The Binding of Glycogen and Phosphorylase*

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It has been calculated from kinetic measurements that the rate of the phosphorylase reaction is half-maximal when the glycogen concentration is 20 mg./100 ml. (1). This $K_n$ (Michaelis-Menten constant) value can be expressed as a molar concentration, if the percentage of terminal glucose units (with which phosphorylase reacts) is known. For a glycogen with 9 per cent end groups, the $K_n$ would be $1 \times 10^{-4}$ M, a concentration much higher than the enzyme concentration ($2 \times 10^{-4}$ M) used in such kinetic measurements.

It seemed of interest to study the combination of glycogen and phosphorylase by the ultracentrifugal separation method in order to determine how the binding constant obtained by this method would compare with the $K_n$ obtained by the kinetic method. For this purpose a glycogen of much larger molecular weight than that of the enzyme was needed. A phytoglycogen prepared from mature sweet corn seemed suitable; it had a mean sedimentation coefficient of 20 S and contained only 1.2 per cent of material of an $s_{20, w}$ of less than 100.1 Phosphorylase $a$ from rabbit muscle has an $s_{20, w}$ of 13. The principle of the method was to centrifuge most of this glycogen from the upper layer in the centrifuge tube, leaving most of the phosphorylase in the upper layer. The extra amount of protein sedimented in tubes which contained glycogen (compared with tubes without glycogen) would be considered to be bound to the glycogen.

EXPERIMENTAL

Protein was determined by measuring the optical density at 280 mμ. To determine glycogen, 1.0 ml. of sample was mixed with 0.1 ml. of concentrated HCl and heated for 3 hours in a boiling water bath in a tube fitted with a reflux condenser. The sample was partially neutralized with 1.1 ml. of 1 N NaOH; neutralization was completed with 0.1 N NaOH, and phenol red as the indicator. Total glucose was then determined by the method of Nelson (2).

The mature corn phytoglycogen was a sample (No. 512) from Corn Products Refining Company, prepared by Dr. T. Schoch. Previous analyses in this laboratory showed that this glycogen had 8.4 per cent end groups (3). 100.0 mg. of air-dried material was weighed out in a 25-ml. volumetric flask, and 0.0015 M Versene (the disodium salt of ethylenediaminetetraacetic acid)-0.02 M sodium glycerophosphate buffer at pH 6.7 was added to volume to give a 0.4 per cent solution. The glycogen dissolved well, except for a few white fibers. Analysis showed that this stock solution contained 0.373 per cent of glycogen (as glucose). For glycogen monohydrate, this would correspond to a purity of 93 per cent.

The crystals of a phosphorylase $a$ preparation which had been recrystallized five times were dissolved in the same buffer as described above and centrifuged at room temperature to remove a small amount of insoluble material. This stock solution contained 3.08 mg. of protein per ml. and 1.5 ml. of it, made up to 2.5 ml. with various amounts of glycogen, were used in the experiment reported in Table I.

The Spinco model L preparative ultracentrifuge with the No. 40 rotor was used. Each of the above solutions was placed in 8 x 50 mm. plastic tubes of about 2.3-ml. capacity. These were seated in nylon holders for placement in the rotor. After centrifugation of the preparation, a drawn out glass tube with an upturned tip was used to remove the upper solution down to 28 mm. from the top of the tube. A preliminary experiment with solutions containing only protein or glycogen, but no mixtures, indicated that a 20-minute centrifugation at room temperature at 40,000 r.p.m. (113,000 x $g$) sedimented all of the glycogen from the upper layer. However, 63 per cent of the protein was sedimented under these conditions, and so in subsequent experiments the solutions were centrifuged for 20 minutes at 28,600 r.p.m. (57,300 x $g$. It can be seen in Table I that only 30 per cent of the protein was sedimented under these conditions. Glycogen was still completely sedimented. In the experiment in Table I, two control tubes (not shown), each of which contained glycogen alone, were included. After centrifugation only 0.4 per cent of the original glycogen was left in the top layer. This amount was disregarded in calculations.

Binding of Phosphorylase—It was noted that a turbidity was produced when the protein and glycogen solutions were mixed, and the extent of turbidity increased from tubes 3 to 9, Table I. By the time all of the solutions were ready for centrifugation, some agglutinated particles were settling out. After centrifugation of the solutions, the upper layers were analyzed for protein, as described earlier. It is well known that the Michaelis-Menten equation also describes certain types of adsorption equilibria which have been studied by Langmuir. Therefore, the data of Table I were treated according to the method of Lineweaver and Burk, i.e. by plotting the reciprocal of protein bound (1/Pb) against the reciprocal of the glycogen concentration (in percentage), as shown in Fig. 1. The slope is equal to $K_{\text{maximal}}$, and $K$ is found to be 270 mg. of glycogen per 100 ml. or 13.5 times the $K_n$ of liver glycogen as determined by the kinetic method.
A number of possibilities were explored which might explain the difference between the two measurements. It seemed desirable to repeat the binding experiment after the corn glycogen had been subjected to further purification. The material was digested with strong NaOH, precipitated several times with alcohol, and then was dried with alcohol and ether. The purified glycogen dissolved easily and completely when made up to a 4 per cent solution and, after acid hydrolysis, it yielded 3.87 per cent of glucose; it was therefore 96.8 per cent pure as glycogen monohydrate. The purification procedure had apparently released some glycogen molecules of lower molecular weight, since 7.3 per cent of the glycogen remained in the upper layer after centrifugation, compared to 0.4 per cent in the previous experiment.

The experiment described in Table II differs from that in Table I mainly in the use of higher glycogen concentrations. Owing to this, up to 75 per cent of the added protein was carried down with the glycogen during centrifugation. It was again noted that a turbidity developed at 1 to 2 minutes after mixing the phosphorylase and glycogen solutions. The amount of turbidity appeared to be less at the higher glycogen concentrations.

In Fig. 2 the amount of phosphorylase a bound to glycogen is plotted against the glycogen concentration, and the same data are also shown in a double reciprocal plot. The K derived from this plot is 210 mg. of glycogen per 100 ml., which is lower than the value obtained in the first binding experiment. It seems possible that the purification of the glycogen resulted in a tighter binding of the protein. Although different enzyme preparations were used in the two experiments, the extrapolated maximal binding of the protein, for nearly equal initial protein concentrations, was almost the same in both instances, being 2.5 and 2.7 X 10⁻⁴ m, respectively.

Another possibility to be considered was that the other substrates of phosphorylase (glucose-1-phosphate and inorganic phosphate) which are present in kinetic experiments might increase the binding of the enzyme to glycogen. The two-substrate case has recently been analyzed by Frieden (4) who has shown that in some instances the experimentally determined dissociation constant for one substrate may be higher than the apparent Michaelis constant determined in the presence of several concentrations of the second substrate. In tube 4, Table II, glucose-1-phosphate and inorganic phosphate were present during centrifugation in concentrations sufficient to

### Table I

#### Data from first binding experiment

<table>
<thead>
<tr>
<th>Materials</th>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tbody>
<tr>
<td><strong>Glycogen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Percentage...</td>
<td></td>
<td>0.0034</td>
<td>0.0067</td>
<td>0.027</td>
<td>0.054</td>
<td>0.081</td>
<td>0.116</td>
<td>0.134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molarity, X 10⁶ (as glucose end group)</td>
<td></td>
<td>0.175</td>
<td>0.35</td>
<td>1.4</td>
<td>2.8</td>
<td>4.2</td>
<td>5.6</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg. per ml...</td>
<td></td>
<td>1.29</td>
<td>1.29</td>
<td>1.276</td>
<td>1.26</td>
<td>1.16</td>
<td>1.07</td>
<td>1.05</td>
<td>0.987</td>
<td>0.95</td>
</tr>
<tr>
<td>Molarity, X 10⁶</td>
<td></td>
<td>2.61</td>
<td>2.61</td>
<td>2.58</td>
<td>2.55</td>
<td>2.34</td>
<td>2.16</td>
<td>2.12</td>
<td>1.99</td>
<td>1.92</td>
</tr>
<tr>
<td>Molarity, X 10⁶ of bound protein</td>
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<td>0.03</td>
<td>0.06</td>
<td>0.27</td>
<td>0.45</td>
<td>0.49</td>
<td>0.62</td>
<td>0.69</td>
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</table>

#### Table II

#### Data from second binding experiment

<table>
<thead>
<tr>
<th>Materials</th>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glycogen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage...</td>
<td></td>
<td>0.07</td>
<td>0.14</td>
<td>0.14</td>
<td>0.42</td>
<td>0.84</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Molarity, X 10⁶ (as glucose end group)</td>
<td></td>
<td>3.6</td>
<td>7.2</td>
<td>7.2</td>
<td>21.7</td>
<td>43.5</td>
<td>72.5</td>
<td></td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg. per ml...</td>
<td></td>
<td>1.35</td>
<td>1.01</td>
<td>0.78</td>
<td>0.75</td>
<td>0.42</td>
<td>0.34</td>
<td>0.33</td>
</tr>
<tr>
<td>Molarity, X 10⁶</td>
<td></td>
<td>2.72</td>
<td>2.04</td>
<td>1.58</td>
<td>1.52</td>
<td>0.84</td>
<td>0.68</td>
<td>0.67</td>
</tr>
<tr>
<td>Molarity, X 10⁶ of bound protein</td>
<td></td>
<td>0.68</td>
<td>1.14</td>
<td>1.20</td>
<td>1.88</td>
<td>2.04</td>
<td>2.05</td>
<td></td>
</tr>
</tbody>
</table>
FIG. 2. The data of Table II are represented as the amount of protein (Pb) bound to glycogen of different concentrations, and the same data are also shown in a double reciprocal plot.

satrate the enzyme, but their addition did not cause an increase in the amount of phosphorylase bound to glycogen as determined by comparison with a control (tube 3).

$K_m$ of Corn Glycogen—There remained the possibility that sweet corn glycogen did, in fact, have a lower affinity for phosphorylase than did liver glycogen. Previous work with another preparation of corn glycogen had established merely the fact that it was a "primer" of the phosphorylase reaction (5), but the $K_m$ had not been determined. The purified sample of corn glycogen (see above) was used for the $K_m$ determination under conditions which approximated those of the binding experiments.

A method for the determination of the $K_m$ of glycogen has been described (1). It involves the determination of the first order velocity constant, $K'$, with 0.016 m glucose-1-phosphate as substrate in the presence of various amounts of glycogen. The phosphorylase reaction in the direction of synthesis involves the elongation of the outer chains of glycogen which is the greater the lower the initial concentration of glycogen. With the elongation of the chains, there also occurs a progressive inhibition of the reaction which is the greater the lower the initial concentration of glycogen. Initial rates can be obtained, however, by plotting $1/K'$ against time. This is illustrated in Fig. 3, which also includes two concentrations of liver glycogen for comparison. From the extrapolated velocities at zero time, the $K_m$ is evaluated by the method of Lineweaver and Burk. When this is done (not illustrated), the $K_m$ for corn glycogen is found to be 27 mg./100 ml. in this experiment as compared to 21 mg./100 ml. for liver glycogen. Therefore, it is clear that the reason for the discrepancy between the apparent dissociation constants derived from the binding studies and the $K_m$ derived from enzyme kinetics is to be sought in other considerations.

Molecular Weight of Corn Glycogen—In order to calculate the number of enzyme molecules that can be bound by 1 glycogen molecule, it is necessary to consider the molecular weight of the latter. A sedimentation diagram of corn glycogen, presented in Fig. 4, shows that this glycogen, like other glycogens, is polydisperse, especially with respect to large sizes, as indicated by the long tail.

The distribution of sizes is illustrated in Fig. 5, in which $dc/ds$ has been plotted against $s_{20,w}$, ...
the glycogen without causing too much sedimentation of the enzyme.

Extrapolation to zero concentration was not done with this glycogen, but with other glycogens it was found that, over a 10-fold range, concentration has very little effect on the value of \( s_{20,w} \). The diffusion coefficient, \( D_{20,w} \), has been determined for a considerable number of glycogens in this laboratory as well as in others, and the partial specific volume of glycogen was found to be 0.65 by Bridgman (6). The glycogen molecule is presumably not rigid enough to justify deductions about its shape from the frictional ratio \( f/f_0 \). In general, this ratio for different glycogens was relatively constant and has been found to be between 1.7 and 1.9 by different investigators.

From the equation for the calculation of the frictional ratio, it follows that \((D_{20,w})^2 \cdot s_{20,w} = C\), where the numerical value of C is determined by the value of \( f/f_0 \). In a series of molecular weight determinations on glycogen subjected to stepwise enzymatic degradation (7), the above relationship was found to obtain; that is, a plot of \((D_{20,w})^2 \) against \( 1/s_{20,w} \) yielded a straight line with C as the slope and with C proportional to

\[
\left(1 - v_{20}\right)
\over
v_{20}
\left(f/f_0\right)^\nu.
\]

From this relationship and a value for \( f/f_0 \) of 1.67, \( D_{20,w} \) of corn glycogen is calculated to be 0.75 \( \times 10^{-11} \). The approximate mean molecular weight of this glycogen would then be 19 to 20 million.

Further Analysis of Binding Experiments—The maximal number of enzyme molecules which can be bound by 1 corn glycogen molecule of \( 20 \times 10^6 \) molecular weight can be evaluated by plotting the data in Table II in the manner shown in Fig. 6. Since 7.3 per cent of the glycogen remained in the upper layer after centrifugation, 92.7 per cent of the actual glycogen concentration was used in the calculation of the molar concentration. \( R \) is the molar ratio of glycogen sedimented to protein bound, and \( C \) is the original molar ratio of total glycogen to total protein, corrected for that amount of protein which sediments independently from the glycogen and that amount of glycogen which does not sediment. In Fig. 6, \( R \) is plotted against \( C \), and it can be seen that the limiting value of \( R \), the ratio of glycogen sedimented:protein bound, is 0.03. This can be interpreted to mean that the maximal number of protein molecules which can be bound to 1 glycogen molecule of \( 20 \times 10^6 \) molecular weight is 33.

Such a glycogen molecule has 10,000 glucose end groups (8.4 per cent of \( 20 \times 10^6/162.3 \)). If it is assumed that each phosphorylase \( a \) molecule has four active centers, then it can be calculated that a maximum of only 1.3 per cent of the glucose end groups on each glycogen molecule is available for binding.

\[
\frac{4 \times 33}{10,000 \times 100}
\]

The rest of the end groups may not be available because of steric hindrance by the relatively large enzyme molecules. With a molecular weight of 500,000 and a partial specific volume of 0.75, 1 molecule of phosphorylase \( a \) would have a volume of 6.23 \( \times 10^9 \) cu. A. If it is assumed that the shape is a prolate ellipsoid of revolution and that the axial ratio is 8 \( (f/f_0 = 1.55) \), this protein molecule would have a length of 424 A and a diameter of 53 A. If it were flat against the surface of the glycogen molecule, it would occupy an area of approximately 1.7 \( \times 10^4 \) sq. A. A glycogen molecule of \( 20 \times 10^6 \) molecular weight and of a partial specific volume of 0.65 would occupy 2.16 \( \times 10^5 \) cu. A. If its shape were assumed to be a sphere, the surface area would be 3.75 \( \times 10^5 \) sq. A. According to these assumptions the maximal number of enzyme molecules which could be bound by 1 glycogen molecule would be 22 (37.5/1.7).

This calculated value is not inconsistent with the value of 33 obtained from experimental results. Steric hindrance may well account for the discrepancies between physical binding measurements and kinetic measurements. The physical binding studies involve a static situation in which steric hindrance limits the effective glucose end group concentration to less than 2 per cent of the total. In the kinetic studies, a dynamic situation ob-

FIG. 6. A plot of \( R \), the molar ratio of glycogen sedimented:protein bound versus \( C \), the original molar ratio of glycogen:protein, calculated from the data of Table II for a molecular weight of \( 20 \times 10^6 \) for glycogen and of \( 5 \times 10^4 \) for enzyme.

<table>
<thead>
<tr>
<th>( R )</th>
<th>( 0.1 )</th>
<th>( 0.2 )</th>
<th>( 0.3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C )</td>
<td>( 0.01 )</td>
<td>( 0.02 )</td>
<td>( 0.03 )</td>
</tr>
</tbody>
</table>

\( \frac{4 \times 33}{10,000 \times 100} \)

\( \frac{4 \times 33}{10,000 \times 100} \)

\( \frac{4 \times 33}{10,000 \times 100} \)

When the molar concentration of the end group is used for the plot in Fig. 6 instead of the molar concentration of glycogen, the limiting ratio, end group sedimented:protein bound, is found to be 300. This could be interpreted to mean that 1 protein molecule occupies 300 end groups and thus leaves room for 33 enzyme molecules on a glycogen molecule with 10,000 end groups.
Electron Microscopic Observations—Some preliminary results were obtained in collaboration with Dr. Joseph Toby in the laboratory of Dr. Robley Williams in the summer of 1956. Very dilute solutions of glycogen in water or in 0.025 per cent serum albumin (dilutions of the order of 0.7 to 1.5 µg. of glycogen per ml.) were sprayed upon collodion-covered screens. The solutions also contained standard polystyrene latex particles (1.0 to 1.5 X 10⁶ particles per ml.) which served as reference particles for the measurement of diameter and height. The preparations were shadowed with uranium. Photographs at magnifications of the order of 20,000 show distinct spherical preparations were seen in any of the preparations. The particles seem soft in texture, and they are all more or less flattened, as indicated by a greater diameter than height. The removal of water molecules from between the chains of glycogen is probably responsible for the flattening process.

The purified preparation of corn glycogen mentioned in this paper showed particles with diameters ranging from 350 to 450 A. A sample of muscle glycogen with an average molecular weight of 2.5 X 10⁶ (as calculated from ultracentrifugation and diffusion measurements) yielded fairly monodisperse particles with an average diameter of 350 A. This glycogen was also examined after the outer chains had been degraded (30 per cent reduction in size) or elongated (20 per cent increase in size) by phosphorylase, but no distinct effect on the diameter of the particles could be detected as the result of this treatment. Because of the difficulty of estimating the degree of flattening with accuracy, it is not possible to make much use of the measured diameters. It is to be mentioned merely that corn glycogen of 20 X 10⁶ molecular weight, if it were completely spherical, would have a diameter of 345 A and the above sample of muscle glycogen, a diameter of 173 A. On the other hand, if the flattening observed would correspond to a prolate spheroid with an axial ratio of 2, the molecular weight corresponding to a major axis of 350 A would be approximately 5 X 10⁶. This emphasizes the difficulties in relating the measured diameters to the molecular weight. It is believed, however, that the technique of molecular weight determination of glycogen by electron microscopy can be improved.

Another problem, investigated more successfully, was to determine whether a combination of phosphorylase and glycogen could be detected. Since the fine droplets of the spray dry almost instantaneously on the screen, and since the solutions used are necessarily very dilute, it was feared that the concentration range which would permit combination would be passed through too quickly. Accordingly, the technique of spraying was modified as follows. The collodion-covered screens were placed for 5 minutes in a closed container in an atmosphere saturated with water vapor. After opening the container momentarily for spraying, the vessel was closed again and allowed to remain for 2 to 3 minutes. The screens were then removed, mounted, and shadowed with uranium. Control procedures consisted of spraying in the same manner phosphorylase alone or muscle glycogen alone, with or without added serum albumin. In the combination experiments, muscle glycogen was present in a concentration of 1 µg. and phosphorylase, in a concentration of 0.3 µg. per ml. The photographs show the presence of large particles with diameters up to 500 A which are distinguished by their much greater density from particles seen on other plates. There were also small particles of lesser density present. It is at least suggestive that the large particles represent glycogen molecules which are covered with phosphorylase protein, since this would account for their greater density and size.

Consideration of Size of Glycogen Molecules—From the structure analysis of corn glycogen, it is known that the average chain length is 11.9 glucose units and the average length of the inner and outer chains, 5.0 and 6.9 glucose units, respectively (3). It can also be calculated that when the molecule has grown to a molecular weight of 20 X 10⁶, there will be about 13 successive layers or tiers of branch points (glycogen units in 1:1 linkage) present. If the growth were entirely regular, that is, if each outer chain had an equal chance of being elongated by phosphorylase and branched by the branching enzyme, the branch points would double in each successive tier, so that 50 per cent of all the branch points would be present in the outermost tier. In such a structural arrangement, which can be compared to that of a multibranched tree, the 13 tiers would have the following approximate linear dimensions. From x-ray data, the length of 2 glucose units along the fiber axis can be estimated to be 10.3 A. For an average of 5 glucose residues per inner chain and 13 tiers, this would amount to 335 A, as compared to a diameter of 345 A for a spherical glycogen molecule of molecular weight of 20 X 10⁶.

The approximate agreement of these figures is not used as an argument that glycogen in solution has a spherical shape. In order to make predictions about shape, the degree of hydration, the extent of hydrogen bonding, the rigidity of the structure, and other factors would have to be known. There is, however, one factor of considerable interest which may explain why a glycogen molecule cannot grow beyond a certain size. From the data given in this paper, one can calculate how much space is available to the terminal glucose residues in glycogens of different sizes. For corn glycogen of molecular weight of 20 X 10⁶, the surface area, assuming a sphere, is 3.75 X 10⁶ sq. A, and this measure, divided by the number of end groups (10,000), allows 37.5 sq. A for each terminal glucose unit. The same calculation for a glycogen molecule of one-fourth the size and with 2,500 end groups yields an area of 50 sq. A available to each terminal glucose unit. It can be noted that the glucose residues become more and more crowded as the structure grows in size. The radius of glucose in terms of an equivalent sphere is 3.6 A and from x-ray data the dimensions are determined as 5 X 7 X 9 A. In either case, the area occupied by 1 glucose residue would be between 35 and 40 sq. A. These considerations suggest that a structure of the type of glycogen may be self-limiting in size.

SUMMARY

The binding of glycogen and phosphorylase was investigated by the ultracentrifugal separation method. A phytylglycogen prepared from sweet corn was used; it had an average molecular weight of 20 X 10⁶, as compared to 5 X 10⁶ for the enzyme. This difference made it possible to sediment most of the glycogen and retain most of the enzyme in the upper layer of the centrifuge.

The photographs will be made available to interested persons.

The linear polymer amylose is known to assume a helical con-

figuration in solution, with approximately 6 glucose residues per turn. The chains in glycogen were too short to form a helix.
tube. The extra amount of enzyme sedimented in tubes which contained glycogen was considered to be bound to the glycogen. The binding constant was evaluated from a Lineweaver-Burk plot and compared with the Michaelis constant derived from enzyme kinetics. The $K_m$ for corn glycogen was 27 mg./100 ml. and a corresponding value for liver glycogen was 21 mg./100 ml. The binding constant was found to be about 10 times larger than $K_m$. The presence of the other substrates (glucose-1-phosphate and inorganic phosphate, as in the kinetic measurements) had no effect on the binding of glycogen and phosphorylase.

From the limiting ratio, glycogen sedimented : phosphorylase bound, it was calculated that 1 glycogen molecule of molecular weight $20 \times 10^6$ could bind a maximal of 33 enzyme molecules. Another calculation, based on the simplified assumptions that the glycogen molecule is spherical and the enzyme flat against its surface, yielded 22 as the maximal number of enzyme molecules which could be bound by 1 glycogen molecule. This type of binding would limit the effective glucose end group concentration to less than 2 per cent of the total, whereas in the kinetic measurements all of the end groups are accessible to the action of the enzyme. Steric hindrance by the relatively large enzyme molecules in a static, as compared to a dynamic situation is considered as the most likely explanation for the difference between the binding and the kinetic measurements.

Preliminary experiments are recorded in which the diameters of glycogen particles have been measured on photographic plates obtained with the electron microscope. Mixtures of glycogen and phosphorylase yielded particles of greater size and density than either glycogen or phosphorylase alone.

Consideration of the structure of glycogen in relation to surface area indicates that the glucose end groups become more and more crowded as the size of the molecule increases. It is suggested that glycogen may represent a self-limiting structure with respect to size.

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