BIOCHEMISTRY OF THE AMINOSUGARS

KENT AND WHITEHOUSE

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AMINOSUGARS

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FOREWORD

RESEARCH upon biochemistry is no longer a trickle; it is a raging mountain stream. More than forty years ago, I heard the physiologist, W. H. Gaskell, speak in some despair of the growth of physiological literature; in 1925, in the foreword to Levene's monograph, the editors, R. H. Aders Plimmer and F. Gowland Hopkins, justified the separate treatment of specialized parts of biochemistry by the increasing importance of the subject in *their* time. One wonders what these earlier giants would have thought of the present day. Yet it seems that the human mind is adjusting itself to the widened horizon—though it may be asked whether the attempt to keep pace with the literature should be made—and it is peculiarly a field in which the present position of research needs an authoritative survey: this is what the authors have presented. Thirty years ago no one would have dared to predict the wide invasion of the most important fields of biochemistry, which has resulted from further study of the aminosugars and the mucosubstances; some chemists might have regarded them as curiosities. An ancient Chinese saying runs: ' Without going out of the door, one can know the whole world.' Study of a tiny corner of biochemistry very soon leads the enquiring worker to consider what the substances are doing, and so to study their part in the organization of the tissues. Hence comes the interpenetration of chemistry and biology in the outlook and training of the modern biochemist. I am indeed glad to have the privilege of recommending this book to all those who from various backgrounds desire a better acquaintance with our current knowledge in this field.

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PREFACE

Some thirty years have elapsed since Levene published his monograph on *Hexosamines and Mucoproteins*, a work which embodied many of his own findings. In the intervening years, this field has expanded enormously and one cannot help but feel that much of this remarkable progress is due to Levene's own pioneer work.

From the small beginnings outlined by Levene, scarcely a field of biological activity can be found in which aminosugars or mucosubstances are not involved. These substances play significant parts, for instance, in processes of immunity and fertility, in the regulation of blood clotting and in some antibiotic activities.

Mucins containing aminosugars are extensively implicated in pathological conditions, such as rheumatic diseases and virus infections, and further advances in the biochemical understanding of these substances may well be expected to elucidate considerably the pathological changes involved.

This book is offered as a contribution that may help to stimulate interest in the subject and with the hope that through the biochemical approach, incorporating the contributions of the biologist and the chemist, a proportionate outlook may be achieved.

We are greatly indebted to many friends and colleagues for the kind advice and encouragement in the preparation of this work. In particular we wish to thank Sir Rudolph Peters, Sir Howard Florey, Drs D. D. Woods, P. Brunet, R. B. Fisher, D. S. Parsons, A. G. Ogston, C. C. Curtain and W. H. Taylor, Lt. C. A. Pasternak, Mr D. B. Hope and Drs Kramer and Windrum. We are also grateful to Mr R. M. Greenway and Dr R. Jeanloz for allowing us to quote unpublished results, to the Director of the Rockefeller Institute, New York, for the Frontispiece and to the following authorities and the authors concerned for permission to reproduce published diagrams: *Nature*, London (*Figure 1*); *Tohoku Journal of Experimental Medicine* (*Figure 3*); the American Chemical Society (*Figure 5*); the Sweizerische Chemische Gesellschaft (*Figure 6*); the Chemical Society (*Figure 7*). Finally we should like to record our gratitude to Rosemary Kent for her patient care in preparing the index.

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CLASSIFICATION AND DISTRIBUTION OF MUCOSUBSTANCES

THE most abundant sugar in nature is D-glucose and in view of its importance as a source of energy for many living species, it is scarcely surprising that more is known of the biochemistry of this sugar than of any other carbohydrate. The course of its metabolic breakdown is known in some detail for a variety of creatures ranged between mammals and unicellular forms and considerable information concerning its conversion into reserve polysaccharides is becoming available.

The unquestioned importance and ubiquity of glucose has tended to overshadow the significance of other sugars which, though present in lesser amounts, nevertheless perform important functions. These must include D-ribose and D-2-deoxyribose the carbohydrates of the nucleic acids, D-galactose, L-fucose, D-fructose, D-mannose and various others. Amongst these also are the 2-aminosugars, glucosamine and galactosamine. These common nitrogenous sugars occur mainly in the animal kingdom where their most usual occurrence is as constituents of carbohydrate polymers—aminopolysaccharides. Such polysaccharides are found in the skeletal substances of many species, in bacterial constitutents, antigens of many types, intercellular cementing agents and in a variety of biological lubricants. Less common types of aminosugars are known to be synthesized by fungi and are present in some antibiotics.

Sufficient experimental evidence is now available concerning the chemical structures of combined aminosugars to enable the biochemist and histologist to study the distribution, metabolism and true biological significance of this group of substances.

Much of the early chemical and enzymic work on aminosugars has been reviewed by LEVENE[12] in his monograph *Hexosamines and Mucoproteins*. Since then, it has been shown that D-glucosamine* (or chitosamine) is 2-amino-2-deoxy-D-glucose and that D-galactosamine* (or chondrosamine) is 2-amino-2-deoxy-D-galactose.

* In this book, the names glucosamine and galactosamine are employed throughout as universally accepted terms. In referring to derivatives, where it is desired to indicate a particular ring form, the full nomenclature is used.

Aminosugars have properties in common with other reducing aldohexoses, *e.g.* reduction of cupric and silver salts, oxidation to hexonic acids, reduction to alcohols and formation of glycosides. Free aminosugars are rarely found in nature, though their presence has been reported in pineapple plant tissue[19], cerebro-spinal fluid[9] and rachitic rat cartilage[21]. Their distribution in various tissues of rat, rabbit and human bodies has been investigated by KUZIN and his co-workers[11]. In general, enzymes acting on neutral hexose derivatives are without action on the corresponding aminosugar derivatives but specific glycosidases and other enzymes are known.

Biosynthesis of glucosamine takes place directly from glucose, or glucose-6-phosphate, glutamine being a suitable donor of ammonia. *N*-Acetylation is accomplished by a transacetylase system. Glucosamine is phosphorylated by a hexokinase-like enzyme so that the aminosugar acts as an inhibitor in the enzymic phosphorylation of glucose in the presence of adenosine triphosphate.

Little is known of the biosynthesis of polysaccharides containing aminosugars, though these polysaccharides have such diverse biological activities both in normal and in pathological conditions.

Nitrogenous polysaccharides in which aminosugars account for all the nitrogen are, the writers feel, best designated *aminopolysaccharides*. The use of the term ' mucopolysaccharide ' for those substances, which are in fact solely polysaccharide in character, is undesirable in face of the use of mucoprotein to denote a protein-containing carbohydrate, and mucolipoid to denote a complex between lipoid, protein, and carbohydrate. The constituent sugars in aminopolysaccharides are glycosidically linked as in nitrogen-free polysaccharides, and there is at present no evidence of other forms of linkage, for instance, involving the amino group. The amino groups are generally understood to be either acetylated, methylated or in one case at least, formylated. Aminopolysaccharides are found in cartilage as chondroitin sulphate, in arthropod integument as chitin and in many other tissues, where they are almost invariably associated with proteins, lipoids or both. One may enquire whether aminopolysaccharides or, in fact, polysaccharides in general, exist in the biological environment as ' pure ' substances.

TYPES OF MUCOSUBSTANCES

For many years examples of substances composed of proteins linked to carbohydrate or lipoids have been known. These complexes were reviewed and classified by KARL MEYER[13] in 1938, and revised by STACEY[20] in 1948. Such is the progress in this field that

the earlier classifications should now be extended and modified to accord with the present state of knowledge.

There is a growing wealth of evidence that in the widest biological environment, conjugate substances known generally as *mucosubstances* or *mucocomplexes* are formed by associations between the major groups of biopolymers—proteins, polysaccharides, and lipoids. Combination can be envisaged at all levels in all ways, and include chemically linked compounds, *e.g.* lipopolysaccharides, comprised of a polysaccharide and esterified lipoid, and absorption and ionic complexes of varying stability, *e.g.* chondroprotein consisting of chondroitin sulphate and protein components.

On this basis, a *mucopolysaccharide* is a polysaccharide–protein (or peptide) complex in which the carbohydrate is the major constituent whilst a *mucoprotein* is composed of the same substances but with protein as the major component. Extension of this classification now logically includes *lipoproteins*, *mucolipoids* and *lipopolysaccharides*. The complete classification is as follows:

Mucocomplex	*Components*	*Examples*
mucopolysaccharide	*polysaccharide* + protein*	*blood group substances*
mucoprotein	*protein* + carbohydrate*	*serum globulins*
lipoprotein	*protein* + lipoid*	*lipovitellin*
mucolipoid	*lipoid* + carbohydrate (+ peptide or protein)*	*Salmonella antigens*
lipopolysaccharide	*polysaccharide + lipoid*	*mycobacterial waxes*

* Major constituent

Nucleic acids are another type of ubiquitous polymer and eventually the complexes formed by these acids will be incorporated into the above classification. Nucleoproteins are a well-known example of such a complex and there is at least one example[18] of a stable deoxyribonucleic acid–polysaccharide complex. Perhaps the latter should be generically termed a nucleopolysaccharide?

It should be emphasized that the carbohydrate moiety of a mucosubstance may or may not contain an aminosugar. The presence of such a sugar is no longer a criterion for a mucosubstance. Certain terms such as glycoids, glycidamines, mucoids, *etc*, it is suggested, have now outlived their usefulness since in fact they have no precise structural or biological meaning. The term *mucin* (L. *mucus*, slime) is legitimate as a general description of viscous, soluble biological products.

COMPARATIVE BIOCHEMISTRY OF MUCOSUBSTANCES

The production of mucins appears to be a biological activity common to very many animal and bacterial species. The chief viscosity-raising agents in most mucins are mucoproteins or mucopolysaccharides. The chemical nature of these substances differs considerably from one species to another, but nevertheless similar biological roles can be ascribed to them. In general, mucins appear to have two main functions; firstly, to lubricate moving parts and, secondly, to provide protective or mechanical supports. This concept is illustrated by examples from many different species. In mammals, for instance, a mucosubstance, hyaluronic acid, is believed to comprise the covering layer of ova and to provide an intercellular cementing substance. A series of enzymes—hyaluronidases—present in spermatozoa and other mammalian secretions specifically hydrolyse hyaluronic acids. Other mucosubstances are associated with cartilage in the mammal and play a significant role in bone formation, whilst yet other substances protect the intestinal mucosa and facilitate the passage of food through the alimentary tract.

The insect integument is comprised partly of chitin–protein complexes of varying composition. The hardening process of this exoskeleton is accomplished without inorganic salts and is achieved by 'tanning', *i.e.* coupling with quinonoid substances. The formation of chitin (a glucosamine polymer) is an essential step in the formation of the exoskeleton (see p. 92). During the moult, chitinases are secreted which disperse the exoskeletal substances.

In snails, *e.g. Helix pomatia*, a similar situation exists. Mucosubstances in the form of a chitin–protein conjugate are laid down in the exoskeleton. Movement of the body within the shell is facilitated by a mucin of which the polysaccharide is composed[2] of the D- and L- forms of galactose. Enzymes hydrolysing amino-sugar compounds have been obtained, however, from snail species.

In echinoderms, the ovum is protected by mucopolysaccharides consisting of about 80 per cent polysaccharide (some nitrogen-free) and 20 per cent of protein. That from the ova of *Echinus esculentus*[17, 23, 24] has a polysaccharide[25] comprised of only L-galactose units esterified with sulphate groups (33 per cent). Similarly, *Echinocardium cordatum* synthesizes a polysaccharide consisting of fucose units whilst in those of *Strongylocentrotus droebachiensis* and *Paracentrotus lividus*[25] fucose and galactose have been found. These mucosubstances, like those in mammals, exhibit marked ion-binding properties and bring about agglutination of the corresponding spermatozoa. These cells contain enzymes, analogous

to hyaluronidases, which cause rapid breakdown[3, 5, 22, 15] of the surface mucosubstances of ova of the same species. Aminosugars do not appear to be involved in these systems.

The exoskeletons of the molluscs and the crustacean group again are hard structures formed by deposition of inorganic substances, mainly calcium carbonate, in a mucocomplex matrix of chitin–protein. Chitin can be regarded as an insoluble mucin synthesized predominantly during certain phases of growth, unlike the usual mucins which are continuously secreted. In the case of squids the exoskeleton is modified to an internal structure. It is colourless, little hardened and consists of large amounts of chitin.

Mucosubstances are secreted by many types of worms, but as yet comparatively little is known of their properties or composition. Epithelial mucins here facilitate the movement of the animal and probably provide a protective layer. Certain of the Annelida secrete mucins which form tubes within which the animals move. DEFRETIN[6] has shown that these have the composition of muco-proteins, and that glucosamine is a common sugar component.

	Glucosamine	*Galactose*	*Fucose*	*Glucuronic acid*	*Mannose*	*Fructose*	*Ribose*	*Arabinose*	*Glucose*
Myxicola sp.	+	+	+	+	+	+	+	–	–
Lanice	–	+	+	–	–	–	–	+	+
Sabella	–	+	–	–	?	–	–	+	+
Pectinaria	–	–	+	–	–	+	+	+	+

Like products[7, 8] from *Hyalinoecia tubicola*, *Leiochone clypeata*, *Petraloproctus terricola*, *Lanice*, and *Spirographis* sp. are hydrolysed by bull testis hyaluronidase, and, therefore, have presumably some resemblance to hyaluronic acid of mammals.

Similarly, capsules formed by certain micro-organisms are frequently extracellular mucosubstances, which may be regarded as a primitive form of skeletal structure. In many ways, they are analogous to the cementing mucosubstances of higher animals. Here, the substances appear to have a much less definite function and, if one has to be sought, it is probable that they form surface layers which are resistant to many hydrolytic enzyme systems and which may regulate ionic and water transport. In the case of

pathogenic organisms, the antigenic activity which capsular materials frequently possess, is, in part, related to the ability of the organism to survive in the pathogenic environment. Capsular substances are of diverse chemical nature and include mucopolysaccharides, proteins and mucoproteins. Enzymes exist which, analogous to the hyaluronidases, bring about the dispersal of these substances. Evidence for these has been found in the study of autolysing cultures where the sequence of enzymic action appears to be in the order, nucleases, peptidases and finally polysaccharases.

It is interesting that particular mucosubstances, *e.g.* hyaluronic acid, occur in bacteria which closely resemble those of higher animals both in enzymic and chemical properties. For the most part, however, these mucocomplexes, like blood group substances, appear to reflect specific metabolic activities of the species or type of organism. Aminosugars and uronic acids are found in some bacterial capsular substances, as for example in the pneumococci[4]. In *Type I* the capsular substance has been isolated as a polysaccharide containing *N*-acetylaminosugar and galacturonic acid, *Type II* is composed of rhamnose, glucuronic acid and glucose, and in *Type III* only glucose and glucuronic acid have been found. *Leuconostoc dextranicum* and *Leuconostoc mesenteroides* produce a group of extracellular polysaccharides composed solely of glucose and known collectively as dextran[10a, 16]. *Bacillus anthracis*, on the other hand, synthesizes capsular material which is purely of a protein nature and it has been shown to be a polypeptide composed[10] solely of poly-D-glutamic acid.

Comparatively few plant species (except fungi) utilize aminosugars. Intercellular cementing substances are comprised largely of pectins (deposited as calcium salts) and similar gums containing uronic acids. In algae, sulphated polysaccharides occur and these are apparently analogous to chondroitin sulphate in providing ionic matrices. The chief structural material of plants, cellulose, is nitrogen free and is deposited in a discrete form as a pure carbohydrate, so that any resemblance to a mucocomplex is rather remote.

The absence of aminosugars in plant cementing and structural substances exemplifies biological economy in species where the supply of nitrogen is a critical nutritional factor. Carbon, being readily available to the plant, is utilized in the relatively static structural and cementing substances, allowing nitrogen to be conserved for vital proteins.

It is evident from a consideration of extant species that the ability to metabolize and utilize aminosugars appears early in the

evolutionary scale. These activities, found in many unicellular species, emerge as major pathways of carbohydrate synthesis amongst the crustaceans and insects, and are retained in some measure in higher forms of life.

MUCOCOMPLEXES IN MAMMALS

The same pattern is evident in many species in that mucocomplexes fall into the broad classes of cementing or protective agents and lubricants. In the mammals, however, these substances play even more roles and appear as constituents of the circulating fluids. Various serum proteins, notably α, β and γ-globulins, fibrinogen 'seroglycoid' and 'seromucoid' are in fact mucoproteins containing small ($\sim$ 2 per cent) amounts of carbohydrate, including aminosugars. Lipopolysaccharides and lipoproteins are of importance in membrane construction as for instance in erythrocytes.

Lubricants in a mammalian body are found in association with the following functions:

anatomical movement	joint fluids ocular fluids
digestive movements	salivary mucin gastric mucins intestinal mucin
parturition	cervical mucins

For the most part, the efficiency of these biological lubricants is dependent on high viscosity and formation of structural gels at low concentrations. It will be noticed that lubricants are secretions into anatomical cavities, *i.e.* have an extracellular environment. Lubricants show a marked degree of stability toward non-specific enzymes. Gastric mucins, in lubricating the passage of digesting foodstuffs in the intestine, require to resist powerful intestinal enzymes if their action is to continue.

Possibly owing to their extracellular distribution, mucosubstances characteristically do not appear to be in a state of rapid 'turnover' (biological equilibrium or steady state involving simultaneous biosynthesis and degradation).

Intracellular components and cementing substances are found in mammalian tissue, in the following situations:

intercellular layers
mesenchymal tissue
envelope of ova
elastic tissue
bone structure and precursors

These substances are subject to biological equilibrium and the enzymes acting on them appear to change during the growth of the tissue. The mucosubstances which comprise mesenchymal components are in a state of slow ' turnover ' and are laid down in association with other cellular components. Thus, in the development of connective tissue, a mucosubstance, chondroitin sulphate, is brought into association with the collagen, and the mechanical properties of this type of tissue have been ascribed to the resulting complex fibre structure. Mucosubstances are polymeric materials, often possessing ionic properties arising from uronic acid constituents[1] or by esterification with sulphuric acid[16a]. The ionic properties of these substances have not been extensively investigated and it is doubtful whether their importance is as yet fully realized. Such acid mucosubstances form ionic complexes with other macromolecules as well as series of salts with cations. In this latter respect, both cementing and lubricating substances display differences in cationic binding, and as a consequence these substances, in selectively binding certain cations, may directly influence the salt metabolic processes. This is reflected in the role of chondroitin sulphate, in the matrix of embryonic bone, which forms a basis for salt deposition. In cartilage and joint fluids, the mechanical properties of the mucosubstances may well be regulated by the nature of the metallic and other cations in the containing medium.

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2

ENZYMIC DEGRADATION OF AMINOCARBOHYDRATES

THE enzymic hydrolysis of polysaccharides possessing aminosugar constituents[58] follows a pattern similar to that which has been worked out for other polysaccharides. The three following broad groups of carbohydrases are involved:

(*a*) Polysaccharases whose action results initially in change in macromolecular properties (*e.g.* decrease in viscosity) then in the extensive cleavage of glycosidic linkages. Enzyme preparations of this type show marked substrate specificity, although similar enzymes from different biological sources are seldom completely alike. The end-product of the hydrolysis is either of an oligosaccharide or disaccharide nature. Purified preparations rarely result in complete hydrolysis of an aminopolysaccharide to monosaccharides. FISHMAN[20], in a systematic review, designated these enzymes *mucopolysaccharases* (or more concisely, *mucases*), though they are in fact no more than specific polysaccharases. This group includes the hyaluronidases, lysozyme, chitinase, the enzymically active influenza viruses, and heparinase.

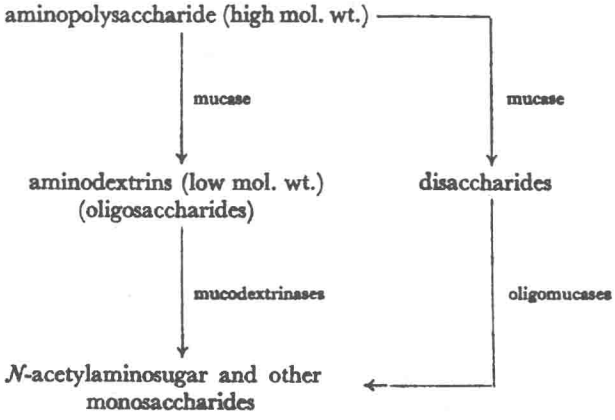
(*b*) The complete hydrolysis of oligosaccharides and disaccharides resulting from the action of the above mucases is brought about by *mucodextrinases* and *oligomucases* (*e.g.* chitobiase) respectively. As yet, comparatively little is known about these enzymes. Oligomucases, however, differ from glucosaminidases in being able to use non-aminosugar glycosides as substrates.

(*c*) Well-known specific glycosidases bring about hydrolysis of α- and β-glycosides of glucosamine. These *glucosaminidases* act only on certain *N*-substituted glycosides and are widely distributed in nature, usually in association with β-glucosidases. Although at the present time α- and β-glucosaminidases are the only aminosugar glycosidases which have been characterized, it is probable that similar specific enzymes act on galactosaminides.

The over-all pattern of enzymic degradation of aminopolysaccharides can be depicted as shown opposite.

Enzymic breakdown of mucocomplexes such as mucoproteins may also proceed in a non-specific fashion, for instance by the

action of a proteolytic enzyme, *e.g.* trypsin. In this case, the protein moiety is hydrolysed to diffusible fragments leaving the carbohydrate relatively undisturbed.



HYALURONIDASES

The hyaluronidases represent a group of enzymes[51, 62] which promote the hydrolytic degradation of hyaluronic acids. These enzymes are widely distributed in mammals (testes[7, 14], skin[10], ocular fluids, spleen, and other tissues[23b]), bacteria[72] (pneumococci[54–56], clostridia[56, 45], *Streptococcus haemolyticus*[11, 15, 55 etc]), *Schistosoma*[42], *Mansonia*, and in snake[8, 19], leech[9] and bee venoms[8]. Hyaluronidases have counterparts in other species, as for example in the spermatozoa of echinoderms. The biological role of hyaluronidases involves tissue penetration and permeability, resistance to bacterial infections and diseases of cartilaginous tissue. Extensive reviews of the subject were published in 1947[51] and 1952[62].

Spreading Factors

In the aetiology of disease, the resistance of the host to pathogenic hyaluronidase-secreting organisms may be correlated with the ability to withstand the action of these tissue-degrading enzymes[45]. In 1928, DURAN-REYNALS[14] observed that experimental infection of rabbits caused by intradermal injection of a virus was enhanced considerably when the virus was mixed with extracts of the testes of various animals. The enhancement was shown to be due to the action of a ' spreading factor ' present not only in testicular extracts but in some snake venoms and extracts of certain pathogenic