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Characterization of components of starch

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CHARACTERIZATION OF COMPONENTS OF STARCH

by

194

Joseph Franklin Foster

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Plant Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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1943

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I. INTRODUCTION

That starch is not a homogeneous material has been recognized since the very earliest investigations of its properties. As to the nature and degree of its heterogeneity, however, there has been much debate, and the history of the investigation of starch down to the last decade is essentially the story of multitudinous, unsuccessful attempts to obtain a definite fractionation on the basis of phosphorus content, solubility, molecular size and other properties of which little was known. This situation is not surprising when we consider that the structure of maltose, which may be considered the building unit of starch, was not fully elucidated until 1926.

Only within the last five years has our concept of the chemical structure of starch reached a point where any scientific explanation for the existence of definite fractions could be made. The introduction of the concept of a branched structure, in contrast to the previous tendency to consider starch as consisting entirely of linear molecules (as in the case of cellulose), made it apparent that there was a possibility of variation in the degree of branching as well as in molecular size. This concept furthermore clarified discrepancies which had previously led to hazy concepts of a micellar structure for starch, involving secondary valences of somewhat questionable nature. It would thus seem justifiable to consider the introduc-

tion of the branching concept as the real "turning point" in the investigation of the structure of starch.

The concept of branching, while fundamental to our present picture of the components of starch, also led to the attitude that there could be no definite, sharp fractions. It seemed more likely that a random distribution would be present, both as to molecular size and as to degree of branching. This point of view ignores the fact that nature is not haphazard but as a rule extremely systematic.

Only very recently two independent techniques have been introduced which appear to give exceedingly sharp separations of starch into two components, one apparently consisting of purely linear molecules, the other of highly branched molecules. In addition the development of a potentiometric iodine titration technique, based on the well known tendency of starch to form a complex with iodine, has provided overwhelming evidence that there is no material intermediate in properties between the two components. It thus appears that for the first time it is now possible to resolve the exceedingly difficult problem of the investigation of the structure of starch into an investigation of two entirely different components, thereby eliminating much of the previous cause of confusion.

This development of an apparently well founded two-component concept of starch would make it seem not only helpful but imperative to characterize these components thoroughly. The manner

in which this is to be done, however, is difficult to choose. At the present time the best evidence for the structure of these components is based on results of methylation, which results, in view of the present methods for separation of the methyl glucoses, are to be accepted with reservation. No other chemical methods are yet available.

Recent advances in the theory of solutions of high polymers indicate the possibility of the use of viscosity and osmotic pressure techniques in this characterization. Perhaps the most striking property of solutions of linear polymers, as contrasted to those of essentially spherical molecules, is their exceedingly high viscosity. It is this approach which has been utilized largely in this investigation. At best the study of high polymer solutions is difficult, even in the case of the simpler polymers such as the normal hydrocarbons. In the case of starch the difficulties are many times multiplied due to such factors as retrogradation (crystallization) and variation in the configuration of even the linear molecules (straight and helical configurations). These difficulties will be discussed later in detail.

An effort has been made to keep this investigation as broad as possible in spite of the numerous points which have tempted more concentrated study. Due to the novelty of our present concepts much effort could easily be wasted in such studies. The wiser course at this stage seems to be to char-

acterize the components of starch as much as possible, thereby providing evidence that definite components do exist, and to defer the more detailed studies until the problem can be attacked with a broader perspective.

II. ADVANCES IN THE THEORY OF SOLUTIONS OF HIGH POLYMERS

With tremendous recent interest in synthetic resins, plastics, rubber and silk, the study of high polymer solutions has taken on a practical as well as a theoretical aspect. The increasing number of theoretical publications on this subject, coming largely from the industrial laboratories, perhaps best emphasizes this trend. These investigations have had the effect, not so much of developing new tools, as of putting the old methods of investigation of high polymers on a new and much firmer basis.

Of the experimental methods which have been employed in the investigation of high polymer solutions the following have, perhaps, been most used and have met with or promise the most success: Osmotic pressure, viscosity, birefringence of flow and other optical properties, rate of diffusion, ultracentrifugal analysis and electron microscopy. Ebulliscope and cryoscopic methods are worthless above molecular weights of 5,000 at the most. Of the available methods, osmotic pressure and viscosity have been used in this investigation and will be discussed in detail below. Ultracentrifugal results, while at the present time somewhat difficult of evaluation, have been very valuable, chiefly in the protein field. The electron microscope, although at present still in a formative stage, promises much in the future and will be somewhat more direct than the other

methods. The last two methods require, of course, expensive and highly specialized equipment which is not at present widely available.

Solution Viscosity

The measurement of the viscosity of their solutions has been a favored method of study of high polymers, not only because of the relative ease of measurement (as compared to osmotic pressure for example) but because of the extremely high viscosity of high polymer solutions.

The first attempt to place the viscosity of solutions and suspensions on a theoretical basis was made by Einstein in 1906 (21). In this derivation Einstein assumed the disperse phase to consist of rigid spheres to which the solvent adhered completely, large in comparison with the free path of the solvent molecules but small in comparison with the dimensions of the apparatus. He further excluded turbulence and inertia effects and ignored any interaction between solute particles, which assumptions would be approximately fulfilled in the case of careful measurements at extreme dilution. By utilizing fundamental hydrodynamic postulates he derived the expression

$$\eta_c = \eta_0 (1 + 2.5 v/v) \quad (I)$$

where η_c is the viscosity at the given concentration, η_0 the viscosity of the pure solvent, v the total volume of dispersed phase and V the total volume of solution. It is customary to

define

$$\eta_R = \eta_c / \eta_0$$

and

$$\eta_{sp} = \eta_R - 1$$

and thus Einstein's equation can be put in the form

$$\eta_{sp} = 2.5 \phi \quad (II)$$

where ϕ is the volume fraction of solute.

It is apparent from this equation that the viscosity of a solution of spherical molecules should depend only on the volume fraction of solute and be quite independent of the size of the individual suspended particles. In other words the viscosity should be independent of the molecular weight of the solute. This has been confirmed experimentally on suspensions of gamboge and sulfur (7,68,22).

This result has been extended by others in an attempt to provide a theory that is applicable to more concentrated solutions and to solutions of ellipsoidal and rod-like rather than spherical particles. This subject has recently been reviewed very adequately by Mark (60), and only a brief summary will be given here.

Numerous equations have been proposed to fit the concentration dependence found experimentally for particular systems, all of these being empirical in nature. Attempts have been made to attach theoretical implications to the various constants but with little success. These equations have the common feature of being readily expandable in a power series expression

for η_{sp} in terms of c . Perhaps the best known are the Arrhenius equations (3,4)

$$\log \eta_R = k \cdot c \quad (\text{III})$$

and

$$\log \eta_R = (k \cdot 100 c)/(100 - nc) \quad (\text{IV})$$

where n and k are constants, and the Fikentscher equation (24)

$$\log \eta_R/c = k + 75 k^2/(1 + 1.5 kc). \quad (\text{V})$$

The various equations predicting concentration dependence have been reviewed by Papkov (70) in addition to the comprehensive review of Mark (60-a).

In contrast to the empirical nature of the equations representing concentration dependence, the extension of the theory to non-spherical molecules has been essentially theoretical in nature. It has long been an experimentally observed fact that the viscosity of solutions of certain high molecular substances is in some way related to molecular weight. As early as 1909 Stobbe and Posnjak (84) employed viscosity to follow the polymerization of styrene. These observations, coupled with the results of Einstein predicting viscosity to be independent of molecular weight in the case of spherical molecules, stimulated attempts to determine the effect of ellipsoidal and linear solute molecules. Perhaps the first successful attempt was that of Kuhn (54) who pointed out that if two spherical particles were attached rigidly (to form a dumb-bell shaped molecule) they would tend to rotate in the

flow gradient about their center of mass. Whereas the spheres, if free, would tend to travel with the velocity of the surrounding fluid, they would now have a small velocity relative to the surrounding solvent due to this rotation. This introduces an extra work term, calculable by means of Stokes' formula, and thus modifies Einstein's equation by an added term. This derivation may be readily extended to the case of a long molecule consisting of many spheres with the result

$$\eta_c = \eta_c(1 + 2.5 \phi + 1/16 \phi f^2) \quad (\text{VI})$$

where f is the axial ratio, or, introducing our previous definition of η_{sp} ,

$$\eta_{sp} = 2.5 \phi + 1/16 \phi f^2 \quad (\text{VII})$$

Other workers have derived similar expressions according to the special assumptions and approximations made. These have been reviewed by Mark (60-a).

Staudinger observed experimentally that the viscosity of solutions of linear polymers increases with increasing chain length and proposed the now well known relationship (79,80,81)

$$\eta_{sp}/c = K_m \cdot M \quad (\text{VIII})$$

where K_m is an empirical constant. He proposed that chain molecules are present in solution as rigid rods and attempted to explain his equation as follows: Due to the rotation of the molecules about their center of mass each will effectively occupy a volume which is proportional to the square of its length. However, the number of molecules present in unit volume

(at a given weight concentration) is inversely proportional to the molecular weight. Therefore, the effective volume of solute per unit volume of solution (ϕ) is proportional to the first power of \underline{M} and, by equation (II), η_{sp} is also proportional to \underline{M} .

Staudinger has tested his equation with a number of synthetic polymers such as polyprene, polyethyleneoxide, polystyrene and others, as well as with natural polymers such as the paraffins, cellulose and starch. In most cases he found K_m to be reasonably constant at high molecular weights, although he did find deviations for the lower members. He emphasized that the relationship holds only for dilute solutions of homopolar compounds in indifferent solvents.

The Staudinger equation has been the subject of much heated debate. In the first place it presupposed the existence of free solute molecules in solution (80) rather than clusters of molecules (micelles) as pictured by most workers at that time. It further portrayed these molecules as rigid rods, which one would not intuitively expect.

According to Kuhn (56) the very fact that this equation does hold so well in many cases is proof that the molecules are not rigid rods in solution. According to his derivation (54) as well as practically all other derivations based on hydrodynamic postulates, the function η_{sp}/c should be proportional to the second power of the molecular weight (or

molecular length) in the case of rigid rods rather than to the first power. He explained Staudinger's results as due to the fact that the molecules are not rigid in solution but, rather, randomly kinked.

Because of the relative ease of viscosity determinations the Staudinger method has been widely used (or, more often, mis-used) for the determination of molecular weights. Care has not been taken to evaluate carefully the K_m constant for different materials as this requires tedious osmotic measurements or some similar independent molecular weight determinations. The value of K_m determined in one solvent has been assumed to hold in other solvents, and, in some cases, the value of K_m determined for one polymer series has even been carried over to other polymers.

Alfrey, Bartovics and Mark (2) have emphasized that K_m depends not only on polymer type but also on the temperature and nature of solvent. The effect of the solvent is quite obvious. In the first place the degree of solvation (or swelling) of the solute molecules will affect their effective volume. This was emphasized by Kuhn (55). Moreover, the extent to which the molecules are extended or kinked depends in large part on the solvent, a good solvent maintaining them in a more extended form (on the average) than a poor solvent. Thus, other factors being the same, viscosity should be higher the better the solvent, and this has been observed experimentally (2).

Since the Staudinger equation predicts the function η_{sp}/c to be concentration-independent it has been customary to determine viscosity at only one concentration. It now appears that this function is not only concentration-dependent but that the magnitude of this concentration-dependence is also a function of the molecular weight, being greater for longer molecules (6).

This abuse of the Staudinger equation has, unfortunately, led to the publication of many false molecular weight values and has caused considerable discredit of the Staudinger method.

Recently still another source of error in the use of the Staudinger equation has been brought to light by Kraemer and Lansing (53), who pointed out that the viscosity method leads to a "weight-average" molecular weight given by

$$M_w = \sum_i f_i M_i \quad (\text{IX})$$

where f_i is the fractional weight of material with the molecular weight M_i , whereas most other methods (osmotic pressure for example) give a simple "number average" molecular weight given by

$$M_n = 1/\sum_i f_i/M_i. \quad (\text{X})$$

This has been verified experimentally (8).

From a qualitative standpoint this simply means that in the case of viscosity longer molecules play a relatively more important role than short molecules, whereas in the case of osmotic pressure they have an equal effect. In case of a

perfectly homogeneous material the two averages are identical; however, if a wide molecular weight distribution exists the two averages are decidedly different. Such a distribution would especially be expected in the case of synthetic polymers or natural polymers which have been partially degraded. This very important point has been quite generally ignored by a large proportion of the workers in the field.

Meyer and van der Wyk (65) consider the Staudinger equation to be much oversimplified. They carried out a series of precise viscosity measurements on the homologous series of normal paraffins, covering the range from C_{17} to C_{34} , and using carbon tetrachloride as solvent, and concluded that the Staudinger function varies linearly with molecular weight in that case but is not directly proportional. In other words there is an additive constant. They emphasized that the viscosity method should be used only for interpolating between two members of a series, not for extrapolating in either direction. Their results were verified in essence by Fordyce and Hibbert (27) on a series of synthetic polyoxyethyleneglycols, and the equation

$$\eta_{sp}/c = K_m \cdot M + \beta \quad (XI)$$

was proposed. The last two mentioned results suffer from the fact that viscosities were run only at one concentration instead of carrying out extrapolation to zero concentration.

Kraemer (52) used the Arrhenius function $(\ln \eta_R)/c$ rather than the Staudinger function and extrapolated to infinite dilu-

tion, defining the limit value $[(\ln \eta_R)/c]_{c=0}$ as the "intrinsic viscosity". He compared the intrinsic viscosities of cellulose and some of its derivatives with molecular weights determined by means of the ultracentrifuge, demonstrating that a simple relationship exists. He furthermore followed viscosimetrically the cyclic conversion of cellulose \rightarrow cellulose acetate \rightarrow regenerated cellulose, providing very definite evidence that these materials exist in solution as true molecules, not micelles.

Kemp and Peters (5), as a result of their investigations on rubber, prefer the Arrhenius function over that of Staudinger, using the equation

$$(\ln \eta_R)/c = 1/K_{cm} \cdot M \quad . \quad (XII)$$

Baker, Fuller and Heiss (5) have recently prepared a series of well-defined and linear self-polyesters from ω -hydroxyundecanoic acid. These were carefully purified and characterized as to molecular weight by means of alkali titration of the free acid end-groups. They found the equation

$$[(\ln \eta_R)/c]_{c=0} = K_w \cdot M_w + B \quad (XIII)$$

to hold very well, K_w being a true constant at least down to molecular weights as low as 5,000. The additive constant, B , was found to be small and proposed to be a function of the solvation and statistical degree of chain kinking.

Flory and Stickney (26) pointed out that the limit of the function $(\ln \eta_R)/c$ is identical with the limit of η_{sp}/c , and

therefore the limit of either function may be equally well used. In other words the equation

$$[\eta_{sp}/c]_{c=0} = K_W \cdot M_W + B \quad (XIV)$$

is identical with equation (XIII). These workers used the logarithmic function, since by plotting η_R on a log scale against concentration they obtained a straight line, thus facilitating the extrapolation.

Huggins (45) has recently provided an excellent theoretical derivation of equation (XIV), following essentially the procedure used by Kuhn (54,56). Using fundamental hydrodynamic postulates he derived, for the case of rigid rod-like molecules, the equation

$$\eta_{sp}/c = \frac{\pi N l^3 a n^2}{24,000} \quad (XV)$$

where π and N have the usual significance, l is the length of each unit of the chain, a its effective radius, and n the number of such units in the chain. Obviously since the molecular weight is proportional to n , this equation predicts η_{sp}/c to be a function of M^2 , confirming the earlier results of Kuhn.

Huggins then assumed molecules which are not rigid, but kinked in a completely random fashion. Eyring (33) has derived an equation which gives, for such molecules, the average value of the square of the distance from the atom at the molecular center to any other atom i , in terms of a power series in $\cos \alpha$, where α is the supplement of the angle between successive units (a fixed value). Using this equation he derived the expression

$$\eta_{sp}/c = \frac{K_e M_0 / 1000 \rho + (\pi N / 45,000) l^2 a B_\infty \beta (n-3+1/n)(1+\alpha^2/448)}{1 - (\pi N / 45,000) l^2 a B_\infty \beta (n-3+1/n)(1+\alpha^2/448)} \quad (XVI)$$

where \underline{N} , $\underline{\pi}$, \underline{l} and \underline{n} have the same significance as in Equation (XV), M_0 is the "molecular weight" of each chain unit, K_e is a constant depending on the relative sizes and shapes of solvent and solute molecules, ρ is the density of pure liquid solute, B_∞ is the function $(1 + \cos \alpha)/(1 - \cos \alpha)$ and β is a complicated function which approaches unity as \underline{n} becomes large. The $1/n$ term can be ignored, thus giving an expression which is essentially the same as Equation (XIV). For large values of \underline{n} , the $\underline{2}$ in the term $(n - 3 + 1/n)$ can also be ignored, and, upon assigning numerical values to the constants, the equation

$$\eta_{sp}/c = 2.94 \times 10^{20} B_\infty l^2 a n \quad (XVII)$$

results. These equations were derived for the case of strong Brownian movement (high temperature) or small velocity gradient. In the case of negligible Brownian motion or large velocity gradient the resulting equation is

$$\eta_{sp}/c = 2.82 \times 10^{20} B_\infty l^2 a n \quad (XVIII)$$

Huggins applied these results to the normal-paraffins.

In this case the values of \underline{l} and \underline{a} are easily chosen, the latter being simply the supplement of the tetrahedral angle. The value of \underline{a} , the "effective radius" of the chain units, is rather uncertain. However, using a value of \underline{a} which appears to be reasonable Huggins was able to check the experimental results of Meyer and van der Wyk (65) with surprising accuracy.

Huggins' work further showed that the viscosity of solutions of rod-like molecules would be strongly temperature-dependent and dependent on velocity gradient, whereas the temperature dependence of randomly kinked molecules would be negligible. These predictions have not been checked experimentally.

More recently (47) Huggins has derived, by a simple modification of his earlier derivation, an expression representing the concentration-dependence of the Staudinger function. This equation, which is identical with one previously stated empirically (78), predicts that for randomly kinked long-chain molecules

$$\eta_{sp}/c = [\eta_{sp}/c]_{c=0} (1 + k' \eta_{sp}) \quad (\text{XIX})$$

which, at low concentrations, can be put in the form

$$\eta_{sp}/c = [\eta_{sp}/c]_{c=0} + k' [\eta_{sp}/c]_{c=0}^2 \cdot c \quad (\text{XX})$$

Huggins points out that most of the expressions previously proposed to represent the concentration dependence of such systems will reduce to this form.

So far no mention has been made of the methods for determining viscosities of solutions experimentally. These methods have been reviewed adequately on numerous occasions, for example by Mark (60-b). Suffice it to say that the many methods can be conveniently grouped under three classical methods: (a) capillary flow, (b) motion of a solid body, usually a sphere, in the fluid, and (c) behavior of the fluid between two concentric cylinders of which one is stationary and the other rotating.

The last named method, that of Couette, is, perhaps, to be preferred from a theoretical standpoint. However, under suitable conditions the methods give essentially identical results and the bulk of the published results on solution viscosities have been determined by the capillary flow method. It is important when using this method that the velocity gradient be small, not only to eliminate turbulence but, in the case of long molecules, to minimize flow orientation. It has been shown (6) that this latter effect is negligible in viscometers of practical dimensions and at low concentrations (below 1% by weight). It has also been found essential, by practically all workers using the capillary method, to filter the solutions before the viscosity determinations in order to eliminate any foreign matter which will impede the flow.

By way of summary, it is apparent that the viscosity method of Staudinger for determining molecular size is essentially empirical in nature. Certain inherent errors and limitations of application have been ignored, leading to much mis-use and misunderstanding. However, recent theoretical work, combined with more careful experimental work, gives confidence that the method is a worthwhile tool for drawing conclusions as to molecular size and shape, provided the limitations are clearly recognized.

Osmotic Pressure

If a solution is placed in contact with pure solvent, separated only by a thin membrane which is permeable to solvent but not to solute, the solvent will show a tendency, due to its greater fugacity, to diffuse through the membrane. This phenomenon, termed "osmotic pressure", is well known. Equally familiar, perhaps, is the van't Hoff relation

$$P = R T C_m \quad (\text{XXI})$$

in which P is the osmotic pressure, R the universal gas constant in appropriate units, and C_m the molar concentration.

In spite of the extreme simplicity of this relation, it has been found to hold very well for dilute solutions of low molecular weight substances¹.

Thermodynamically, the exact expression for the magnitude of the osmotic pressure is

$$P \bar{V}_1 = -(\bar{F}_1 - F_1^0) = -\Delta \bar{F}_1 \quad (\text{XXII})$$

where all the quantities have the usual thermodynamic significance, the subscript 1 referring to the solvent component. If the solution under consideration is ideal we have the further restrictions

$$\Delta \bar{H}_1 = \bar{H}_1 - H_1^0 = 0 \quad (\text{XXIII})$$

1. See any standard textbook of Physical Chemistry, for example Getman and Daniels, "Outlines of Theoretical Chemistry", John Wiley & Sons, New York, Sixth Edition, 1937, pp. 174-180.

and

$$\Delta \bar{S}_1 = \bar{S}_1 - S_1^0 = -R \ln N_1 \quad (\text{XXIV})$$

where N_1 is the mol-fraction of solvent. In other words the solution is both athermic and has the ideal entropy of mixing. The latter assumption requires that any molecule be completely interchangeable with any other molecule in the solution.

Since

$$\Delta \bar{F}_1 = \Delta \bar{H}_1 - T \Delta \bar{S}_1 \quad (\text{XXV})$$

one can write, in view of (XXIII) and (XXIV)

$$P \bar{V}_1 = -\Delta \bar{H}_1 + T \Delta \bar{S}_1 = R T \ln N_1 = + R T \ln (1-N_2) \quad (\text{XXVI})$$

or, expanding the logarithmic function in powers of N_2 ,

$$P = RT/\bar{V}_1 (N_2 + N_2^2/2! + \dots) \quad (\text{XXVII})$$

In very dilute solutions all but the first power of N_2 can be ignored and, moreover, \bar{V}_1 approaches the molar volume, V , of the solution. The resulting equation

$$P = RT/V \times N_2 = RTQ/VM_2 \quad (\text{XXVIII})$$

where Q is concentration in gm. per liter and M_2 the molecular weight of the solute, is obviously equivalent to that of van't Hoff.

It is desirable to extend the use of the osmotic pressure method of molecular weight determination to molecules of high molecular weight. Whereas ebulliscope and cryoscopic methods are worthless above molecular weights of 5,000 at most, osmotic pressure, due to the much greater effect per mole of solute,

is sensitive enough for use up to molecular weights of about 500,000. This statement, of course, depends on the validity of van't Hoff's law for such molecules. The extension of any physical property from the low molecular to the high molecular realm is to be carried out only with extreme caution.

At first thought it might seem that a long molecule would behave, with respect to osmotic pressure, not as a unit, but as a number of small units. For example, if a molecule consisted of two spheres attached non-rigidly together, one might expect these to act more or less independently so that the osmotic molecular weight would be that of the separate spheres, not of the unit.

Experimental determinations carried out by numerous workers (63-b, 18, 15) indicate that the function P/C , in the case of high polymers, shows a steep positive slope when plotted against concentration. However, it appears that even in this case the limit of the function at infinite dilution fulfills van't Hoff's relation completely (63-b). Apparently an equation of the type

$$P = RTC/VM + BC^2 \quad (XXIX)$$

is necessary.

Meyer points out (63-b) that the quantity B should involve not only heat of mixing effects (ignored in the derivation given above) but also deviations from the simple statistical nature of entropy of mixing, implied in Equation (XXIV). The

heat of mixing may be either positive or negative, producing either positive or negative deviations from the van't Hoff ideal.

It should be noted that Equation (XXIV) for the calculation of the entropy of solution is valid only for systems in which the molecules of both components are of the same size and shape, i.e., completely interchangeable. This is obviously far from true in solutions of high polymers.

Meyer (63-b) considers the deviations from ideal entropy to be the much more important term. He has analyzed qualitatively the process of solution of a high polymer, predicting that due to this anomalous entropy the plot of P/C vs. C for such systems should show a strong positive slope, dependent on the polymer, solvent, and temperature, but independent of the chain length. That is, various members of a given polymer-homologous series, under similar conditions, should give plots which are parallel, but differ in intercept.

Meyer's concept has been placed on a quantitative basis by the recent statistical treatments of Huggins (46) and of Flory (25). The problem has also been attacked by Eyring and coworkers from the flow-segment point of view (72), their conclusions being essentially the same. It thus appears that the osmotic method is applicable for the determination of molecular weights of chain polymers. It is imperative, however, that several points be determined at low concentration so that extrapolation can be carried out, since the deviations from perfect

behavior are so great that even at the lowest practicable concentrations anomalously high pressures are observed.

The older molecular weight values reported on the basis of osmotic pressure should be critically analyzed with this point in mind. In some cases it has been considered sufficient to determine values at only one low concentration.

In contrast to the situation which was observed in the case of solution viscosities, the experimental determination of osmotic pressure is extremely difficult. This is especially true in case non-aqueous solvents are used. These difficulties are chiefly in the construction of a suitable cell, and in the choice and preparation of suitable membranes.

Innumerable osmotic cells have been designed, practically every worker in the field modifying in some way those previously used. Perhaps none has been perfectly satisfactory. The older cells were of the static type, the solvent being permitted to diffuse through the membrane until equilibrium between the osmotic pressure and the hydrostatic pressure of the solution was attained. This method is extremely slow since the rate of diffusion becomes progressively slower as the equilibrium point is approached. Recently Bourdillon (13) has constructed a micro adaptation of this type of cell which is much faster due to the smaller volumes involved. He claims equilibrium to be attained in about four hours.

An entirely different type of cell, the so-called dynamic

type, was designed by Van Campen (85), based on the counter-pressure principle of Berkeley (12). In this method diffusion through the membrane is prevented by either the application of pressure to the solution or suction to the solvent. The magnitude of the pressure or suction required to maintain equilibrium (zero flow) is, of course, the osmotic pressure. The volume of solution used need not be large since practically no dilution occurs. Furthermore equilibrium is reached much more rapidly than in the other method.

The equilibrium point can be determined most rapidly by determining the diffusion rate at various pressure settings, and interpolating to zero flow rate. This is best done by observing the meniscus of either the solvent or solution in a glass capillary which is attached to the cell.

Satisfactory cells working on this principle have been used by others. Carter and Record (18) used as solvent and solution chambers two glass bells of about 5 ml. capacity, between which the membrane was clamped. The latter was further supported by perforated brass discs, a very important point in this type of instrument since any motion of the membrane will cause complications in the apparent flow rates. Also in this cell the solution compartment was equipped with an electromagnetic stirrer to speed attainment of equilibrium. As an added precaution against leakage, the joint between the glass bells was surrounded by a collar containing mercury under

approximately one atmosphere pressure.

Buchner and Samwell (15) clamped the membrane between two similar brass plates, which were grooved to accommodate the liquid. In this way large membrane areas were possible with a small volume of solution. They immersed the instrument in a thermostat controlled to 0.05° C., presumably to prevent variations in diffusion rate due to temperature variations. This accurate control of temperature has not been found necessary by other workers (71,66). Perhaps the biggest objection to this cell was the use of rubber gaskets to seal the membrane.

Montonna and Jilk (60) used a cell patterned after that of Buchner and Samwell, but eliminating the use of rubber gaskets. However, their cell contained rubber connections and numerous ground glass stopcocks which were lubricated with grease so that it was still not free from objection when used with organic solvents.

Perhaps the most satisfactory type of cell yet designed is that of Hepp (42). A block of glass or leucite is drilled to take the glass capillary. The solvent chamber consists simply of a small disc of bolting silk which is soaked with solvent and laid on the block, over the capillary, and covered with the membrane. This is then clamped down with a ring which also holds the solution (only about one ml.). Equilibrium is attained in this cell in less than an hour, the secret of its speed lying in the extremely small volume of solvent.

Peters and Saslow (71) have checked the performance of this type of cell on solutions of horse serum albumin finding it very satisfactory. No rubber washers or stopcocks come in contact with the solution or solvent, so that from this standpoint the cell is satisfactory for use with organic solvents.

For use as an osmotic membrane a material must be porous enough to permit measurable diffusion of the solvent and still have no openings of a size large enough to permit passage of the solute. The latter problem obviously is more difficult the smaller the solute particles.

In the case of aqueous solutions the commercially obtainable Zsigmondy cellulose filters have been found satisfactory by several workers (71,13). Ordinary cellophane has been found to have too low a porosity (71,13) but McBain and Stuewer (58) have shown that the porosity can be increased and controlled by swelling in water-alcohol of varying concentrations. Membranes of this type have been found satisfactory by Carter and Record (18) for solutes of molecular weights as low as 3,000. Other workers (66,15) have used collodion membranes with aqueous solutions and denitrated collodion membranes with organic solvents. These membranes are, however, extremely difficult to prepare in a manner which will yield reasonably uniform porosity.

It is thus apparent that the primary obstacle to the determination of molecular weights osmotically is experimental.

From the theoretical standpoint it is, perhaps, one of the most straightforward and reliable methods yet available in the high molecular-weight range.

III. DEVELOPMENT OF OUR PRESENT TWO-COMPONENT CONCEPT OF STARCH

No attempt will be made in the present review to include all of the voluminous literature on starch. Numerous extensive summaries are available; in particular should be mentioned the more recent reviews of Hanes (32), Meyer (63-c,62), and Hixon and Rundle (44). An attempt will be made here to summarize that work directly relating to the present two-component picture of starch. For this purpose it will be sufficient to go back only to about 1930 and take up the work with the first intensive efforts to elucidate the molecular size and shape of starch.

The Discrepancy Between Apparent Chemical and Physical Molecular Weights and Introduction of the Branching Hypothesis

Early work on the methylation and subsequent hydrolysis of starch indicated an almost quantitative yield of 2,3,6-trimethyl glucose (40,48) and it was largely on the basis of this evidence that the α -1:4-glucosidic linkage (as in maltose) was proposed. In 1932 both Haworth (35) and Irvine (49) reported the presence of a small amount of tetramethyl glucose in the hydrolysates from methylated starch. Haworth pointed out that this derivative would be expected to arise from glu-

ose units occurring at the non-reducing ends of chains, and that a determination of the relative proportion of this derivative would provide a measure of the average chain length.

This method has been much used, not only with starch, but with cellulose and other carbohydrates. In the case of starch the results have been strikingly uniform, practically all workers reporting values of 25-30 glucose units (35,49,43,41,36,33,29). This uniformity is all the more surprising in that these results were obtained on starches from widely different sources, and even on the "glutinous" or "waxy" varieties which have properties so markedly different from ordinary starches. The methods of estimating the tetramethyl glucose can be strongly criticized; nevertheless there can be little doubt as to the correctness of the order of magnitude.

These extremely low values for the apparent molecular weight are in strong contrast to the molecular weights predicted from other properties. First might be mentioned the low reducing value of undegraded starch. Whereas for a free chain of 25 glucose units a reducing value equivalent to about 8% that of maltose would be expected, the observed values for starch are as a rule less than 0.5%. Similarly the molecular weights estimated from physical properties are very high. Osmotic pressure studies, for example, have indicated values of about 100,000-200,000 (75,19).

Haworth and coworkers have attempted to explain these

discrepancies by postulating a fundamental building unit of about 25-30 glucose molecules linked by an α -1:4-glucosidic bond, these fundamental units, in turn, being held in aggregates through some secondary bond (32). According to this picture the differences between different starches, starch fractions and partially degraded starches are due principally to differences in the degree of this aggregation.

These results should be contrasted with those obtained in the case of cellulose for which similar molecular weight values, ranging from about 100 to 1000 glucose units, have been obtained by methylation, reducing value and physical measurements (59). This striking difference between two polymers so closely related as starch and cellulose would hardly be explainable in the manner attempted by Haworth.

The way out of this dilemma was shown by the very excellent work of Staudinger and coworkers. In an attempt to apply the viscosity method to starch (82) it was shown that K_m was not constant but varied between the approximate limits 0.9×10^{-4} and 2.7×10^{-4} for a number of dextrans and modified starches. A value of 1×10^{-4} was more or less arbitrarily assigned on this basis. It was pointed out that K_m in the cellulose series is reasonably constant and of a magnitude about 8-10 times the value assigned in the case of starch. As a possible explanation of the lower magnitude of K_m , a helical configuration of the starch chains was suggested; this

would not, however, explain the variations between different fractions.

A much better explanation was suggested in a later paper by Staudinger and Husemann (83). They pointed out that the relatively constant amounts of tetramethyl glucose isolated from methylated starch could not be harmonized with the Haworth chain formula. Using formamide as solvent, they carried out viscosity and osmotic pressure determinations on several fractions prepared by mild acid hydrolysis of starch. These fractions were then acetylated and similar determinations carried out on the acetates in various organic solvents. Finally the acetates were saponified to give the regenerated starch fractions and measurements again made in formamide. The molecular weights checked in the three cases. Moreover, the value of K_m was approximately the same for both the original and regenerated materials.

These results cannot be reconciled at all with a micellar structure; on the contrary, a macromolecular structure is indicated. To explain the high yield of tetramethyl glucose these authors suggested a branched structure for starch. In this case the proportion of end-groups found would be a measure not of the molecular size, but of the degree of branching. It was further pointed out that dimethyl glucose, which would arise at the branching points, had usually been found in quantities approximately equivalent to the tetramethyl glucose.

A branched structure had previously been suggested for glycogen in order to explain the high yield of dimethyl glucose obtained (38,39). In that case the yield of both tetramethyl and dimethyl glucose is higher than in the case of starch, indicating a higher degree of branching.

Young and coworkers showed that starch could be disaggregated by mild hydrolysis with oxalic acid to a molecular weight of about 20,000 without any change in the endgroup analysis (10). From the kinetics of the hydrolysis it was concluded that the bonds broken were covalent and not hydrogen-bonds as had been formerly thought possible. They also investigated the dimethyl glucose obtained (8), showing, by means of the tosylation and sodium-iodide replacement reaction, which is specific for primary hydroxyl groups, that the dimethyl derivative obtained was the 2,3-isomer. This would indicate the branching to occur on the sixth carbon.

Freudenberg and Boppel (30) were similarly led to the conclusion that carbon six is the site of branching. They concluded that although both 2,3- and 2,6-dimethyl glucose are present in the hydrolysates from methylated starch, the former is the characteristic derivative, while the latter comes largely from degradation of 2,3,6-trimethyl glucose during the hydrolysis. They further concluded that the branching linkage is α -glucosidic.

Obviously the complexity of the starch system is greatly

increased if branching is present. One might expect not only a complete gradation of molecular sizes, but also a distribution as to degree of branching, ranging from perfectly straight molecules to very highly branched. Evidence that this is not the case will be brought out in the ensuing discussion of the fractionation of starch.

Fractionation of Starch

Perhaps the first fractionation of starch was that of de Saussure (20) who in 1819 isolated, from a starch paste which had stood for two years, a cellulose-like substance, insoluble in hot water, which he termed *ligneux amylicé* or "starch lignin".

Innumerable attempts have since been made to separate starch into definite components through fractional solution, electrophoresis, aging of pastes etc. The work has been handicapped from lack of methods for characterizing the fractions obtained. A complicating factor has been the phenomenon of retrogradation whereby the originally more soluble component becomes extremely resistant. The net result of this work has been a large mass of disorganized, and in some respects conflicting, data. This work up to about 1927 has been reviewed by Samec (75).

K. H. Meyer has recently investigated very carefully and

thoroughly the fractionation of starch on the basis of solubility in hot water (62,63-c). This method, first used by A. Meyer, divides starch roughly into resistant and soluble portions. These fractions have been variously termed alpha- and beta-amylose by A. Meyer, and amylopectin and amylose by Maquenne and Roux.

This method of fractionation consists essentially in heating a starch paste (usually 2%) to a temperature of about 70° C. and holding at constant temperature with slow stirring for approximately one hour. After allowing the insoluble material (amylopectin) to settle out, the clear supernatant liquid is kept under refrigeration for several weeks, the amylose crystallizing out of solution. By this method, the yield of amylose varies between the approximate limits 4 and 15%.

K. H. Meyer and coworkers have been able, on the basis of the newly suggested branching concept, to distinguish and characterize these components quite completely and, apparently, quite unambiguously. Through application of the methylation procedure these workers have shown that the yield of tetramethyl glucose from amylopectin is very nearly the same as from total starch, about 4%. The molecular weights as determined osmometrically and by reducing value, on the other hand, are very high, indicating a high degree of branching. The amylose fraction, however, yields only about 0.5-1.0% of end-

groups, and this yield checks reasonably well with the molecular weights determined by other methods. This fraction is hence concluded to consist essentially of straight chains. The properties of these fractions, tabulated by Meyer (63-c), are given in Table I.

Table I
Properties of Amylose and Amylopectin

Property	Unbranched fraction (amylose)	Branched fraction (amylopectin)
Molecular weight	10,000-60,000	50,000-1,000,000
Proportion in starch	10-20%	80-90%
End-group determination shows	1 end-group per molecule	1 end-group per 20-30 glucose units
Phosphorous content	Phosphorous free	Cereal amylopectin is phosphorous free; potato contains some chemically bound
Films of acetyl and methyl derivatives	Elastic, solid; like cellulose films	Brittle
Behavior toward water	Solid and liquid phases separate	Forms paste
Action of β -amylase	Up to 100% hydrolyzed	Up to 60% hydrolyzed leaving a "limit dextrin"
Reaction with iodine	Blue color	Violet to red-violet color

The low degree of digestion of amylopectin by β -amylase is explained by the branched structure. The enzyme, being apparently specific for the α -1:4-glucosidic linkage, would not be expected to attack the linkage at the branching point

(probably α -1:6). A short treatment with α -glucosidase of the residual dextrin so formed renders it susceptible to further β -amylase digestion. From this Meyer concludes amylopectin to have a highly ramified net-like structure, rather than a "herring-bone" type of branching.

The essentially amorphous nature of amylopectin and its inability to retrograde may be regarded as further evidence of a branched structure. Amylose, on the other hand, shows a tendency to form definite, anisotropic spherocrystals.

The effectiveness of the hot water method of fractionation is subject to question. It seems quite possible that much of the amylose might be so tenaciously held in the granule that it would not be extracted readily. This is indicated by the extreme variation in yield obtained by different workers and even by the same worker.

Recently new and apparently much more satisfactory techniques for carrying out this fractionation have been developed. Passu and Mullen (69) found that amylose is selectively adsorbed from a starch paste by such adsorbants as activated carbon, fuller's earth and alumina. Best results were obtained, however, with cotton. The adsorption complex is formed instantaneously in the cold but can be readily broken by means of hot water.

Schoch has shown (76) that through saturation of a dilute,

autoclaved starch suspension with n-butanol, the amylose can be precipitated as strongly birefringent spherocrystals. It now seems apparent that both this and the adsorption methods give much sharper fractionations of starch than the hot water extraction method.

Evidence that the Fractions of Starch are Sharp and Distinct - The Development of an Iodine Titration Method for the Determination of Amylose

Meyer's work, while of inestimable value in the investigation of starch, still does not answer the question of whether or not starch consists of two definite components, the one entirely linear in structure, the other highly branched. Rundle has shown (9) that if the synthesis of starch were entirely random, giving a complete statistical distribution of molecular species, both as to molecular size and degree of branching, only about 1.7% of unbranched molecules would be expected at most, in strong contrast to the amount found experimentally. Nevertheless, it can still be argued that there is a statistical distribution in the branching, and that the fractionation procedures used simply tend to separate the less highly branched from the more highly branched molecules. The large variation of the amount of amylose obtained by hot water extraction under different conditions might be offered as evidence for this concept.

However, there has recently been developed a powerful tool for actually determining the amount of amylose in a given material. This method, developed by Bates, French and Rundle (9), indicates beyond question of doubt that the amylose is an entirely distinct component, sharply different in its behavior toward iodine from the bulk of the starch.

In this method a given starch sample is dispersed in dilute alkali and the solution neutralized with hydriodic acid. If this solution is then titrated with dilute iodine in potassium iodide (of the same concentration as the iodide in the starch solution) it is found that the iodine-iodide potential remains essentially constant as long as there is free amylose in solution. In other words, the amylose tends to take up iodine at a characteristic iodine activity. Amylopectins, on the other hand, show much less tendency to take up iodine and do not give a flat region in the titration curve.

Using this method these workers have been able to compare the purity of amylose and amylopectin samples prepared by different methods. An amylose prepared by a combination of the Meyer and Schock techniques, the so-called "crystalline amylose" of Kerr (51), has been found to give the longest flat region and has hence been taken as a standard of purity. The amyloses prepared by butanol precipitation appear to be about 90% as pure as "crystalline amylose". With the amylopectins

it was found that in most cases a small percentage of amylose was left after butanol precipitation; however, one passage through a cotton column removed this very sharply.

Another very interesting point brought out by this work is the fact that the amyloses from various starches take up iodine at different characteristic potentials, and this variation has been ascribed to variations in chain length. Furthermore, if the amyloses from potato and corn are mixed they show two definite breaks, which would indicate that there is no overlap in the molecular size distributions of these materials. A much higher degree of homogeneity is indicated than one would ordinarily expect in such a polymer.

The amount of amylose present in various native starches, as shown by this method, varies from none in the case of the waxy starches to about 34% in the starch from lily bulbs. Most of the common starches, such as tapioca, corn, potato, rice and wheat, fall in the range 17-24% amylose. The absence of amylose in the waxy starches explains, at least in part, their markedly different properties.

Whether or not the waxy starches are simply pure amylopectin cannot be stated definitely. They show even less tendency to take up iodine than the amylopectins, their titration curves lying nearer to glycogen. This would indicate a higher degree of branching in the waxy starches than in the amylopectins.

The sharp difference in the iodine colors of amylose and amylopectin (9) should also be mentioned. The ordinary starch-iodine color is decidedly purplish - neither pure blue nor red. Amylose, however, prepared by the butanol method, gives a clear blue iodine color, with no trace of red. Amylopectins prepared by the butanol method give an essentially red color, with perhaps a trace of purple, but if they are purified by passing through cotton every trace of blue staining material is removed and a pure red staining material obtained. Waxy starches, too, stain pure red with iodine.

The evidence given can leave little doubt that amylose is a distinct component of starch. Moreover, it is apparently fairly homogeneous as to molecular weight. The homogeneity of the amylopectin fraction, however, with regard to degree of branching or chain length, cannot be considered established.

The Helical Configuration of Starch and its Relation to the Starch-Iodine Complex

A helical configuration of starch molecules was suggested by Hanes (32) to explain the preponderance of six-membered dextrans produced by α -amylase, as well as the formation of six- and seven-membered cyclic dextrans by the enzyme of B. Macerans. He further pointed out that dextrans of at least six units were necessary for the production of the typical iodine color.

Freudenberg (31), assuming a boat-shaped glucose ring, constructed models of starch chains, finding them to be essentially helical, with the hydroxyls directed outward. Essentially a tube with a hydrocarbon lining is formed. According to Freudenberg the blue color of the starch-iodine complex is to be explained by the binding of iodine within these tubes in an essentially hydrocarbon medium. Caesar and Cushing also constructed a model of an amylose chain consisting of 12 glucose units, showing its pronounced tendency to take on a helical configuration (16). In contrast the beta-linkage (as in cellulose) gave a zig-zag chain.

Bear (11) has recently shown a close resemblance between the X-ray diffraction pattern of the starch-iodine complex and the so-called "V" pattern, which is exhibited by starches and amyloses precipitated with alcohols. He further suggested that these patterns might result from a helical structure.

Further evidence of the close relationship existing between the "V" configuration and the iodine complex is given by the much greater tendency of materials in the "V" configuration to take up iodine vapor (74).

The strong dichroism of flow exhibited by solutions of starch-iodide (73) indicates an orientation of the iodine molecules in the direction of the major axis of the starch molecules in solution. Presumably the orientation is due to the lining up of the iodine molecules in the helices. Amylose-

iodide showed a strong dichroism of flow, while amylopectin-iodide showed none, indicating an essentially linear structure for the former and spherical structure for the latter.

That the amyloses precipitated by butanol exist in the helical configuration has been indicated by a study of their optical properties (74). By the same method of investigation, the crystallites of granular starch appear to exist in a linear configuration. Apparently iodine and alcohol play an important role in causing the chains to assume the helical configuration. That phenylhydrazine may also be bound in a manner analogous to iodine has recently been suggested (57).

IV. EXPERIMENTAL AND DISCUSSION

Materials Used

Butanol Fractions.

Amylose and amylopectin fractions from corn, potato and tapioca starch were prepared and supplied by T. J. Schoch. Their preparation and properties have recently been described (77).

As shown by iodine titration the amyloses were all approximately 90% pure (9). The potato amylopectin showed no trace of amylose in its iodine titration curve; corn and tapioca, however, showed some contamination.

For viscosity studies the amylopectins from corn and potato were purified by passing through cotton according to the method of Pacsu. This removed all traces of blue staining material. The corn amylopectin so purified was checked by iodine titration in the usual way, its titration curve indicating no trace of amylose.

The butanol precipitated fraction from lily bulb starch was prepared by F. L. Bates following the procedure of Schoch. Its purity was approximately the same as the other amyloses so prepared.

Crystalline Amylose from Corn Starch.

This material was prepared by R. W. Kerr and has been

described by him (51). It was considered to be pure amylose.

Hot Water Fractions from Corn Starch.

Corn starch was fractionated by extraction with hot water, following the procedure of K. H. Meyer (64). The starch (200 gm.) was pasted in one liter of water and added to nine liters of water at 66° C. The temperature was held at 65-66° C. for one hour and slow stirring maintained. At the end of this time the suspension was allowed to settle over night. The bulk of the supernatant liquid was siphoned off and the remainder filtered through a fluted filter. The residue was dried on a suction filter with methanol and ether, ground in a mortar and finally dried two days in vacuo at 60° C. The yield was 165 gm.

The entire filtrate was stored at approximately 10° C. for six weeks, being protected from microbiological action by a layer of toluene. Some precipitate formed, amounting to only 4.0 gm., or 2% of the total starch. The solution remained very turbid but no more precipitate formed, even upon repeated freezing and thawing.

This yield of amylose is very low, being about half the value reported by Meyer in his first work. The yield could doubtless be increased by carrying out the extraction at a higher temperature.

These fractions were checked for purity by means of iodine titration. The amylose took up the same amount of iodine as

"crystalline amylose" and was considered pure. The titration curve for the amylopectin fraction showed a flat portion approximately as long as in the case of starch itself, indicating that most of the amylose remained unextracted.

Waxy Maize Starch.

This starch was from Iowa Waxy 939 hybrid corn, milled February 7, 1942 by B. E. Starr.

Amylodextrin Fractions.

The action of strong acid on granular starch in the cold yields high-reducing dextrans which show a strong tendency to crystallize. Apparently in the hydrolysis of granular starch the amorphous regions (presumably containing all of the branching points) are preferentially attacked leaving the crystalline regions intact (28). The resulting dextrans hence contain practically no branching linkages.

F. L. Bates fractionated the amylopectin from corn starch by means of butanol and methanol. A first fraction was obtained by saturating the autoclaved solution with butanol alone, and successive fractions by progressively increasing the solubility of butanol by adding methanol.

Synthetic Starch.

By the action of the enzyme phosphorylase on glucose-1-phosphate, fairly high molecular weight glucose polymers are obtained with some properties approaching those of starch. The sample used in this investigation was supplied by W. Z.

Hassid and a description of its properties has recently been published (34).

Methylation results indicate this material to consist of long, straight chains or of loops, the yield of tetramethyl glucose being very low (34). Results of iodine titration indicate it to be amylose in nature, though the slope of the curve indicates considerably more heterogeneity than in the natural amyloses (9). Further evidence of its linearity is its tendency to retrograde and the fact that it is quantitatively hydrolyzed by β -amylase.

Glycogen.

The sample used was a commercial preparation, C.P. grade, obtained from the Pfanstiehl Chemical Co.

Limit Dextrin from Waxy Maize.

The limit dextrin was prepared by C. G. Caldwell and has been described by him (17). It was prepared by twice digesting waxy maize starch with β -amylase and precipitating in 60% alcohol. The recovery amounted to 39.5% of the original starch on the dry basis.

Schardinger Beta-Dextrin.

This material was prepared and ten times recrystallized by D. French.

Acetylated Derivatives.

All acetylations were carried out by means of pyridine and acetic anhydride which have been shown to produce no

degradation of starch (83). In the earlier preparations the reactants were merely placed in a glass-stoppered flask and allowed to stand until complete dispersion was obtained. This required from a few days to several weeks depending on the material.

Recently there has been proposed a modification of this method in which the carbohydrate is first dispersed in azeotropic pyridine, the azeotrope replaced by adding anhydrous pyridine and distilling, and then the acetylating agent added (67). The actual acetylation is carried out at the boiling point of pyridine (115° C.), at which temperature complete reaction is obtained in a few minutes. This method was tried. However, since the treatment with azeotropic pyridine is designed primarily to disrupt the granules of the starch and as most of the materials here used were no longer granular, it was found possible to eliminate this step.

The carbohydrate was simply placed in a flask equipped with ground-glass reflux condenser, dry pyridine added (about 15 parts by weight) and heated to boiling. The acetic anhydride (in slight excess) was then added dropwise through the condenser and reflux continued until complete dispersion was obtained. The entire process required only about two hours, the reaction itself only about 30 minutes.

Isolation was carried out in the usual manner, namely, by pouring the reaction mixture into cold water with stirring,

filtering and washing. In all cases products completely soluble in chloroform were obtained by this method, and in yields of 90% theoretical or better. The method was also found satisfactory with granular wazy maize starch.

Solution Viscosities

Experimental Procedure.

Solvent. Obviously for the determination of any solution property, true molecular dispersion is the first requirement. In the case of starch few good dispersing media are available. Meyer recommends hydrazine hydrate and ethylenediamine hydrate (63-c). Formamide has also been used.

Ethylenediamine was chosen for these studies. However, in order to reduce the complexity of the systems involved, it was deemed wise to use the anhydrous solvent rather than the hydrate. This introduces complications from the practical standpoint due to the extreme affinity of anhydrous ethylenediamine for both H_2O and CO_2 . Great care was exercised at all times to prevent more than momentary exposure of solvent and solutions to the atmosphere.

Eastman ethylenediamine (95-100%) was dried, first by refluxing over anhydrous KOH to remove the bulk of the water, then over metallic sodium until practically no reaction could be observed in the cold (there is apparently some slow reaction between sodium and ethylenediamine). The solvent was then

distilled from sodium in an all glass apparatus, completely enclosed and protected from the atmosphere by means of a calcium chloride tube. This dehydration was carried out in quantities just large enough to prepare a series of solutions (usually four to six) with enough left over for the determination of the time of flow of the solvent (t_0).

In spite of the precautions taken in dehydration it was found that the time of flow of the solvent varied as much as one per cent from sample to sample, hence the necessity for determining the t_0 each time.

Preparation of Solutions. The carbohydrate samples were dried for 12-18 hours in vacuo at 60° C. and weighed directly into glass-stoppered volumetric flasks (10 or 25 ml. capacity). The dehydrated solvent was added to volume, working in a transfer box containing an atmosphere of dry nitrogen. After stoppering, the flasks were placed in a desiccator over P_2O_5 and permitted to stand until solution was complete.

The time required for solution varied considerably for different materials. All of the amyloses gave clear solutions inside a few days, potato amylose being the slowest to dissolve. Synthetic starch went in rapidly, but a small amount of flocculent material was left unattached (probably negligible). Glycogen went in very rapidly as did the limit dextrin. Corn amylopectin required a few days but gave clear solutions. Waxy maize starch was very slow (probably due to its granular

structure) requiring two or three weeks. The resulting solutions were only slightly opalescent. The amylopectins from potato and tapioca starch did not give clear solutions, even after standing for months, a small amount of residual gelatinous material remaining which made the determination of their viscosities impossible.

Filtration. It was found imperative, in order to obtain consistent results, to filter the solutions just before determining the flow time. This filtration was at first carried out in the nitrogen chamber but later this was found unnecessary if the solution was protected from the atmosphere during the filtration by means of a tube containing calcium chloride and soda-lime. By using a large test tube (4X24 cm.) as filtering flask the filtrate could be caught directly in the viscosity pipette.

In the case of the amyloses the choice of a filter was no problem, almost any fritted-glass filter serving. Medium grade Pyrex crucibles served excellently. With the amylopectins, however, the filtration was difficult. Medium grade Pyrex filters were extremely slow. Porous porcelain filters were tried, the medium grade being too slow and the course apparently not fine enough, irregular flow rates being observed. A Jena G-4 filter was the most satisfactory of any tried. Waxy maize starch and tapioca and potato amylopectin solutions could not be filtered satisfactorily through any medium.

Determination of the Time of Flow. All measurements were made at atmospheric pressure in a standard Ostwald viscosity pipette having the approximate capillary dimensions 0.05X9 cm. and total capacity about 4 ml. The time of flow for anhydrous ethylenediamine in this pipette was approximately 3.0 minutes. Measurements were made at approximately 30° C. in a water thermostat, insulated from vibration and maintained constant to $\pm 0.04^\circ$ C. by means of a mercury thermoregulator and relay.

The pipette was clamped in the bath and vertical alignment assured by sighting from two directions at vertical members in the water bath. Both outlets of the pipette were closed with tubes containing calcium chloride and soda lime to protect the solution from the atmosphere during the measurements. The drying tube on the capillary side was attached through a two-way stopcock to an aspirator bottle. In this way the solution could be drawn up and then permitted to fall by simply turning the stopcock. The drying tubes did not exert enough resistance to air flow to affect the time of flow appreciably as shown by checks without them.

The time of flow was measured by means of a stop-watch graduated in hundredths of a minute, and the values were estimated to thousandths of a minute. It was found wise to discard results unless at least three checks within 0.1% were obtained. This required numerous runs in some cases, especially with the amylopectins. Deviations of more than 0.1% from the

mean value were always in the positive direction (i.e., greater flow time). This is to be expected since all of the experimental difficulties tend to slow the flow rate. Hence in case two or even more checks were obtained but another lower value found, the checks were discarded and more runs made.

Results and Discussion.

The results are tabulated in Table II and shown graphically in Figures 1,2,3,4,5 and 6.

Concentration Dependence of Viscosity. The extreme dependence of the function η_{sp}/C on concentration is apparent from a study of the figures. Moreover the magnitude of this concentration dependence is not constant but increases, in general, with increasing intercept. Obviously it is essential to carry out careful extrapolations to infinite dilution in any attempt to correlate these results with molecular weights. The Arrhenius function, $(\ln \eta_R)/C$, though better than the Staudinger function in this respect, is still far from constant.

The relationship between slope and intercept in the amylose series is especially interesting. In Figure 7 the slope of the first portion of the η_{sp}/C vs. C curves (Fig. 3, up to concentration 0.3%) is plotted as a function of the intercept. The experimental points can be well fitted by a straight line, represented by the equation

$$B = 0.15 A - 0.96 \quad (XXX)$$

where B is the slope and A the intercept of the viscosity-con-

centration curve. Substituting into the equation

$$\eta_{sp}/C = A + BC \quad (XXXI)$$

the expression

$$\eta_{sp}/C = A + (0.15 A - 0.96) C \quad (XXXII)$$

is obtained. By means of this equation the limiting value of η_{sp}/C could be estimated by determining its value at one concentration below 0.3%.

Equation (XX), which was derived on a theoretical basis by Huggins, predicts B in equation (XXXI) to involve the square of the limiting value (i.e., $[\eta_{sp}/C]_{C \rightarrow 0}^2$) rather than the first power. The experimental points in Figure 7 can apparently be equally well fitted by an equation of this type as shown by the dotted curve. Figure 3 shows excellent agreement with Equation (XIX) which is merely a more general form of Equation (XX). Most of the values of k' computed from the graph lie between about 0.5 and 0.7. For the lower curves the values spread rather badly as shown by Table III. However, in this region the experimental error is relatively high and a very slight change in either the slope or intercept will greatly affect the value of k' .

Table II

Summary of Viscosity Results

Fraction	Conc.	η_R	$(\ln \eta_R)/c$	η_{sp}/c
Potato Amylose (Butanol method)	0.0576	1.165	2.63	2.85
	0.110	1.341	2.67	3.10
	0.134	1.441	2.73	3.29
	0.200	1.728	2.74	3.64
	0.326	2.353	2.63	4.15
	0.626	4.254	2.31	5.20
Tapioca Amylose (Butanol method)	0.0648	1.150	2.16	2.32
	0.126	1.324	2.23	2.57
	0.291	1.857	2.13	2.94
	0.640	3.161	1.80	3.38
Lily Amylose (Butanol method)	0.117	1.155	1.23	1.33
	0.183	1.263	1.27	1.44
	0.373	1.599	1.26	1.61
	0.565	1.969	1.20	1.72
	0.785	2.382	1.11	1.76
Corn Amylose (Butanol method)	0.0560	1.053	0.922	0.947
	0.0968	1.095	0.941	0.982
	0.356	1.432	1.01	1.21
	0.398	1.479	0.983	1.20
	0.696	1.944	0.956	1.36
	0.996	2.504	0.923	1.51
Corn Crystalline Amylose (Kerr's method)	0.0568	1.030	0.521	0.528
	0.161	1.084	0.501	0.522
	0.388	1.215	0.501	0.555
	0.638	1.343	0.463	0.538
Corn Amylose (Meyer's method)	0.152	1.050	0.321	0.329
	0.207	1.058	0.272	0.280
	0.370	1.109	0.279	0.295
	0.450	1.129	0.270	0.287
	0.486	1.172	0.327	0.354
	1.155	1.398	0.290	0.345

Table II (Continued)

Fraction	Conc.	η_R	$(\ln \eta_R) / c$	η_{sp} / c
Corn Amylose (Prepared by KOH dispersion)	0.472	1.627	1.03	1.33
	0.775	2.099	0.958	1.42
	0.165	1.175	0.979	1.06
Fraction #3 of Amylodextrin	0.137	1.017	0.121	0.124
	0.216	1.029	0.131	0.134
	0.492	1.072	0.142	0.146
0.960	1.143	0.139	0.149	
Fraction #4 of Amylodextrin	0.139	1.017	0.119	0.122
	0.306	1.034	0.108	0.110
	0.442	1.051	0.113	0.115
0.800	1.094	0.113	0.117	
Mixture of 62.5% Cryst. Amylose & 37.5% Amylodextrin Fraction #3	0.175	1.067	0.371	0.382
	0.449	1.181	0.371	0.403
	0.664	1.275	0.366	0.414
Synthetic Starch	0.130	1.029	0.218	0.223
	0.259	1.064	0.241	0.248
	0.332	1.111	0.317	0.324
0.516	1.164	0.295	0.318	
0.723	1.298	0.361	0.412	
Corn Amylopectin	0.066	1.079	1.16	1.20
	0.160	1.218	1.23	1.36
	0.322	1.492	1.24	1.53
0.460	1.789	1.26	1.72	
0.560	2.049	1.28	1.87	
0.777	2.896	1.37	2.44	
Waxy Maize Starch	0.0913	1.115	1.19	1.26
	0.249	1.343	1.18	1.38
	0.508	1.931	1.29	1.83
Glycogen	0.180	1.057	0.308	0.317
	0.483	1.095	0.189	0.196
	0.864	1.101	0.113	0.118
Waxy Limit Dextrin	0.102	1.017	0.164	0.167
	0.376	1.067	0.171	0.178
	0.822	1.157	0.177	0.191

Table II (Continued)

Fraction	Conc.	η_R	$(\ln \eta_R)/c$	η_{sp}/c
Schardinger β -Dextrin	0.173	1.013	0.074	0.075
	0.479	1.035	0.072	0.073
	0.604	1.038	0.062	0.063
	1.245	1.069	0.054	0.055

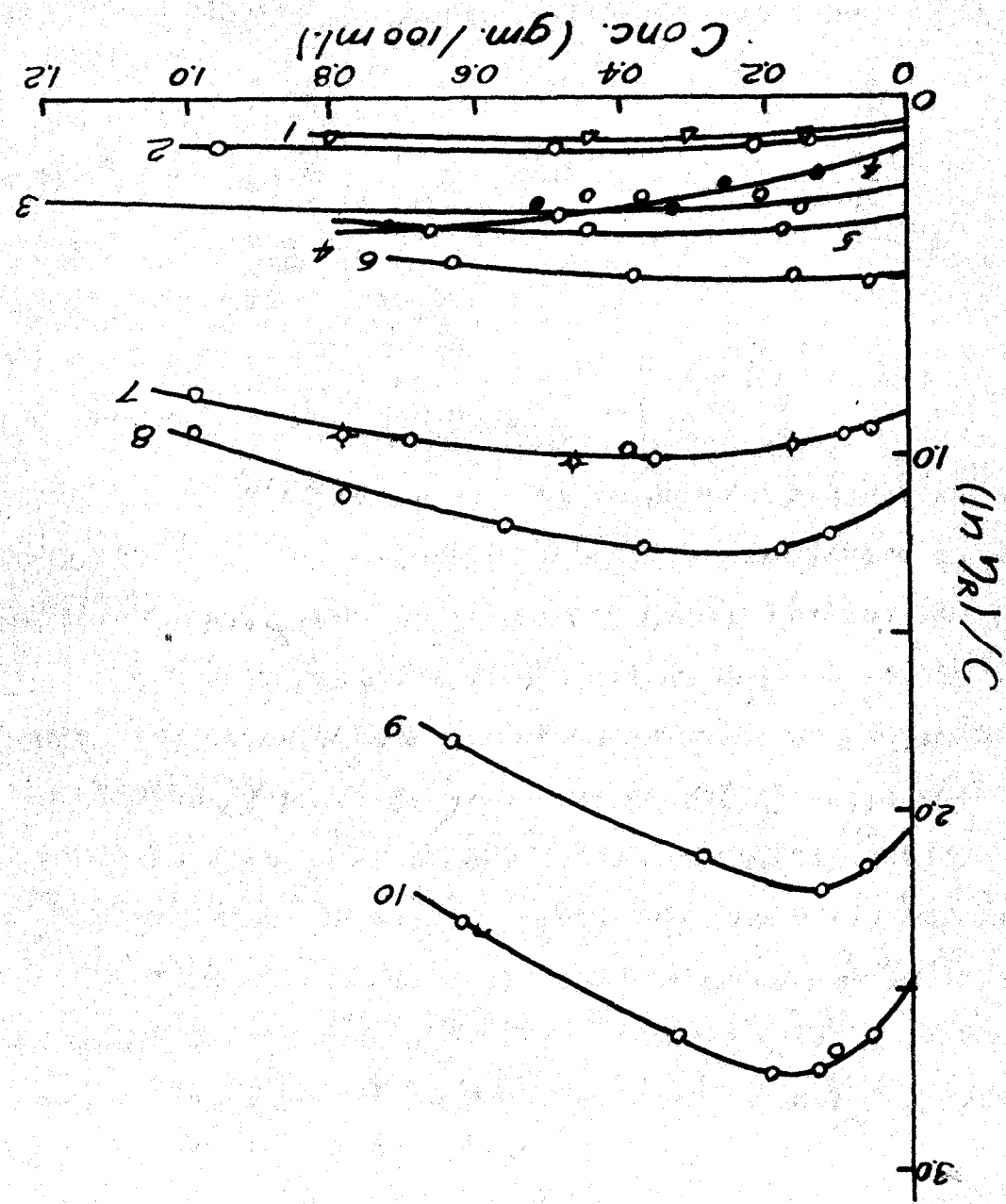
Table III

Value of the Constant k' in Huggins' Equation

Material	Limit of η_{sp}/c	Slope	k'
Potato amylose	2.58	1.60	0.62
Tapioca amylose	2.11	1.42	0.67
Lily amylose	1.24	0.64	0.52
Corn amylose	0.91	0.64	0.70
Crystalline amylose	0.53	0.08	1.51
Mixture	0.37	0.10	0.27
Meyer's amylose	0.31	0.10	0.32
Amylodextrin Fraction 4	0.11	0.10	0.91
Amylodextrin Fraction 3	0.12	0.20	1.67

1, amylopectin fraction #1; 2, amylopectin fraction #3; 3, corn amylose (Meyer's method); 4, synthetic starch; 5, mixture of crystalline amylose and amylopectin; 6, crystalline amylose; 7, corn amylose (butanol); 8,illy amylose (butanol); 9, tapioca amylose (butanol); 10, potato amylose (butanol); \diamond = corn amylose prepared by KOH dispersion; \bullet = synthetic starch; ∇ = amylopectin fraction #4.

FIG. 1. Concentration dependence of the Arrhenius function for the amyloses



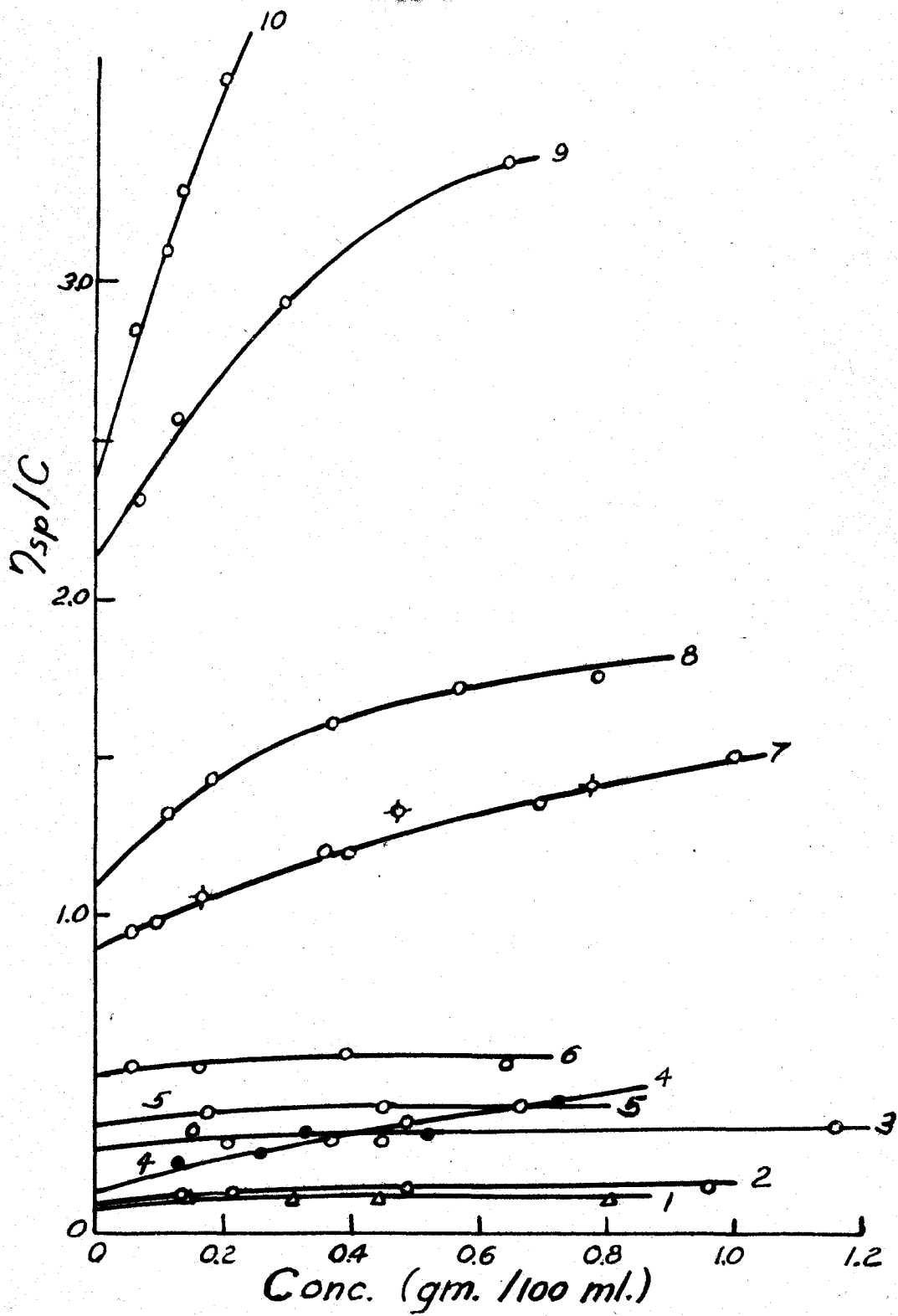


Fig. 2. Concentration Dependence of the Staudinger Function for the Amyloses

Designation of curves and experimental points is the same as in Fig. 1.

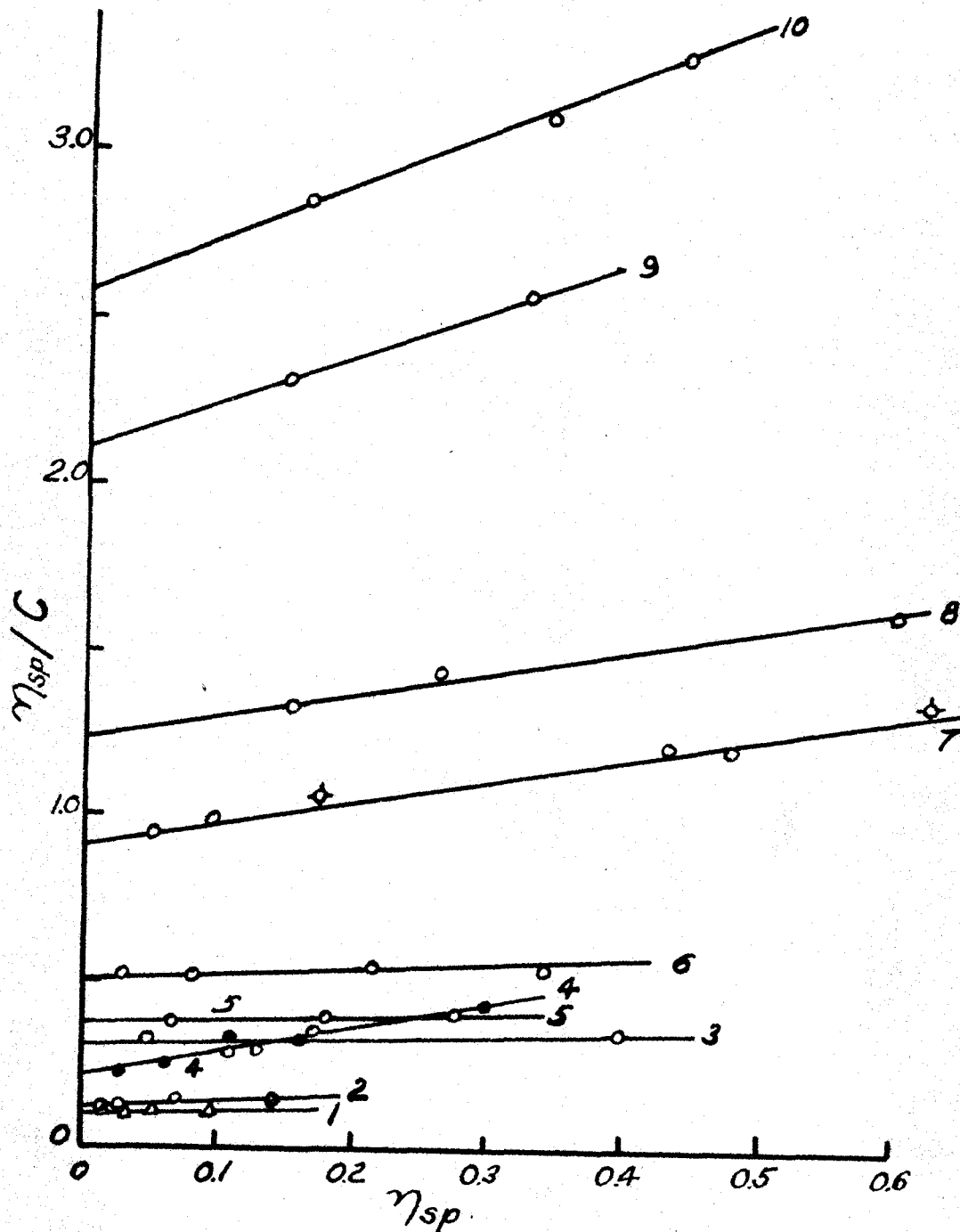
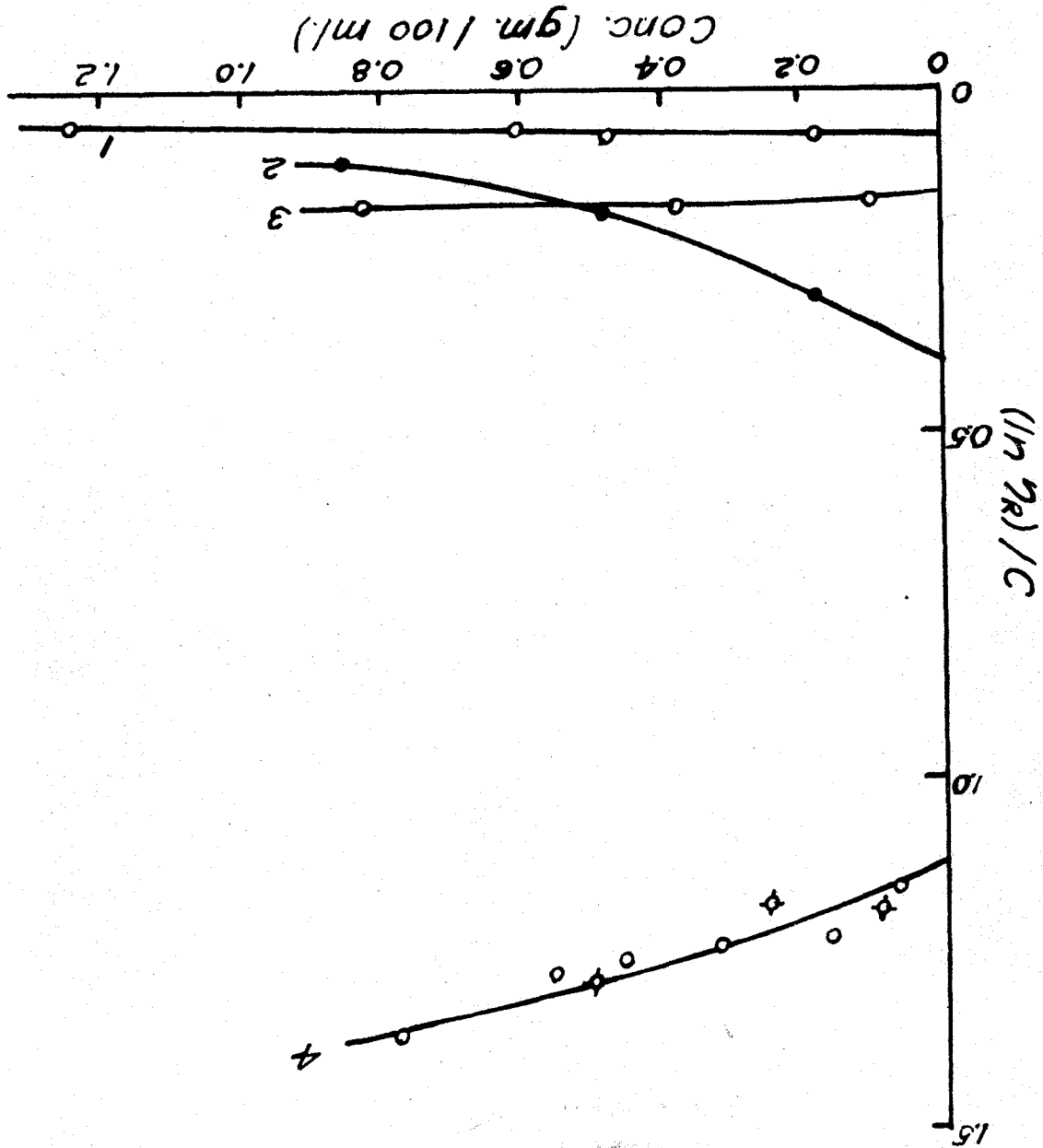


Fig. 3. Relationship between η_{sp}/c and η_{sp} for the Amyloses

Designation of curves and experimental points is the same as in Fig. 1.

FIG. 4. Concentration Dependence of the Arrhenius Function for some Non-Linear Carbohydrates

1, Schardinger β -dextrin; 2, Glyogen; 3, Limit dextrin; ϕ = waxy maize starch; \bullet = Glyogen; 4, corn amylopectin and waxy maize starch.



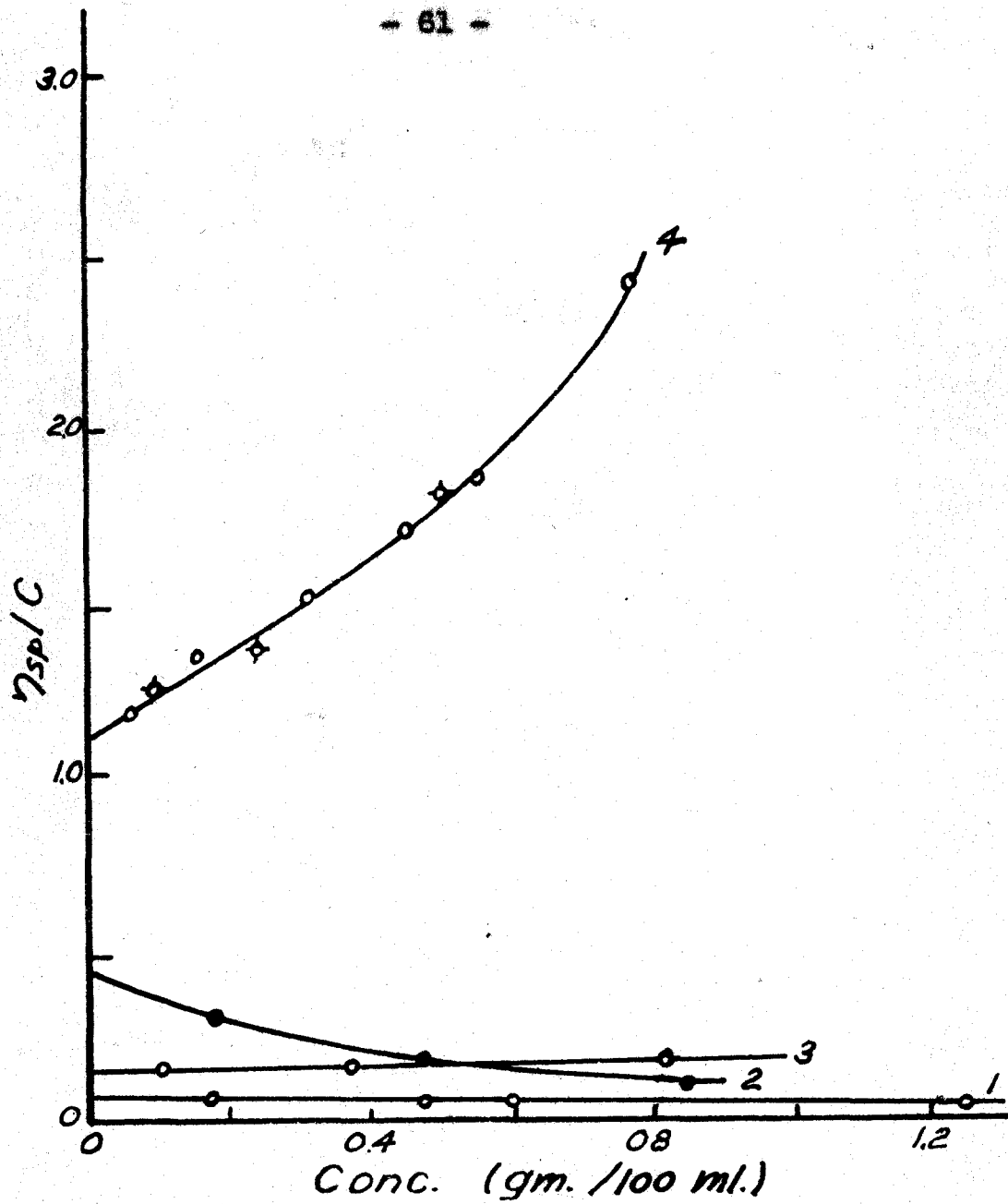


Fig. 5. Concentration Dependence of the Staudinger Function for some Non-linear Carbohydrates

Designation of curves and experimental points is the same as in Fig. 4.

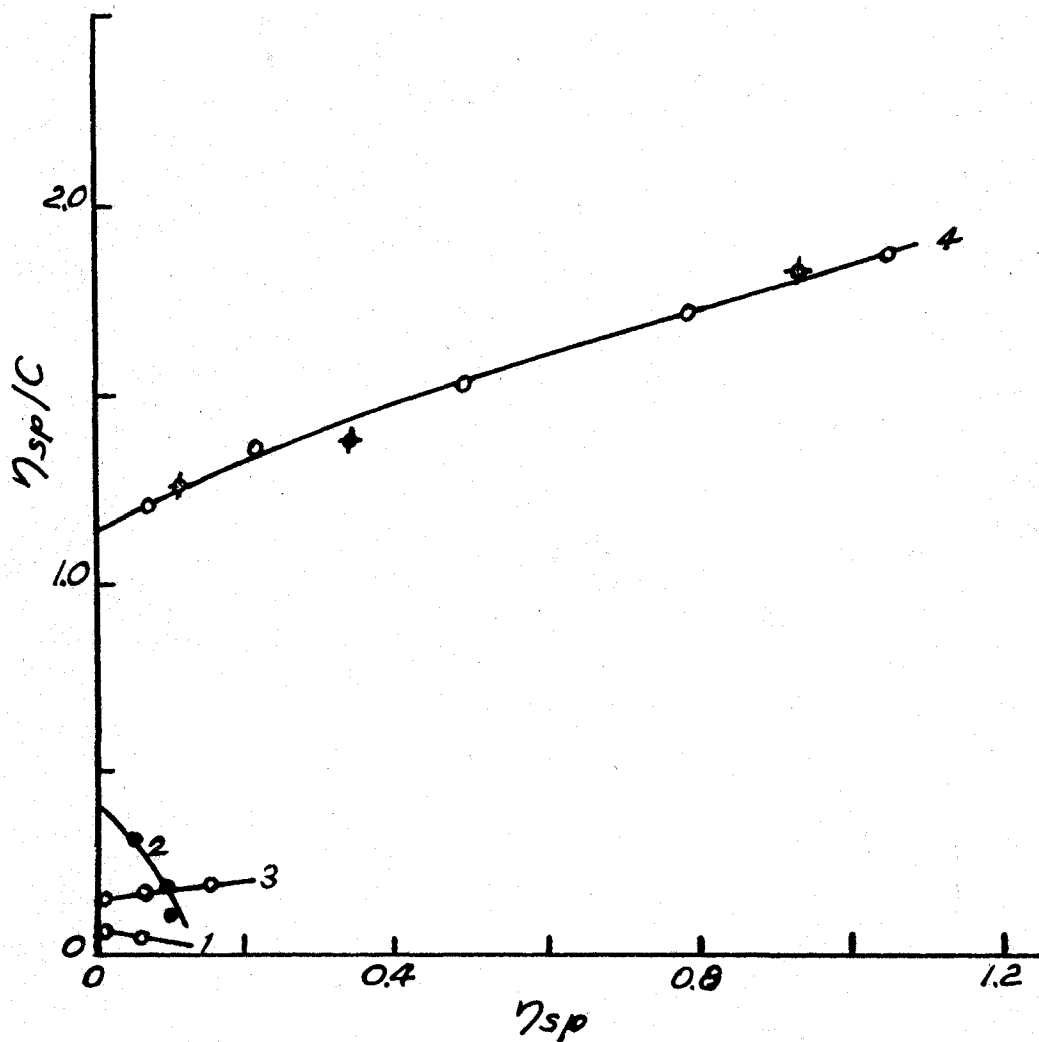


Fig. 6. Relationship between η_{sp}/C and η_{sp} for some Non-linear Carbohydrates

Designation of curves and experimental points is the same as in Fig. 4.

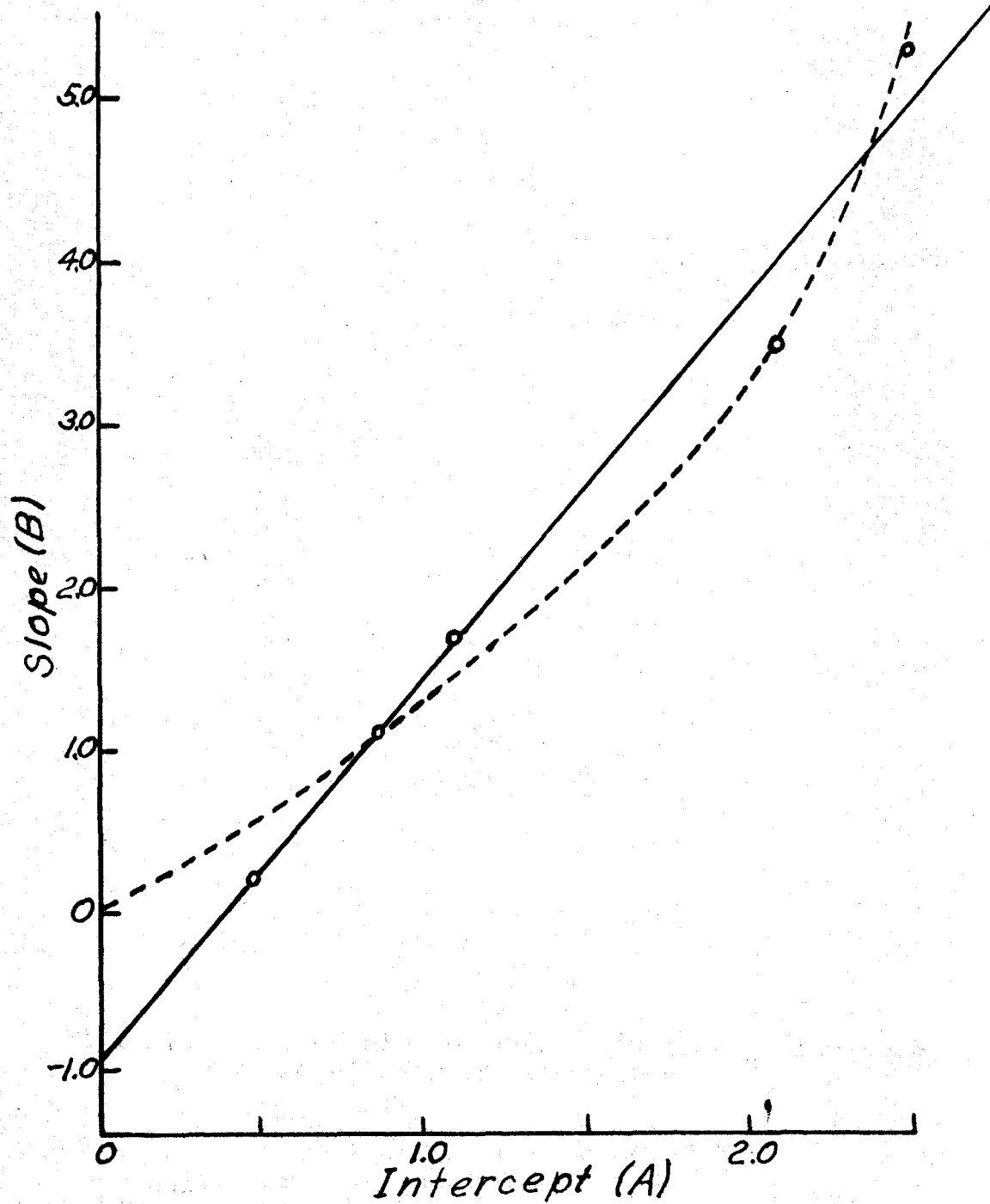


Fig. 7. Relationship between Slope and Intercept of the Graph of η_{sp}/c vs. c for the Amyloses (taken from Fig. 2)

Anomalous Slope of the Synthetic Starch Viscosity Curve.

From an inspection of Figures 1, 2 and 3 it is apparent that the concentration dependence of synthetic starch is greater than for a pure amylose of similar intercept. In view of the previously mentioned evidence from iodine titration that synthetic starch is far more heterogeneous than the amyloses, this anomalous slope might be attributed to the heterogeneity. However, in view of the preceding section, the slope depends only on the intercept which in turn is presumably a function only of the weight-average molecular weight for a linear polymer (6). If this is true the lack of homogeneity should have no effect on the slope. To confirm this prediction a mixture consisting of 62.5% of "crystalline amylose" and 37.5% of the third amyloextrin fraction was run, the slope conforming with the pure amyloses.

Another possible explanation is the presence of a small amount of branched material. The results of iodine titration, however, indicate almost complete absence of amylopectin. It is conceivable that branched material is present in which the branches are so long as to titrate similarly to amylose. This possibility should not be overlooked.

A third possibility, and the one which seems most tenable at present, is that extraneous groups which increase the polymer-polymer interaction are present in synthetic starch. In

view of the method of preparation (from glucose-1-phosphate) phosphate groups would be expected and it would not be unlikely that these groups would lead to increased interaction. However, the phosphorous content reported for this material, namely 0.09% (34) would correspond to only one phosphate group to about 200 glucose units.

Significance of the Limiting Viscosities of the Amyloses.

The question of absolute molecular weights of the amyloses will be discussed in a later section. However, it is interesting at this point to note and speculate on the wide variation in the limits of the function η_{sp}/C or $(\ln \eta_R)/C$ for amyloses from various starches, and from the same source when prepared by different methods.

The differences in limits for the various corn amyloses is especially interesting. Extraction of starch granules with hot water according to the Meyer method would be expected to remove preferentially the shorter, more soluble chains. This is reflected in the low limiting viscosity for this material. It should be remembered that this sample consisted of only about 10% of the total amylose present in the starch.

In the same way, butanol would be expected to precipitate longer chains first and the amylose so prepared has a relatively high viscosity. "Crystalline amylose", prepared by a combination of the other two techniques, has an intermediate viscosity as would be predicted.

It is interesting to speculate on whether this heterogeneity of molecular sizes is present in the amylose as it occurs naturally, whether it is a result of the milling processes, or possibly a result of the fractionation process itself. In the usual milling process for corn SO_2 is used as a preservative during steeping. Possibly this produces some degradation and any random degradation would produce heterogeneity. Similarly it is quite conceivable that the autoclaving of an unbuffered starch paste or even extraction of starch with hot water might cause a small amount of hydrolysis. In terms of the per cent of linkages hydrolyzed the amount of hydrolysis necessary to reduce greatly the mean molecular size of a long glucose chain is very small. However, the amylose prepared by dispersing the starch with KOH rather than autoclaving shows the same viscosity within the experimental error, indicating the hydrolysis during autoclaving to be negligible.

The possibility of the naturally occurring amyloses from various sources being identical as to chain length should be considered. It would simplify the starch picture if amylose could be considered as such an entity. It seems extremely unlikely, though, that the differences between the materials studied could be due to differences in treatment. The previously mentioned evidence from iodine titrations that the size distributions of potato and corn amylose do not overlap would

almost eliminate this possibility. However, it should be noted that the butanol precipitated amyloses seem to fall into two groups, potato and tapioca being much more viscous than lily and corn, and the possibility of there being two definite amyloses should not be ruled out. The difference between corn and lily amyloses, for example, could be due to random hydrolysis. The steeping with SO_2 was not used in the preparation of the lily starch and could easily account for its slightly higher viscosity.

Equality of the Staudinger and Arrhenius Functions at Zero Concentration. An inspection of Figures 1 and 2 shows a close relationship between the limiting values of the Staudinger and Arrhenius functions, η_{sp}/C and $\ln \eta_R/C$. It has been pointed out that their limits are identical though no proof was given (36). Since the reason for this equality is not readily apparent from the nature of the functions it does not seem out of place to demonstrate equivalence here.

Since

$$\eta_R = \eta_{sp} + 1$$

then

$$\ln \eta_R = \ln (\eta_{sp} + 1) .$$

The logarithm can now be expanded in a McLaurin series in powers of η_{sp} about the point $\eta_{sp} = 0$ (this is legitimate since as the concentration goes to zero η_{sp} also goes to zero).

Hence

$$\begin{aligned} \ln \gamma_R &= [\ln (\gamma_{sp} + 1)]_0 + [1/(\gamma_{sp} + 1)]_0 \gamma_{sp} \\ &\quad + 1/2! [-1/(\gamma_{sp} + 1)^2]_0 \gamma_{sp}^2 + \dots \\ &= 0 + \gamma_{sp} - 1/2! \gamma_{sp}^2 + \dots \end{aligned}$$

$$\therefore \lim_{c \rightarrow 0} \ln \gamma_R = \lim_{c \rightarrow 0} \gamma_{sp}$$

and $\lim_{c \rightarrow 0} (\ln \gamma_R)/c = \lim_{c \rightarrow 0} \gamma_{sp}/c$

Osmotic Pressure Studies

Membranes.

As previously mentioned the choice of suitable membranes is perhaps the biggest single problem in carrying out osmotic measurements. In this work several membranes have been tried with more or less success, none proving perfectly satisfactory.

The commercial Zeigmondy ultracellafilters¹ were found satisfactory for aqueous solutions of high molecular weight materials such as modified starches. They were somewhat permeable, however, to low molecular weight dextrans and were entirely unsatisfactory in the case of organic solvents, showing a tendency to wrinkle badly.

1. These were obtained from Pfaltz and Bauer, Inc., New York, diameter about 7 cm., designated ultrafine-medium, 25-30 minutes.

Numerous attempts were made to prepare collodion membranes, both simple collodion films as described by Montonna (66) and collodion-impregnated cellulose films. The former were extremely hard to prepare free of wrinkles and defects. The permeability of both types to water was low and no films could be prepared which were completely impermeable to low molecular weight dextrans. For use with organic solvents these films must be denitrated. However, since films of satisfactory and uniform porosity could not be obtained the denitration was not attempted.

Ordinary cellophane¹ was found to be fairly satisfactory as a membrane material. Untreated it has an extremely low permeability to organic solvents. Swelling in water or water and alcohol greatly increases its permeability as previously pointed out (58). However, upon standing a few hours in contact with organic solvents the permeability seemed to fall off considerably. Another fault of these membranes was their extreme tendency to wrinkle as they lose solvent by evaporation during the assembling of the cell. In spite of these difficulties a number of measurements were made using cellophane.

1. Cellophane samples were supplied by the Cellophane Research Division of E. I. du Pont de Nemours and Company, Wilmington, Delaware, in three sizes, numbers 300, 450 and 600. This material contained glycerol as a plasticizer.

By far the most satisfactory membrane material found was ordinary parchment paper. The permeability of this material is much higher than that of cellophane giving much greater flow rates and more rapid attainment of equilibrium (usually less than one hour as compared to four to eight hours with cellophane). In spite of its increased permeability to solvent this material appeared in most cases to be completely impermeable to the solute molecules used in this investigation. A few parchment discs did seem to be somewhat permeable to the lower molecular weight materials such as the acetates of the amyloses and limit dextrin. This material is sufficiently rigid to eliminate the difficulty caused by wrinkling exhibited by the other materials.

Procedure.

The osmotic cell is described in detail in Appendix I. It worked on the counter pressure principle and was patterned after the previously mentioned cell of Hepp. The pressure system consisted of two leveling bulbs, one mounted on a clamp for coarse adjustment and the other on a screw device which provided a fine adjustment. The range of pressures available was from about plus 60 mm. of water to about minus 250 mm. Pressures were read by means of a water manometer attached to the pressure system. This whole device was mounted permanently on a wooden frame.

Prior to assembling the cell the membrane to be used was thoroughly soaked in the solvent (usually chloroform). The cell was placed in position on the wooden frame and the capillary attached to the pressure arrangement by means of a short piece of pressure tubing, using a little glycerol as lubricant.

The cell was filled by running solvent into the upper end of the capillary with a medicine dropper and drawing it back and forth several times by varying the pressure, in order to eliminate air bubbles. A disc of bolting silk, slightly smaller in diameter than the upper brass ring and soaked in solvent, was then placed on the brass plate inside a penciled guide line. The membrane was quickly layed in place and clamped down with the brass ring by means of six 1/4" machine bolts. These bolts were pulled up quite tightly in order to prevent leakage around the membrane.

When using chloroform as solvent it was necessary to pour a large excess on the brass plate and silk before clamping, due to the extremely rapid evaporation. This was not necessary with higher boiling solvents such as dioxane and tetrachloroethane; however, they did not prove to be such effective solvents for the acetates as chloroform and hence all the results herein reported were determined in the latter solvent.

After clamping the membrane in place the upper part of the cell was about half filled with solvent, the membrane covered with a disc of bolting silk and the grooved brass weight placed in position. The latter served to minimize upward motion of the membrane. Solvent was added until it just began to ooze out around the edge of the brass weight. The entire upper chamber was now covered with an inverted glass funnel fitted with a solvent trap that was designed to minimize evaporation¹.

No thermostat was used, all measurements being made at room temperature (25-27° C.). This deviation in temperature would produce a maximum variation in the observed pressure of not over one per cent, less than the experimental error. It was found expedient to shelter the entire apparatus from drafts which tended to cause irregularities in flow.

The zero point was determined shortly after assembling. In general it would rise somewhat, consequently the solvent was left in the cell until the reading remained constant for a period of about an hour. Without dismanteling the cell the solvent was now removed with a medicine dropper, the membrane, cell, silk disc and brass weight dried with filter paper and the cell filled with solution.

Flow rates were determined by following the meniscus with

1. In the later measurements this trap was replaced with a capillary tube of small bore which proved to be just as effective, if not more so.

a low power microscope equipped with a cross hair and mounted on a micrometer which could be read to 0.01 mm. The average flow rate over a period of five to ten minutes was usually taken in order to iron out any irregularities. The difference between zero reading and equilibrium point when filled with solution was taken as the osmotic pressure. Figure 8 shows an example of the flow curves obtained. In some cases, especially with cellophane membranes, the curves were S shaped rather than linear, apparently due to resistance of the membrane to flow.

Results and Discussion.

Results of the osmotic pressure measurements are summarized in Table IV and in Figure 9. The experimental data there given represent only a small proportion of the values determined. Numerous values were discarded because the cell was obviously not functioning properly. In addition, however, many values were discarded solely on the basis of not correlating with values found at other concentrations. The fact that there is no good criterion for keeping or discarding data coupled with the fact that so many erratic values are obtained constitutes the chief uncertainty in these measurements.

Table IV
Summary of Osmotic Pressure Results

Material	Conc. ^a	P ^b	P/O	Membrane
Corn Amylose Acetate	0.237	9	39	Parchment
	0.474	21	45	"
	0.86	46	53	Cellophane
	0.86	49	57	"
	1.44	92	64	"
	2.40	214	89	"
	2.48	234	94	"
Corn Amylopectin Acetate	0.200	4	20	Parchment
	0.402	13	32	"
	0.402	18	45	"
	0.402	17	42	"
	0.600	28	47	"
	0.600	32	53	"
	1.00	72	72	"
	1.00	67	67	"
	1.00	74	74	"
1.00	73	73	"	
Waxy Maize Acetate	0.198	5	25	"
	0.397	19	48	"
	0.599	34	57	"
	0.999	76	76	"
Tapioca Amylose Acetate	0.197	4	20	Cellophane
	0.197	4	20	Parchment
	0.996	33	33	"

^aConcentration in gm./100 ml.; ^bPressure in mm. H₂O

Substituting numerical values into van't Hoff's equation (XXI) and solving for M the relation

$$M = 8480 T [O/P] \quad (XXXIII)$$

is obtained, in which the quantity enclosed in brackets is

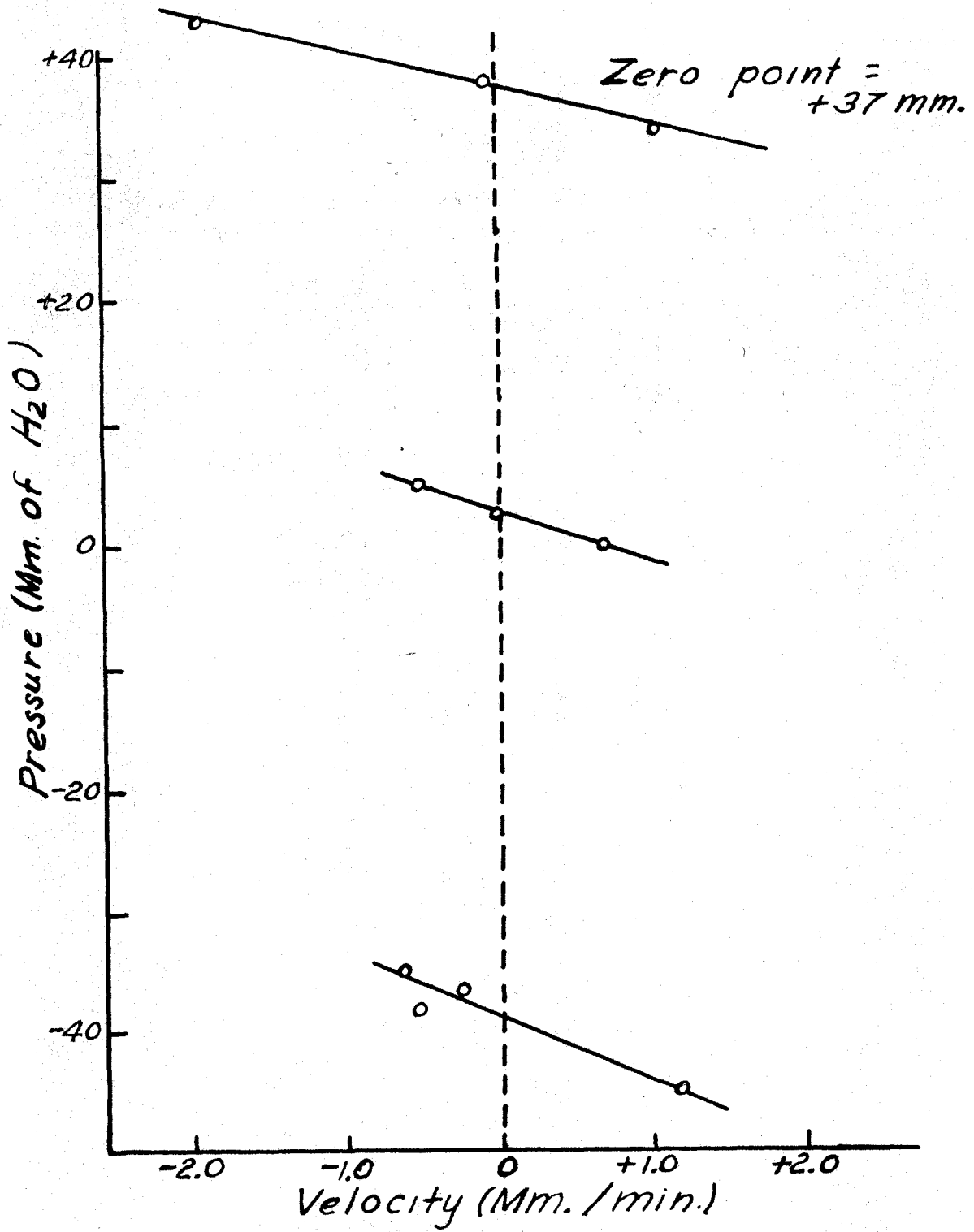


Fig. 8. Example of the Flow Rate Curves Obtained with the Modified Hepp Micro-Osmometer

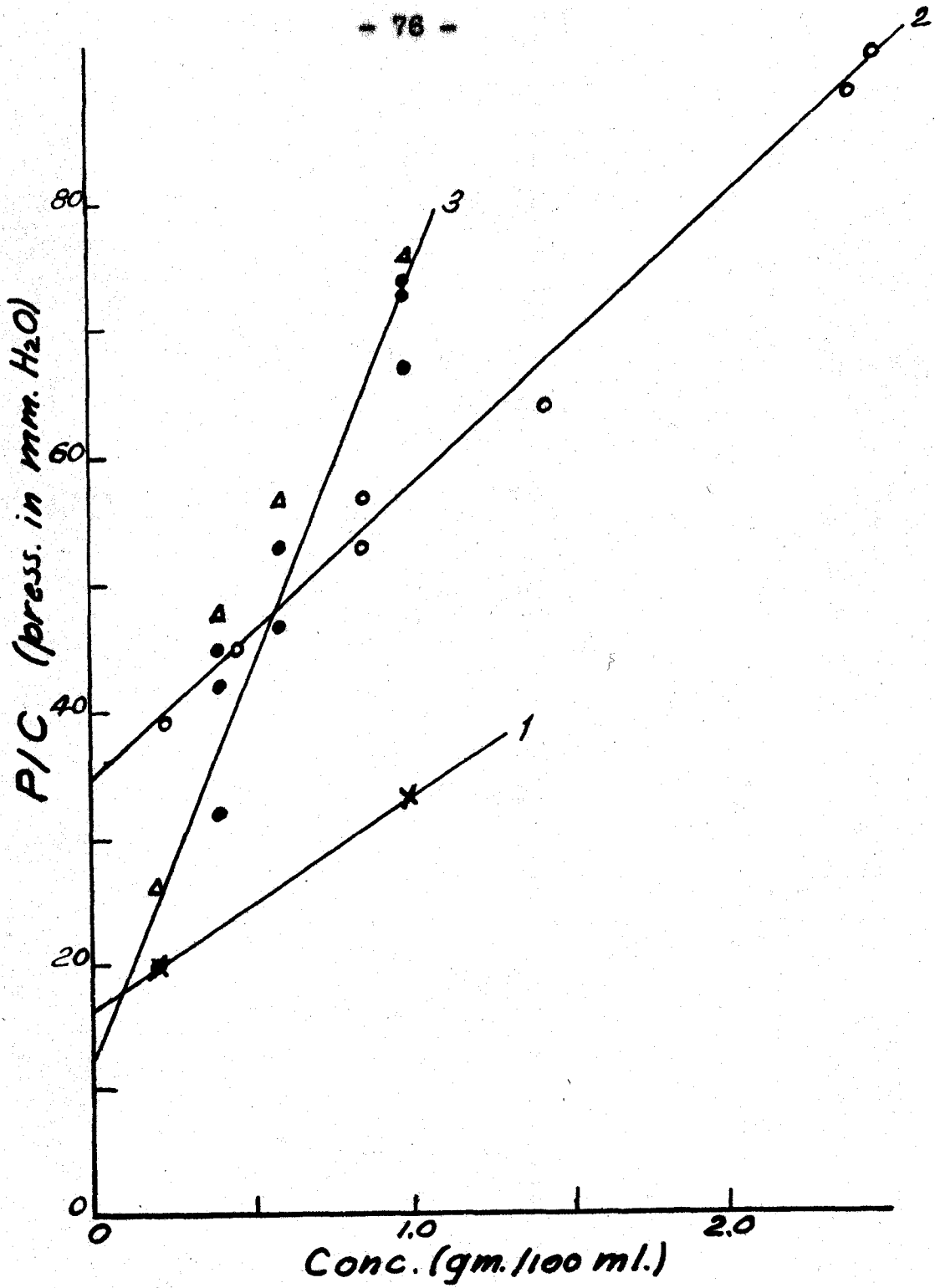


Fig. 9. Concentration Dependence of the Concentration-Reduced Osmotic Pressure

1, tapioca amylose; 2, corn amylose; 3, corn amylopectin and waxy maize starch; x = tapioca amylose; Δ = waxy maize starch.

simply the reciprocal of the limiting value of the function P/C , P being in mm. of water and C in gm. per 100 ml.

Substituting into this relation the value of the intercept for acetylated corn butanol precipitate, the molecular weight is found to be approximately 73,000, corresponding to 250 glucose units. The possible error is difficult to estimate due to the previously mentioned uncertainties but is probably not over about 5%. The value is surely of the correct order of magnitude.

The molecular weight of corn amylopectin is almost impossible to estimate from the graph since it seems to show curvature. However, it is almost certainly at least three to four times as large as the amylose and possibly larger. A reasonable minimum would probably be 300,000 for the acetate or about 175,000 for the amylopectin itself.

The concentration dependence of the function P/C will be discussed in connection with the discussion of the structure of amylopectin.

The Linear Nature of Amylose

The viscosity results give excellent added evidence that amylose is essentially, if not entirely, a linear polymer. In the first place the extremely high viscosities in proportion to the fairly low molecular weights (high K_m constant from the

point of view of the Staudinger equation) indicate an essentially linear structure.

Even better evidence of the linear nature is the close correspondence between the concentration behavior of these materials and that of the known linear polymers studied by Baker, Fuller and Heiss (5). The previously discussed agreement with Equation (XX) which was derived by Huggins for linear polymers is also important. However, Figure 6 indicates that corn amylopectin and waxy maize starch may also give linear plots of this type. Unfortunately the theory has not yet been worked out for branched molecules.

Probably the most striking indication of the linear character of amylose is the fibrous nature of the acetates. These precipitated from water as stringy, cotton-like mats and could not be pulverized in a mortar. In contrast the amylopectin acetates were easily pulverized. Only linear molecules would be expected to give such fibers.

The Structure of Amylopectin and Waxy Starches

Evidence for the Branched Structure.

Excellent evidence is given from both the viscosity and osmotic pressure results that amylopectin is much more highly ramified than amylose. The limiting viscosity of corn amylopectin is only slightly higher than that of amylose in spite

of its much higher molecular weight. This has been previously pointed out by Meyer (63-c) as evidence of a branched structure for amylopectin. His results were not, however, as striking as the results given here since his amylopectin was doubtless impure, containing probably some of the longer amylose molecules. The increased concentration dependence of amylopectin is also no doubt due to the ramified structure although the phosphorous content might also play a role.

It is apparent that glycogen has a much lower viscosity than corn amylopectin. This is not surprising since glycogen is much more highly branched and the molecule is doubtless much more compact. Amylopectin can be regarded as a very loose sponge in solution, glycogen as more nearly a solid sphere. No explanation can be given at present for the peculiar concentration dependence exhibited by glycogen.

The very much greater slope of the P/C vs. \underline{Q} curve for amylopectin shown in Figure 9 is considered by Mark to be excellent evidence of a branched structure (61). Alfrey, Bartovics and Mark (1) prepared polystyrene at three temperatures, 60°, 120° and 180° C. Those prepared at higher temperatures had lower K_m constants indicating a higher degree of branching. Moreover the plot of P/C vs. \underline{Q} was steeper the higher the temperature of polymerization. This was true even when fractions having similar molecular weights (same intercepts) were compared.

This increased slope in the case of branching would probably also be expected from the theoretical standpoint. As previously pointed out this slope is due to anomalous entropies of solution caused by a decrease in the number of possible arrangements of solute particles entering the solution. This decrease would doubtless be even greater in the case of branching.

The Waxy Starches.

Evidence that waxy maize is practically identical with corn amylopectin is given by the viscosity and osmotic pressure results. In both cases the experimental data for waxy maize fit the corn amylopectin curves within the experimental error. Unfortunately only a few viscosity values are available for waxy maize due to the great difficulty encountered in filtering its solutions¹. However, the evidence seems to be reasonably conclusive.

Shape of the Limit Dextrin Molecule in Relation to the Structure of Amylopectin.

The properties of the limit dextrans are of special interest with regard to the structure of amylopectin. (The limit dextrin from waxy maize was used in this work simply

1. A sample of waxy maize was gelatinized by autoclaving in water and recovered by alcohol precipitation. Its solutions did not appear to filter appreciably better than in the case of the ungelatinized material so that the difficulty is apparently not due to the granular structure.

because of its availability.) Important insight into the type of branching present in amylopectin could be had from a knowledge of the structure of the limit dextrans since they are apparently residues formed from amylopectin by digesting off the free branches down to the branch points.

The extremely low viscosity of the limit dextrin (Figures 4, 5 and 6) can be explained in one of two ways. In the first place the dextrin might be a linear polymer of low molecular weight, not over 50-60 glucose units. This type of dextrin would be expected if the branching in amylopectin is of the herring-bone type. (There would be short stubs of two or three glucose units scattered along the chain which would not affect the viscosity results appreciably.) This type of limit dextrin seems to be ruled out since the molecular weight in that case would be only about 10,000. The yield of limit dextrin obtained from waxy maize (about 40%) would indicate a molecular weight of at least 70,000 in view of the minimum value of 175,000 concluded for amylopectin and waxy maize.

The other possibility is that the dextrin is essentially spherical and quite compact. Only in that case could the low viscosity be reconciled with the required high molecular weight. It seems safe to conclude that the limit dextrin is such a molecule, thus confirming in essence Meyer's concept of amylopectin (63-c). The inner portion of the molecule would appear to be extremely compact, apparently even more so

than glycogen.

An attempt was made to investigate the limit dextrin further by means of osmotic pressure. Unfortunately consistent results could not be obtained. There is some possibility that the dextrans might have been passing through the membranes. However, several values of P/C were obtained over the concentration range 0.3 to 1.0%, all falling between 15 and 35 indicating molecular weights of the order 70,000 to 170,000. More interesting is the fact that there seemed to be no increasing trend of the function with increasing concentration. This would be expected if the molecule is essentially spherical. For example, the plot of P/C vs. C is practically flat in the case of hemoglobin, an essentially spherical molecule (63-a).

Homogeneity of Amylopectin.

It should be emphasized that there is still no definite evidence that amylopectin is a well defined homogeneous component of starch. On the contrary some evidence indicates heterogeneity. Schoch (77) has found that whereas practically all of the amylopectin from potato starch migrates in an electrophoretic cell only about 80% of that from corn starch migrates. There seems to be some correlation between the power to electromigrate and the phosphorous content of a fraction, the migrating fraction of corn amylopectin containing about four times as much as the non-migrating fraction. However, the

colloidal nature of amylopectin in water should not be disregarded and it seems unsafe at present to conclude that phosphorous really plays a definite role in its molecular structure.

Some interesting observations were made during this investigation on the solubility of various fractions in 40% hydrazine hydrate. Tapioca amylopectin was dispersed completely in a few hours giving a turbid solution. Corn and potato gave residues, amounting to about 50% and 15% respectively, which were apparently unattacked. Granular waxy maize was practically unattacked as was potato amylose. The fact that those materials giving the best solutions in ethylenediamine are the least soluble in this solvent is somewhat puzzling and might be explained by phosphorous content. Phosphate groups might not tend to increase solubility in anhydrous ethylenediamine but would in the aqueous solvent. However, a more plausible explanation seems to lie in the relative crystalline forces involved or in the size of the crystallites. The possibility of using this relatively weak solvent as a fractionating agent for amylopectin should be further investigated.

Relationship between Molecular Weight and
Limiting Viscosity in the Amylose Series

Staudinger's Constant for Amylose Calculated from Huggins' Theory.

It is of considerable theoretical interest to calculate the value of the constant, K_m , in Staudinger's equation by utilizing the previously mentioned equation of Huggins relating the function η_{sp}/C to molecular weight. In its simplified form, applicable to long chains (in which case the additive constant can be ignored), this equation takes the form

$$\eta_{sp}/C = 2.94 \times 10^{20} E_{\infty} l^2 a n$$

for the case of strong Brownian movement and low velocity gradient.

For the evaluation of the various constants in this equation a section of an amylose chain consisting of six glucose units was constructed using Fischer-Hirschfelder models. Planar glucose rings were used although there is some question as to whether this is the proper configuration. The chain showed a marked tendency toward a helical configuration as has been previously pointed out (31,16).

For l , the length of the unit chain segments, the distance between the centers of successive oxygen atoms in the chain was chosen. This distance as measured on the models was 4.0 cm., representing 4.0 \AA . in the actual molecule.

The value of \underline{a} , the effective radius of the chain segments, is somewhat uncertain, even in the case of the simple hydrocarbons. However, Huggins has suggested that there is reason to assign to \underline{a} the value of the radius of a sphere having the same surface area as a chain segment, considered as a cylinder.

As a first approximation the volume of the glucose unit in the crystalline state could be taken. From the X-ray measurements of French (28) the volume occupied by the glucose unit in maltose is approximately 200 \AA^3 . (It would be better to use data from amylose if it were available.)

A cylinder of length \underline{l} (4 \AA .) having a volume of 200 \AA^3 would have a surface of 96 \AA^2 , and a sphere of the same surface area would have a radius of 2.8 \AA . This value was assigned to \underline{a} .

To compute \underline{B} the value of the angle between chain units is needed. Inspection of the amylose model showed that the angle between successive glucose units could be varied by slight rotation about C-O-C bonds over a wide range, the limits being about 100° to 120° . In a powerful solvent such as ethylenediamine the chain might be expected to take on its most extended configuration, hence the mean value of the angle would probably be close to 120° . The supplement of this angle,

60°, is α and $\cos \alpha$ is 0.50. Hence

$$B_{\infty} = (1 + \cos \alpha) / (1 - \cos \alpha) = 1.5 / 0.5 = 3$$

Substituting in Huggins' equation it is found that

$$\begin{aligned} \eta_{sp}/C_{gm} &= 396 \times 10^{-4} \text{ n} \\ &= 2.5 \times 10^{-4} \text{ M} \end{aligned}$$

or

$$K_m = 2.5 \times 10^{-4} \text{ .}$$

This is surprisingly close to the observed value for corn amylose as computed using the osmotic molecular weight, namely 3.5×10^{-4} . This agreement is probably fortuitous as will be seen.

In Huggins' derivation fixed angles are assumed between chain segments, with complete freedom of rotation about the bonds. In the amylose chain the situation is reversed, the bond angles being variable but with very much limited rotation. It would be better to consider both glucose units and bonding oxygens as chain segments in which case the valence angles would be fixed. It would then be necessary to use average values of some sort for the constants.

A calculation of this type was also carried out. The angle between the free valence bonds on C₁ and C₄ is somewhat difficult to ascertain precisely since extensions of these bonds do not intersect. However, it is approximately 45°. This value was averaged with the bond angle of oxygen,

110° giving a value for α of 103°. The other constants were averaged in a similar manner. Details will not be given here but the resulting value for K_m was considerably higher than in the previous case, approximately 13×10^{-4} .

The approximations and assumptions made were very crude and these calculations are of interest only as to order of magnitude. However, they do bring out the striking difference between the amylose chain and such a chain as a simple hydrocarbon. There is obviously no reason to expect an equation such as that of Staudinger to apply to the amylose series simply because it appears to hold for the simpler polymers.

An Expanded Form of Staudinger's Equation for the Amylose Series.

In the first calculation of the preceding section a fixed angle of 120° between successive glucose units was assumed. From the nature of the model it is found that fixing this angle automatically fixes the chain in a rigid configuration. Nevertheless in a powerful solvent such as ethylenediamine the chains would probably tend to approximate this rigid state. Hence the assumption on which Huggins' equation is derived, namely complete randomness of kinking, is immediately violated.

As was previously pointed out Kuhn has predicted the Staudinger function to be related to M^2 rather than \underline{M} for a rigid chain. In the case of amylose it might be expected that a proportionality would exist with neither \underline{M} nor M^2 , but

rather with some intermediate power. Simha has recently suggested the relationship

$$f = D \cdot M^p \quad (\text{XXXIV})$$

between axial ratio, f , and molecular weight of a chain molecule, D and p being constants. For a perfectly rigid rod-like molecule p would be unity; actual molecules would have somewhat smaller values of p , depending on the freedom of kinking.

If Equation (XXXIV) is substituted into the previously mentioned equation of Kuhn relating specific viscosity and axial ratio, namely

$$\eta_{sp} = 2.5 \phi + 1/16 \phi f^2 \quad (\text{VII})$$

the equation

$$\eta_{sp}/\phi = 2.5 + 1/16 D^2 M^{2p}$$

results. Since ϕ is the volume fraction it is desirable to convert this equation to a weight concentration basis and also replace M by the number of chain units n giving

$$[\eta_{sp}/c] = A + B n^{2p} = A + B n^k \quad (\text{XXXVI})$$

This may be regarded as a more general form of the Staudinger equation, the latter applying only to completely randomly kinked molecules in which case p takes on the value $1/2$ (or $k = 1$).

It is of considerable interest to apply the data herein presented to this equation and thereby attempt to evaluate the constants A , B and k for the case of the amylose series. Since A is the constant in Einstein's equation it can be

evaluated from results on a spherical carbohydrate. The results obtained on the Schardinger β -dextrin, namely 0.07, will serve admirably.

To evaluate B and k two independent molecular weights are needed. The only reliable value available is that for corn, 250 glucose units. However, Schoch has reported alkali numbers on these materials (77), the value for potato amylose being almost exactly half that of corn. It may be objected that this method is empirical. However, the alkali number is apparently a function of the number of aldehyde groups present and it is not inconceivable that any inconsistencies would enter fairly equally in both amyloses since they are both of high molecular weight. Hence, a value of 500 glucose units will be assumed for potato amylose.

Using these values in conjunction with the limiting values of η_{sp}/c (taken from Figure 3) the numerical values of the constants turn out to be $k = 1.6$ and $B = 1.2 \times 10^{-4}$. Hence, Equation (XXXVI) takes the form

$$[\eta_{sp}/c] = 0.07 + 1.2 \times 10^{-4} n^{1.6} \quad . \quad (XXXVII)$$

In Table V the molecular weights of the various fractions are summarized. The values seem to be reasonable. Fraction number 4 of the amylo-dextrin was found to have a molecular weight¹ of about 40 glucose units by R_{cu} reducing value and

1. These values were determined by F. L. Bates. The iodine method has been shown to give correct molecular weights for the lower dextrans (57).

60 by iodine reducing value (Kline and Acree method). However, some retrogradation apparently took place, especially in the iodine oxidation. This would tend to increase the apparent molecular weight so that the lower value may be more nearly correct.

The value for synthetic starch checks very well with the value of 80 to 90 units reported for this material on the basis of end-group analysis (37).

Table V

Comparison of Molecular Weights and Characteristic Iodine Potentials^a of the Amyloses

Material	Molecular Size (Glucose Units)	Characteristic Potential
Potato Amylose	500	0.197
Tapioca Amylose	450	0.200
Lily Amylose	310	0.202
Corn Amylose	250	0.203
Corn "Crystalline Amylose"	175	0.205
Corn Amylose (Meyer's method)	115	-
Synthetic Starch	85	0.204
Amylodextrin Fraction #3	44	0.218
Fraction #4	31	-

^aThese values were taken from the data of Bates, French and Rundle (9) and are the potentials at the midpoints of the titrations, corrected for slight variations in iodide concentration.

Equation (XXXVII) could be replaced by an equation of the type

$$\eta_{sp}/C = A + Bn + Cn^2 \quad (\text{XXXVIII})$$

involving the same number of constants. This form would have the advantage of being more readily amendable as the experimental data is refined since terms in higher powers of n could be added. However, Equation (XXXVII) is to be preferred in that physical significance can be more readily attached to the constants. The additive constant A gives a measure of the actual volume occupied by the solute molecules (not effective volume) and corrects for such factors as immobilization of solvent through solvation. The constant B involves the specific length of the chain units while the exponential constant k is a measure of the statistical degree of kinking of the chain. It is satisfying to note that k falls between 1 and 2 as predicted.

This equation should prove extremely useful in future investigations of starch. Obviously, however, it must be used only for unbranched materials in which case weight-average molecular weights of the correct order of magnitude may be assured. In comparing these with values determined by other methods the homogeneity of the material must always be kept uppermost in mind.

Nature of the Amylose-Iodine Complex

Relationship Between Iodine Potentials and Molecular Weights.

An inspection of Table IV shows that some definite relationship between the characteristic iodine potentials and chain lengths must exist, thereby confirming the postulate of Bates, French and Rundle (9). The one material falling out of line in Table IV is synthetic starch. The explanation of this doubtless lies in the manner in which the characteristic potentials were chosen. The values are simply the potentials at the midpoint of the titration. In the case of fairly homogeneous materials (in which case the iodine curve is quite flat) it makes little difference at what point the potential is taken. Synthetic starch, however, is apparently very heterogeneous, its iodine curve being fairly steep. Hence, since the viscosity results give a weight-average molecular weight, correlation could hardly be expected unless the potential were taken at a point in the titration curve which corresponds to the titration of material having that molecular weight.

It is of interest to ask how the molecular weight of the amylose can regulate the affinity for iodine. One possible explanation is on the basis of simple diffusion out of the ends of the amylose helices. Another, and seemingly much better, explanation is that the stability of the complex is due, at

least in part, to interaction between iodine molecules. On this basis it might be expected that the longer the helix and the more iodine molecules that could be oriented end to end therein the greater would be the stability.

This possibility was suggested by the close analogy to the conjugated polydienes. Branch and Calvin have recently discussed the color of these compounds (14) showing that the wave length of the absorption maximum shifts according to the equation

$$\lambda = 2\pi c / \sqrt{k/nm} \quad (\text{XXXIX})$$

where c is a constant. In other words these chain compounds seem to behave as linear harmonic oscillators, the effect of the number of units conjugated approximately having the effect of increasing the mass of the oscillator by a factor n .

This shift in wave length would help to explain the observed shift in absorption wave length of the iodine complex which leads to the blue color. But in addition a slowing of the characteristic vibration frequency would probably have the effect of stabilizing the complex (resonance stabilization) and would thus explain the dependence on chain length of the affinity for iodine.

The following experiment was designed to prove definitely whether the stability of the iodine complex is due primarily to interaction between iodine and amylose as has been assumed

previously or to interaction between the iodine molecules themselves.

Precipitation of Amylose Iodide.

A 0.034 gm. sample of potato butanol precipitate was dispersed in 50 ml. of 0.2 N. KOH with boiling and the solution neutralized to phenolphthalein with HI.

A little over one-third the calculated amount of 0.001 N. iodine for complete precipitation was now added and the solution immediately centrifuged. The complex came down very nicely leaving the supernatant liquid perfectly clear. The same amount of iodine was again added to the supernatant liquid and another precipitate of complex collected. A third addition of iodine gave somewhat less complex and the supernatant liquid no longer gave any blue color with iodine, showing precipitation to be complete.

This fractional precipitation was predicted on the basis that the stability of the complex is due to interaction between iodine molecules. There is an obvious tendency, once an iodine goes into an amylose helix, for the helix to fill up completely. In other words the second and subsequent iodines go into the complex more easily than the first.

This result is not at all compatible with the concept that the stability is due to interaction between iodine and amylose and is limited by diffusion out the ends of the

helices. In that case the amyloses would tend to fill up uniformly with iodine, none becoming saturated until all were very nearly so.

Color of Iodine in Organic Solvents.

Freudenberg's concept, previously mentioned, that the blue color of starch-iodide is due to the hydrocarbon lining of the helices does not seem likely. The color of iodine in hydrocarbons is not by any means the pure blue of amylose-iodide as shown by the following experiment:

A few milligrams of resublimed iodine were dissolved in each of the following solvents: petroleum ether, benzene, toluene, xylene, carbon tetrachloride and cyclohexane. Most of the solvents were sodium dried. All of the solutions had a distinctly red color by reflected light. By transmitted light the solutions did appear somewhat blue, especially when the concentration was high.

To obtain an accurate comparison these solutions were observed in a colorimeter along side a solution of amylose iodide. At comparable intensities the amylose complex appeared pure blue and the iodine in organic solvents pure red. The contrast was very striking.

A shift in absorption frequency due to resonance between iodine molecules seems to be a much more plausible explanation than the hydrocarbon medium for the blue color.

An Attempted Quantitative Relationship.

In as much as data is now available on the molecular sizes of the amyloses together with their affinities for iodine (characteristic potentials) it is interesting to attempt a quantitative explanation, even though several assumptions are necessary.

Assume a reaction of the type



For the reaction as written

$$\Delta F = \Delta F_n^0 + RT \ln \frac{A_{\text{complex}}}{A_{\text{I}_2}^n \times A_{\text{amylose}}}$$

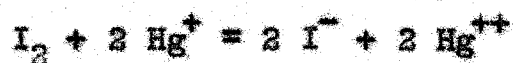
and at equilibrium

$$\Delta F_n^0 = - RT \ln \frac{A_{\text{complex}}}{A_{\text{I}_2}^n \times A_{\text{amylose}}}$$

However, since the complex precipitates out of solution (in the presence of excess KI) as fast as formed its activity can be considered unity so that

$$\Delta F_n^0 = nRT \ln A_{\text{I}_2} + RT \ln A_{\text{amylose}}$$

In the iodine titration using a calomel half-cell the potential measured is that corresponding to the reaction



or

$$E = E^0 - RT/2f \ln \frac{A_{\text{I}^-}^2 \times A_{\text{Hg}^{++}}^2}{A_{\text{I}_2} \times A_{\text{Hg}^+}^2}$$

and since the activities of Hg^+ and Hg^{++} are constant

$$E = E^0 - RT/2f \ln A_{I^-}^2/A_{I_2}$$

and

$$RT \ln A_{I_2} = 2fE - 2fE^0 + RT \ln A_{I^-}^2$$

Substituting this into the expression for ΔF_n^0 obtained above it is found that

$$\Delta F_n^0 = n [2fE - 2fE^0 + RT \ln A_{I^-}^2 + RT \ln A_{\text{amylose}}] \quad (\text{XL})$$

Assume now that iodine behaves as a simple harmonic oscillator of mass m and restoring constant k so that its characteristic vibration frequency is given by

$$\nu_{I_2} = 1/2\pi\sqrt{k/m}$$

and that the frequency of iodines lined up in the helix is given by

$$\nu_{I_2 \text{ in complex}} = 1/2\pi\sqrt{k/nm}$$

There is an equation derived from statistical mechanics¹ which gives the free energy of vibration of an oscillator as

$$F_{\text{vib}} = RT (\ln u - u/2 + u^2/24 - u^4/2880 - \dots)$$

which for small values of u becomes approximately

$$F_{\text{vib}} = RT (u/2 - 1)$$

1. See for example Mayer and Mayer, "Statistical Mechanics", p. 444, John Wiley and Sons, New York (1940)

where

$$u = h\nu/kT$$

Upon factoring and substituting for u this becomes

$$F_{\text{vib}} = Nh/2 (\nu - 2)$$

and substitution for ν gives

$$\Delta F_n = Nh/4\pi \sqrt{k/m} (1/\sqrt{n} - 1)$$

for the process of a free iodine molecule going into the complex, and for n iodines going into the complex the free energy change would be n times as great.

Substitution in Equation (XL) and solving for E gives

$$E = Nh/8\pi f \sqrt{k/m} 1/\sqrt{n} - Nh/8\pi f \sqrt{k/m} + E^0 - RT \ln A_{\text{I}}^2 - RT \ln A_{\text{amylose}}$$

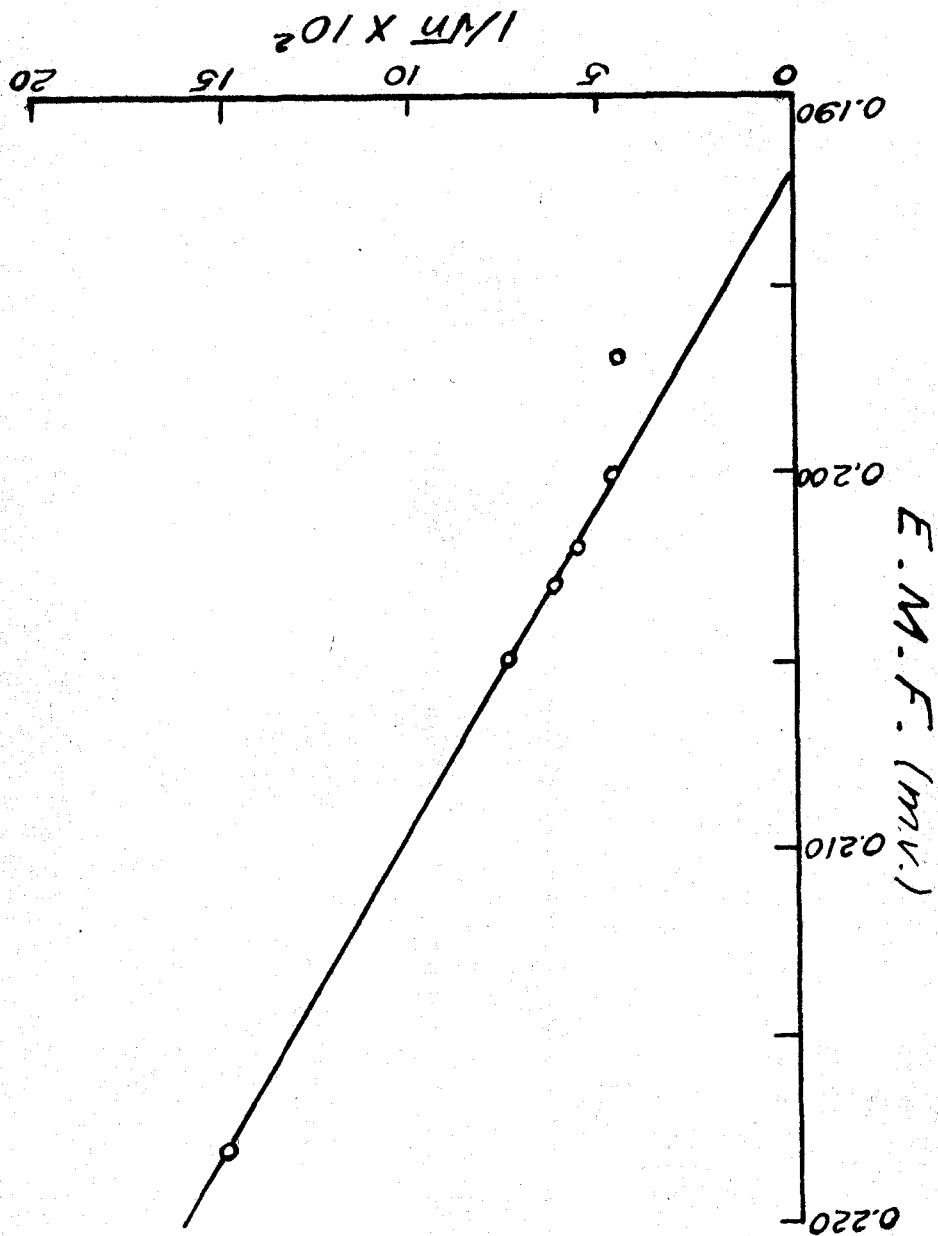
Considering everything except E and n constant this equation takes the form

$$E = A + B 1/\sqrt{n} \quad (\text{XLI})$$

where n is the number of iodine molecules in the helix. However, since the number of iodines which can be lined up in the helix is also proportional to the number of glucose units in the amylose the form of Equation (XLI) would be unchanged if n were so taken. This has been done in Figure 10. It is interesting that the results are remarkably linear in conformity with Equation (XLI) with the one exception of potato amylose. However, this result may be purely fortuitous¹.

1. R. Baldwin of this laboratory has investigated the shift in absorption maxima of the iodine derivatives of the amyloses studied here. The shift does not seem to be nearly as great as would be expected from the derivation given.

FIG. 10. Relationship between the characteristic iodine potentials and polymerization degree of the Amyloses, showing the agreement with Equation XI



V. SUMMARY AND CONCLUSIONS

1. An extensive investigation of the solution viscosities of the amyloses over the concentration range 0-0.8% is made. The intrinsic viscosities vary widely for the amyloses from various sources. Amyloses prepared from corn by different methods also vary but fall in the order expected from the method of preparation. Possible explanations of this apparent heterogeneity of corn amylose are discussed.

2. The close correspondence between the concentration dependence of the amyloses and that of known linear polymers lends added evidence that they are such molecules. Further evidence is given by the strikingly fibrous nature of the amylose acetates.

3. Corn amylose prepared by butanol precipitation is found by osmotic pressure studies to have a polymerization degree of 250 glucose units. An equation is derived relating degree of polymerization and intrinsic viscosity in the amylose series. This is a more general form of the Staudinger equation and allows for the statistical degree of kinking of the chain. It is pointed out that there is no reason to expect the highly idealized Staudinger equation to hold for the amyloses no matter how well it may hold for the simpler polymers.

4. The molecular weight of amylopectin cannot be ascertained definitely at present due to possible curvature of the plot of P/C vs. C . However, a minimum value of 175,000 can be accepted.

5. Amylopectin is much more highly ramified than amylose as shown by both its lower K_m constant and the steeper P/C vs. C curve.

6. Waxy maize starch is essentially identical with corn amylopectin as shown by the close correspondence between the concentration dependence of both the viscosity and osmotic pressure of these materials.

7. The extremely low viscosity of its limit dextrin indicates waxy maize (and hence amylopectin) to have an essentially spherical, three-dimensional, net-like rather than herring-bone type of branched structure.

8. A definite relationship exists between the molecular size of amylose and its affinity for iodine. To explain this a new concept for the stability of the amylose-iodine complex is given, namely that the governing force is due to resonance interaction between the iodine molecules oriented in the amylose helix, rather than to forces between iodine and amylose. This concept is confirmed by a simple experiment which shows that amylose helices tend to saturate successively with iodine instead of taking it up uniformly as would be expected according to the previous idea.

This new concept has the advantage of explaining qualitatively not only the iodine potentials but also the shift in absorption maxima which causes the blue color of the complex. The blue color cannot be due to the hydrocarbon lining of the helices since the color of iodine in hydrocarbon solvents is not by any means the pure blue of the amylose-iodine complex.

An attempt is also made to put this theory on a quantitative basis. Agreement with the iodine potentials is excellent but the equations do not appear to fit the observed shifts in absorption maxima. The derivation is purely speculative but indicates a possible method of attack for relating these functions.

9. The osmotic pressure studies are preliminary in nature. They do, however, indicate the possibility of utilizing this tool far more extensively than just as a means of determining molecular size. It is possible to obtain important information regarding degree of branching and general molecular shape from the relationship between P/C and C .

10. Although amylose appears to be a definite fraction of starch, amylopectin still cannot be regarded as a definite homogeneous entity. Much additional evidence is needed before this question can be settled.

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VIII. VITA

Joseph Franklin Foster was born in Marion, Indiana, May 17, 1918, the fifth child of Grace May (Cameron) and DeWitt Lee Foster. His early education was in the public schools in Des Moines, Iowa and New Providence, Iowa and in the New Providence High School. In 1936 he entered Iowa State College, Ames, Iowa and in 1940 received the degree of Bachelor of Science with major in Chemistry and minors in Mathematics and Physics. He continued his work in the Chemistry Department of Iowa State College, investigating the molecular structure of starch under the direction of Dr. R. M. Hixon. During his graduate work he held the position of Research Graduate Assistant in the Plant Chemistry Subsection of the Agricultural Experiment Station. In 1940 he was married to Ruth Elizabeth Hobson of New Providence, Iowa.

Appendix 1

Description of the Osmotic Cells

Modified Hepp Cell.

The cell used for all the determinations reported in this investigation was a modification and simplification of the so-called "micro-osmometer" of Hepp (42). With the exception of the glass capillary it was constructed entirely of brass. The original construction involved a seal of de Khotinsky cement between the glass capillary and the brass cell, thereby excluding any possibility of using organic solvents which attack the cement. The cell was jacketed to provide for temperature control through the circulation of water. In this form it seemed to be perfectly satisfactory for use with aqueous solutions.

Early in this investigation it became desirable to use organic solvents rather than water. Consequently the de Khotinsky seal was eliminated and a soldered joint made between glass and brass. This was accomplished by first platinizing¹ the glass and then soldering to the platinum. Great difficulty was encountered in obtaining an air tight

1. The platinizing was carried out according to directions given in Handbook of Chemistry and Physics, 22nd. edition, p. 1955, Chemical Rubber Publishing Co., Cleveland (1937).

seal although this was apparently finally accomplished. The reason for this difficulty was that the available surface for making the seal was not great enough, due to the construction of the cell. Nevertheless a number of measurements with organic solvents were made with the cell in this form.

In an attempt to improve the glass to brass seal the cell was completely rebuilt as shown in Figure 11. A solid brass block was soldered to the underneath side of the brass plate and drilled at a right angle, vertically down from the top and horizontally to meet the glass capillary. The latter was sealed into a brass cylinder with Wood's metal. Care was taken to obtain as uniform as possible an opening into the capillary. This was done by inserting a greased piece of wire through the opening in the brass cylinder and into the capillary. The Wood's metal was then permitted to run through the opening and solidify so that removal of the wire left a fairly smooth channel. The brass cylinder was ground to fit into a hole in the brass block, and was clamped therein by means of a large brass nut¹.

The water jacket was dispensed with entirely in this cell as it had been previously found superfluous. Clamping of the

1. A joint of this type was used on a cell by Herzog and is described by Meyer (63-e).

membrane was at first accomplished by means of three thumb screws. However, difficulty was encountered with leakage so these were later replaced by six 1/4" machine bolts which could be drawn up tightly with a wrench.

Montonna Cell.

A cell patterned after that used by Montonna and Jilk (66) was also designed and constructed. It consisted of two similar brass plates with three concentric grooves, each 1/4" wide and 1/8" deep, the overall diameter being about 3". These dimensions are considerably smaller than those used by Montonna. Only four bolts were used for clamping which may account for the fact that leakage around the membrane could not be completely eliminated.

The capillary tubes (one each for solution and solvent) and two dropping funnels (for filling) were attached to the cell by short rubber connections. This is a disadvantage in working with organic solvents. Furthermore, it is almost impossible to change the solution in this type of cell without completely dismantling it. This usually renders the membrane unfit for further use.



Unassembled



Assembled

Fig. 11. The Modified Hepp Micro-Osmometer

Appendix 2

Some Difficulties Encountered in the Determination of Osmotic Pressure

This section, though entirely irrelevant to the development of this thesis, is here included in the hope that it may prove of value to those utilizing this important tool in the future.

The membrane problem was perhaps sufficiently discussed in the section under that heading. It is still not satisfactorily solved by any means.

Another point which has been a constant source of trouble and uncertainty is the difficulty of checking the calculated zero point experimentally. This value should simply be an applied pressure sufficient in magnitude to counteract the head of solvent and solution in the cell plus the capillary force of the solvent, both of which act in the same direction. Actually the experimental zero point was usually less than this. If it could be ascertained definitely that this zero point once determined remains fixed, no error would be involved. However, this is not at all certain. On the contrary on some occasions when the zero point was redetermined after the cell had been filled for a time with solution it was found to have risen.

This difficulty has apparently not been recorded by other workers; on the contrary it appears that some have made no effort to determine the value experimentally, using instead the calculated value.

The best explanation for this difficulty which can be offered at present seems to be loss of solvent around or through the edges of the membrane. The effect of the solutions in stopping this on some occasions might be due to solute molecules being deposited in the capillaries of the membrane, effectively cementing them. The magnitude of the discrepancy varies from trial to trial with the same membrane. For a time it was thought due to entrapment of air bubbles in the vertical part of the capillary (which would reduce the effective head) but this does not seem likely at present.

Values obtained with a low zero point were as a rule either discarded or rechecked, especially in case of solutions of low concentration where a small error would be greatly magnified.

At times the flow in the capillary appeared to be jerky or inhibited. This may be due to air bubbles trapped at the 90° bend in the capillary (the borings in the brass block may not meet perfectly). The cell would be greatly improved by eliminating this sharp angle, either by drilling on an angle or by using a bent glass capillary which is joined to the brass at the top of the cell.