] J. E. BOURDEAU and F. A. CARONE, Nephron *13*, 22 (1974).
[80] C.-S. WRIGHT, M. C. DODD, J. A. BASS and N. G. BRANDT, Anat. Rec. *115*, 457 (1953).
[81] A. LEE, Proc. Soc. exp. Biol. Med. *128*, 891 (1968).
[82] M. H. KNISELY, Anat. Rec. *65*, 131 (1936).
[83] M. BESSIS, *Corpuscles* (Springer-Verlag, Berlin 1974).
[84] D. M. BISSELL, L. HAMMAKER and R. SCHMID, J. Cell Biol. *54*, 107 (1972).
[85] S. GOLDFISCHER, A. B. NOVIKOFF, A. ALBALA and L. BIEMPICA, J. Cell Biol. *44*, 513 (1970).
[86] D. M. BISSELL, L. HAMMAKER and R. SCHMID, Blood 40, 812 (1972).
[87] H. GANS and J. T. LOWMAN, Blood *29*, 526 (1967).
[88] M. J. BARNHART, D. C. CRESS, S. M. NOONAN and R. T. WALSH, in *Fibrinogen: Structural, Metabolic and Pathophysiologic Aspects* (Eds. P. A. OWREN, K. M. BRINKHOUS, I. S. WRIGHT, H. R. ROBERTS, S. HINNOM and T. H. KIESSELBACH; F. K. Schattauer Verlag, Stuttgart 1970), p. 143.
[89] V. BOCCI and A. PACINI, Thromb. Diath. haemorrh. *29*, 63 (1973).
[90] V. BOCCI, T. CONTI, M. MUSCETTOLA, A. PACINI and G. P. PESSINA, Thromb. Diath. haemorrh. *31*, 395 (1974).
[91] P. T. OTIS and S. I. RAPAPORT, Proc. Soc. exp. Biol. Med. *144*, 124 (1973).

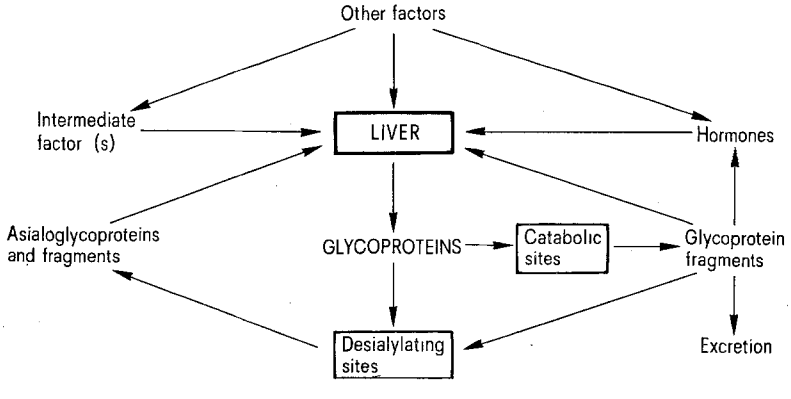


Fig. 2. Probable mechanisms regulating the synthesis of plasma glycoproteins.

by reticuloendothelial cells, or by an asialofragment, or by a major hormonal increase, remains uncertain.

The role of hormones (growth hormone[92], ACTH[93], cortisol[94] alone or associated with insulin[95]) is well documented in vivo and in vitro, but again the role of substances eliciting an increased hormonal response remains uncertain. There is another impressive number of factors, the most significant and similar deriving from leukocytes (leukocytic endogenous mediator, LEM[96–98]; endogenous pyrogen, EP[99,100]; supernatant fraction of DARCY[101]) that also, most likely indirectly, are strongly influencing the synthesis of 'acute phase' glycoproteins.

Thus the regulation of synthesis of glycoproteins can be envisaged at present as regulated in at least two ways: a protein-specific regulatory function could be accomplished by the same protein fragment that, if desialylated, could reach its target more promptly and influence the basal synthesis, while a second aspecific mechanism may variably influence the synthesis in emergency conditions.

[92] K. N. JEEJEEBHOY, A. BRUCE-ROBERTSON, U. SODTKE and M. FOLEY, Biochem. J. *119*, 243 (1970).
[93] A. C. ATENCIO and L. LORAND, Am. J. Physiol. *219*, 1161 (1970).
[94] K. N. JEEJEEBHOY, A. BRUCE-ROBERTSON, J. Ho and U. SODTKE, Biochem. J. *130*, 533 (1972).
[95] E. E. GRIFFIN and L. L. MILLER, J. biol. Chem. *249*, 5062 (1974).
[96] R. S. PEKAREK, R. W. WANNEMACHER JR., F. E. CHAPPLE, M. C. POWANDA and W. R. BEISEL, Proc. Soc. exp. Biol. Med. *141* 643 (1972).
[97] R. F. KAMPSCHMIDT, L. A. PULLIAM and H. F. UPCHURCH, Proc. Soc. exp. Biol. Med. *144*, 882 (1973).
[98] R. F. KAMPSCHMIDT and H. F. UPCHURCH, Proc. Soc. exp. Biol. Med. *146*, 904 (1974).
[99] D. M. MOORE, P. A. MURPHY, P. J. CHESNEY and W. S. WOOD, J. exp. Med. *137*, 1263 (1973).
[100] A. PACINI, G. P. PESSINA, M. MUSCETTOLA and V. BOCCI, Boll. Soc. ital. Biol. sper. *49*, 18, Abstract 78 (1973).
[101] D. A. DARCY, Br. J. exp. Path. *49*, 525 (1968).

# SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

## Roquefortine and Isofumigaclavine A, Metabolites from *Penicillium roqueforti*

P. M. SCOTT[1], MARIE-ANNICK MERRIEN and JUDITH POLONSKY

*Institut de Chimie des Substances Naturelles, C.N.R.S., F–91190 Gif-sur-Yvette (France), 1 September 1975.*

*Summary.* The structures of two metabolites from *Penicillium roqueforti*, designated roquefortine and isofumigaclavine A, have been determined by chemical and spectroscopic studies.

Extraction of mycelium from 2-week-old cultures of *Penicillium roqueforti* (strain CS1) on yeast extract-sucrose medium[2] has yielded 2 crystalline nitrogen-containing metabolites. The major metabolite (20–30 mg/l) is designated roquefortine and assigned structure I on chemical and spectroscopic evidence. The minor substance is a stereoisomer of fumigaclavine A (II)[3].

Isolation of the compounds from chloroform-methanol (9:1, v/v) extracts of lyophilized mycelium was accomplished by partition from ethyl acetate into 0.5 *N* hydrochloric acid followed by addition of ammonium hydroxide and re-extraction with chloroform. The metabolites were separated on a column of Florisil by elution with chloroform-methanol (97:3, v/v).

The more polar product, roquefortine, crystallized from methanol-water as colourless needles, m.p. 195–200° (dec.), $[\alpha]_D^{22} - 703°$ (*c* 1.0, $CHCl_3$). The molecular formula was $C_{22}H_{23}N_5O_2$[4] (found: C, 65.33; H, 6.29, N, 16.87. Calc. for $C_{22}H_{23}N_5O_2 \cdot CH_3OH$: C, 65.54; H, 6.46, N, 16.62%). UV- and IR-spectral properties were $\lambda_{max}$ (95% EtOH) 209 (log $\varepsilon$ 4.47), 240 (log $\varepsilon$ 4.21), and 328 (log $\varepsilon$ 4.43) nm; $\nu_{max}$ ($CHCl_3$) 3430, 3380, 3190, 1685, 1665, and 1608 $cm^{-1}$. The $^{13}C$ NMR-spectrum (off-resonance decoupled)[2] (Table) showed the presence of 2 $CH_3$, 1 $CH_2$, 2 CH, 1 $CH_2$=, 8 CH=, 2 $sp^3$ and 4 $sp^2$ fully substituted C, and 2 C=O groups and accounted for 20 protons. Only 2 of the remaining 3 protons were observed as $D_2O$ exchangeable NH protons in the $^1H$ NMR-spectrum.

Catalytic reduction ($H_2$/Pt/acetic acid) of roquefortine yielded 19,20-dihydroroquefortine, $C_{22}H_{25}N_5O_2$[4], m.p. 185–187°, $[\alpha]_D^{22}$ – 740° (*c* 0.15, $CHCl_3$), whose mass spectrum showed a strong peak at *m/e* 320 (M-71). The suggested isoprene unit in roquefortine (M-69 fragment ion) was shown to be an inverted $\gamma,\gamma$-dimethylallyl group by comparison of the relevant portions of the $^1H$ NMR-spectra of roquefortine (I) and 19,20-dihydroroquefortine

[1] Present address: Health Protection Branch, Health and Welfare Canada, Ottawa, Canada K1A 0L2.

[2] Acknowledgements. We thank S. MOREAU for mycelium of *P. roqueforti* and J.-C. GRIPON and Mlle E. ZISSMANN for additional cultures; B. SEPTE for $^{13}C$ nmr-spectra: S. K. KAN for the 240 MHz $^1H$ nmr-spectrum of roquefortine; Mme LE PHAN DIÊP for amino acid analyses; and S. WILKINSON and P. G. MANTLE, respectively, for samples of fumigaclavine B and agroclavine.

[3] J. F. SPILSBURY and S. WILKINSON, J. chem. Soc. *1961*, 2085.

[4] By high resolution mass spectroscopy, courtesy of P. VARENNE, W. B. TURNER and W. F. MILES. We thank B.C. DAS for helpful discussion.