Pharmacology of Hydroxyethyl Starch

John Milton Mishler IV

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Use in therapy and blood banking

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Foreword

by

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Those with experience of other colloids for clinical use may very well ask 'why bother with hydroxyethyl starch?' Compared with other colloids, hydroxyethyl starch offers a wide spread of molecular weight with more nearly spherical molecules. There is thus a lower viscosity for any given molecular weight. Molecular size and shape influence the distribution of colloid molecules in the body. Hydroxyethyl starch is slowly hydrolysed by α-amylase present in plasma, the rate of hydrolysis depending upon the number and pattern of hydroxyethyl group substitution in the molecule. In addition, there is the very interesting but not wholly understood physical property of protecting red cells against the damaging effects of freezing and thawing. Clearly, hydroxyethyl starch has fascinating possibilities which go beyond those of other colloids yet investigated.

Dextran and gelatin have been widely used as plasma volume expanders and this has led to much scientific effort to understand fully the nature of these materials and their biological behaviour. This is particularly so in the case of dextran which must be one of the most widely researched colloids. Hydroxyethyl starch has been used as a plasma volume expander but perhaps its most exciting uses are for the protection of red cells against freezing and for the separation of leucocytes from blood.

This book brings together for the first time all that is known about hydroxyethyl starch. The first chapter on the molecular structure and chemistry brings out in detail the features referred to above, while appendices give full experimental information on the analytical methods used. The chapter on catabolism, excretion and tissue storage of hydroxyethyl starch is a comprehensive review of the large literature on this aspect. Japanese work on hydroxyethyl starch has been more extensive than is generally realized and due attention has been given to it. Other chapters deal with all aspects of the effects of hydroxyethyl starch infusions in man and animals. It is noteworthy that in man the plasma α-amylase concentration rises after the infusion of hydroxyethyl starch and it seems possible that this is due to the adsorption of α-amylase onto the hydroxyethyl starch molecule with the complex remaining in the bloodstream, instead of the α-amylase being excreted in the urine. Use of hydroxyethyl starch as a plasma volume expander is fully covered. Details are given of the separation of leucocytes from blood and there is much information on the use

of hydroxyethyl starch for storing whole human blood in the frozen state.

Dr Mishler has personally done research on the various applications of hydroxyethyl starch in several countries including the USA, Britain, and Germany. His comprehensive account will be read with great interest by all concerned with the many applications of hydroxyethyl starch.

Foreword

by

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Plasma expander, cryoprotective compound, diluent for heart-lung machines, agent in cytapheresis and in the production of leucocyte-free red blood cells; so the list of uses of the hydroxyethyl starches has grown steadily since first introduced by Wiedersheim in 1957. Probably the full potential of these safe and effective volaemic colloids has not yet been fully realized, but by now a very substantial literature of hydroxyethyl starch has accumulated and the collection of this into a comprehensive work of reference is both timely and desirable. We should be grateful to John Mishler, himself the author of much modern experimental work on hydroxyethyl starch, who has now provided such a welcome monograph, prepared in a thorough and scholarly fashion.

The sequence and organization of the chapters of this book are logical and the detailed subdivisions most helpful. The reader may choose to study only those sections relevant to his own interest and these can be identified with ease and facility. Each section can be read as an isolated entity, without recourse to the rest of the text (except where expressly directed to some other section for more detail or for clarification) making this a convenient and valuable source of information. The literature has been surveyed comprehensively, providing a lucid synthesis from a complete and up-to-date bibliography. As a point of departure for all types of medical or biological applications, ranging from clinical practice to studies at the molecular level, this work is truly outstanding.

The safety and efficacy of hydroxyethyl starch in animals and man no doubt derives in part from the compact, highly branched structure of the molecules, so reminiscent of natural glycogen. Such ready tolerance is emphasized by work reported here, showing hydroxyethyl starch neither to elicit antibody formation, nor to cause significant release of histamine. Under these circumstances, increasing application of hydroxyethyl starch to blood-related problems seems highly likely and workers in this area, faced with the design of experimental procedures or clinical protocols, will now be able to operate from the vastly improved standpoint that is provided by John Mishler's complete and critical review. As a consequence of the availability of this monograph, it is clear that the pace of scientific enquiry will inevitably quicken.

To my son Joshua Evan Mishler, and my wife Sigrid Ruth Elisabeth Fischer-Mishler.

Success is never final and failure never fatal. It's courage that counts

Quoted in *Success Unlimited*

Preface

Poetically speaking '*a journey of one-thousand miles begins with a single step*', and so it may be said realistically that the first step in the development of the volaemic colloid hydroxyethyl starch began with the early discoveries of Ziese (1934, 1935). His work elucidated the inhibitory effect of hydroxyethylation on α-amylase mediated catabolism of starch. This initial or first step in the development of hydroxyethyl starch lay dormant for over 20 years, however, until Wiedersheim (1957) saw the practicality of using a less rapidly degraded form of starch as a means to restore a diminished plasma volume in cats subjected to haemorrhagic shock. The studies of Wiedershiem thus married together for the first time the theory of hydroxyethylation of starch and its logical extension, its use as a volaemic colloid.

When starch is hydroxyethylated, two variables – namely molecular weight (MW) of the parent starch molecule and the degree of hydroxyethyl group substitution (MS) – can be exploited in developing volaemic colloids that survive in blood for varying lengths of time. It was these two variables of hydroxyethyl starch and their application, first in animal models and later in man, that were investigated next by Walton and his colleagues in the early 1960s. This group of investigators 'fine-tuned' hydroxyethyl starch, testing various combinations of MW and MS to establish the most desirable characteristics required in a volaemic colloid. They also studied the effect of this material on organ function, coagulation, antigenicity, and rheology. From these investigations conducted by Walton and his colleagues, the first species of hydroxyethyl starch (HES 450/0.70) was developed for testing in man.

Since these early studies, numerous investigators have reviewed the efficaciousness of several species of hydroxyethyl starch in volume resuscitation (Fiala 1979; Gryszkiewicz 1978; Köhler 1978; Mishler 1980*a*, *d*; Polushima 1980; Thompson 1974, 1978). Although these reviews are useful, they do not systematically detail the many facets of the pharmacology of the various species of hydroxyethyl starch. I have, therefore, attempted in this monograph to complete the next '*950 miles of the journey*' in our understanding of the many features of this most fascinating material. I have attempted to update our knowledge on the *in vitro* and *in vivo* catabolism and excretion of hydroxyethyl starch with special emphasis on its storage in various tissues. In the subsequent chapters, I have isolated the effect of dose of material injected with corresponding host response. In this manner, it should be possible to predict the risk to the host as it relates to the dose of hydroxyethyl starch injected.

Hydroxyethyl starch was originally developed to increase and sustain a deficient plasma volume, but over the past decade this material has found use in several areas of interest to blood bankers. These new applications are also dealt with in this monograph.

The formidable task of synthesizing our present knowledge of hydroxyethyl starch would have been impossible without the assistance of colleagues. Trevor Greenwood and Donald Muir have, with their extensive knowledge of the physicochemical properties of hydroxyethyl starch, prepared Chapter 1. Edward D. Allen, Joseph C. Fratantoni, David M. Robinson and Brenda J. Slade skilfully reviewed portions of the monograph, and I thank them for a job well done. I should also like to extend my warm thanks to colleagues who furnished reprints of their work on hydroxyethyl starch: E.D. Allen, H. Bergmann, D. French, M. Fujimori, Y. Goto, J.P. Hester, B. Hölscher, H. Köhler, F.J. Lionetti, W. Lorenz, H.G. Merkus, K. Messmer, D.E. Pegg, W. Richter, P. Safar, M.W. Scheiwe, J.A.R. Smith, W. Sonntag, and C. Watzek.

My secretary Ms Kathy White completed the task of putting this monograph together, and without her skill and good humour, I would have faced an impossible task. The editorial staff of the Oxford University Press have given much-appreciated support in bringing this work to press.

Bethesda, Maryland
25 September 1981

JMM IV

Contents

1. The structure and chemistry of hydroxyethyl starch

1.1. INTRODUCTION

Methods are now available to produce a very wide range of material which falls within the generic definition of hydroxyethyl starch. The most important properties of hydroxyethyl starch, the level of substitution and the viscosity, are controlled by methods which are readily applicable. Also, within certain limits, the position of hydroxyethylation on the polymer backbone may be manipulated at will. However, it is equally clear that polysubstitution reactions occur with whatever reaction scheme has been used to date, and that the level of polysubstitution increases disproportionately at higher overall levels of substitution (Merkus *et al.* 1977).

Hydroxyethyl starch has been widely characterized by several techniques. It is now feasible not only to measure *molar substitution*, but also *amount* and *pattern* of *substitution* by accurate and elegant analytical techniques. More recent advances have made possible routine and detailed analysis of the chemical structure.

Similar advances in analytical technique allow ready evaluation of not only the molecular weight and viscosity of samples of hydroxyethyl starch but also the molecular weight distribution. Perhaps the most important physical attribute of hydroxyethyl starch is that its branched structure results in a relatively low viscosity for a given molecular weight. Thus, the hydroxyethyl starch molecule has advantages over dextran in many biological applications.

The control of the enzymic degradation of hydroxyethyl starch may be achieved by two independent mechanisms. That is, by control of degree of substitution and also, but to a lesser extent, by manipulation of the substitution pattern.

The technology and quality control methods are now available to produce a very wide range of molecular species. The choice must now be made by the clinician or scientist either to adopt a compromise or to opt for different specialist types of hydroxyethyl starch in much the same way that various types of dextran are available. This chapter will deal with the physicochemical preparation of hydroxyethyl starch. In subsequent chapters, the various clinically-tested species of hydroxyethyl starch will be described in detail in studies in both man and animals.

1.2. PREPARATION OF HYDROXYETHYL STARCH

Hydroxyethyl starch is a relatively simple polymer to prepare; it is formed by

the reaction between ethylene oxide and amylopectin in the presence of an alkaline catalyst (Ziese 1934, 1935). In aqueous solution the reaction probably occurs by either one of the nucleophilic substitution sequences shown below:

$$\underset{\text{Starch}}{\text{R-O-H}} \xrightarrow{\text{base}} \text{R-O}^- + \text{H}^+ + \underset{\text{Ethylene oxide}}{\text{CH}_2\text{-CH}_2\text{O}} \xrightarrow{\text{base}} \underset{\text{Hydroxyethyl starch}}{\text{R-O-CH}_2\text{CH}_2\text{OH}} \quad (1.1)$$

$$\underset{\text{Starch}}{\text{R-O-H}} \xrightarrow{\text{base}} \text{R-O}^{\delta-} \text{---} \text{H}^{\delta+} + \underset{\text{Ethylene oxide}}{\text{CH}_2\text{-CH}_2\text{O}} \xrightarrow{\text{base}} \underset{\text{Hydroxyethyl starch}}{\text{R-O-CH}_2\text{CH}_2\text{OH}} \quad (1.2)$$

Irrespective of the two reaction schemes (1.1) and (1.2), partial or total ionization of the hydroxyl groups on the polymer backbone is a necessary prerequisite for the reaction to occur. Side-reactions may also occur, the most important one being the hydrolysis of ethylene oxide to ethylene glycol:

$$\underset{\text{Ethylene oxide}}{\text{CH}_2\text{-CH}_2\text{O}} + \text{H}_2\text{O} \xrightarrow{\text{base}} \underset{\text{Ethylene glycol}}{\text{HOCH}_2\text{-CH}_2\text{OH}} \quad (1.3)$$

Ethylene glycol is toxic and must be removed from hydroxyethyl starch by repeated solvent extraction (Schoch 1965). Despite such precautions, small quantities of ethylene glycol have been detected in hydroxyethyl starch intended for clinical use (de Belder *et al.* 1976).

Hydroxyethyl starch is an extremely complex polymer because: (i) the parent amylopectin molecule is heterogeneous both in chemical and physical properties; and (ii) hydroxyethylation can proceed at several different sites on the amylopectin molecule, influencing blood-persistence characteristics (see Section 2.2.2).

1.2.1. STARCH MATERIAL FOR THE PREPARATION OF HYDROXYETHYL STARCH

The starch most commonly used for the preparation of hydroxyethyl starch is a waxy species of either maize or sorghum. Starches exist as semicrystalline, individual granules of polymeric material. In waxy starches, the predominant glucan polymer is *amylopectin* (*c.* 98 per cent), but the amount may vary between species, and even between cultivars of the same species (Banks *et al.* 1973).

The predominant linkage between the glucose residues of the amylopectin chains is the α-1:4-bond, but chains of between 16 and 25 residues (depending on the source of the amylopectin) are attached by α-1:6-bonds. The resultant

highly branched structure is thought to be essentially random (Meyer *et al.* 1941) and of the form shown schematically in Fig. 1.1. Doubts have been cast on the adequacy of this model (Gunja-Smith *et al.* 1970), but conclusive evidence is not yet available. Notwithstanding these doubts concerning the chemical structure of amylopectin, the physical form assumed by the polymer in solution is also unusually complex. There is, for example, strong evidence that the polysaccharide structure is much more extended than would be expected from studies of its more highly branched analogue glycogen. For example, the viscosity of amylopectin is about 15 times that of glycogen of comparable molecular weight (see Banks and Greenwood (1975) for an extensive review of the hydrodynamic behaviour of amylopectin).

Fig. 1.1. A schematic representation of the randomly branched structure of amylopectin. Non-reducing chain end (Φ); reducing chain-end (●); α-1:4 bond (○ – ○); and α-1:6-bond (○ → ●).

1.2.2. THE HYDROXYETHYLATION REACTION

This occurs at the three sites on each (unbranched) glucose residue in the amylopectin chain that are *initially* available for substitution, i.e. the hydroxyl groups at carbon atoms 2, 3, and 6 (C2, C3, C6) of the glucose ring (Fig. 1.2).

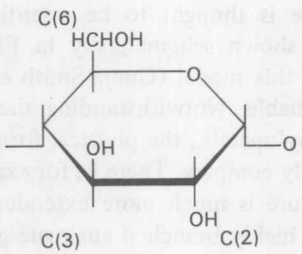

Fig. 1.2. Anhydroglucose residue in hydroxyethyl starch. The numbering convention is clockwise as shown.

In addition, each newly introduced hydroxyethyl group also carries a hydroxyl group, which may itself react further:

$$R\text{-}OCH_2CH_2OH + CH_2\text{-}CH_2 \xrightarrow{\text{base}} R\text{-}OCH_2CH_2OCH_2CH_2OH \quad (1.4)$$

Hydroxyethyl starch

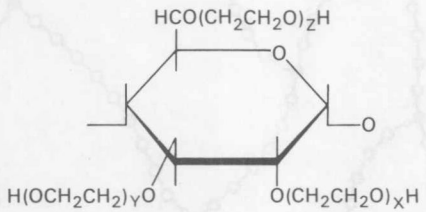

Fig. 1.3. The general structure of hydroxyethyl starch (x, y, and z have values between zero and infinity).

The general structure for internal chain residues in hydroxyethyl starch is shown in Fig. 1.3, where x, y, and z may have a value between zero and infinity. The values of x, y, and z are determined solely by the rate constants for reactions at C2, C3, and C6 respectively, and by the rate constant for the polymerization reaction (eqn. 1.4).

1.2.3. MODEL FOR THE REACTION

It is convenient to use the following mathematical model (Banks *et al.* 1972) to understand the manner in which the substitution pattern of hydroxyethyl starch is controlled. Each glucose residue has a hydroxyl group available for reaction at C2, C3, and C6. If it is assumed that the reaction at any given site is independent of the status of other reactive sites, and that the rate constant

is proportional to the number of unreacted sites, e.g. a first-order reaction, we may write (Spurlin 1939):

$$\frac{dS}{dt} = k(1 - S) \tag{1.5}$$

where S is the fraction of available sites which have reacted and k is the rate constant for the substitution reaction. Integration of eqn (1.5), with the constraint that $S = 0$ when $t = 0$, gives:

$$\ln (1 - S) = kt, \tag{1.6}$$

or, expressed in exponential form

$$S = 1 - e^{-kt}. \tag{1.7}$$

Each of the available hydroxyl groups on starch (or its derivatives) will react in the manner shown by eqn 1.7, but there is no reason to suppose that the rate constants for all three sites will be identical. We may recognize this fact by writing three equations:

$$S_2 = 1 - e^{-k_2 t} \tag{1.8}$$

$$S_3 = 1 - e^{-k_3 t} \tag{1.9}$$

$$S_6 = 1 - e^{-k_6 t} \tag{1.10}$$

where S_2, S_3, and S_6 are the extent of substitution at C2, C3, and C6, respectively, and k_2, k_3, and k_6 are the appropriate rate constants.

The rate of the polymerization reaction (eqn 1.4) is proportional to the fraction of hydroxyl groups already substituted. Therefore,

$$dS_p = k_p (S_2 + S_3 + S_6)\, dt. \tag{1.11}$$

S_2, S_3, and S_6 may be replaced from eqns 1.8, 1.9, and 1.10 to give

$$dS_p = k_p [(1 - e^{-k_2 t}) + (1 - e^{-k_3 t}) + (1 - e^{-k_6 t})]\, dt. \tag{1.12}$$

If, for the moment, S_3 and S_6 are ignored, then

$$dS_p = k_p S_2\, dt = k_p (1 - e^{-k_2 t})\, dt. \tag{1.13}$$

Integration then yields the relation

$$S_p = k_p \left(t - \frac{1}{k_2} e^{-k_2 t} + C\right) \tag{1.14}$$