deoxyadenylate and deoxyguanylate residues, and the second strand contains alternating deoxycytidine and thymidine residues.

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[1] Abbreviations used: A, deoxyadenosine; T, thymidine; C, deoxycytidine; G, deoxyguanosine; dATP, dCTP, dGTP, and dTTP, the deoxynucleoside triphosphates of adenine, cytosine, guanine, and thymine, respectively; dTp, dGp, dAp, and dCp, the 3′-phosphates of the corresponding deoxynucleosides; $dT_{11}$, undeca-thymidylic acid bearing a 5′-phosphate group and $dA_7$, deoxyadenosine heptanucleotide bearing a 5′-phosphate group. d-$(TC)_5$ and d-$(TC)_8$ and d-$(AG)_5$ refer to the oligomers containing, respectively, thymidylate and deoxycytidylate, and deoxyadenylate and deoxyguanylate in alternating sequence. These oligomers lacked phosphomonoester end groups. The high-molecular-weight polymer deoxypolyadenylate and deoxypolythymidylate is designated simply as poly dA:dT, and the polymer containing alternating deoxythymidylate and deoxycytidylate in one strand and deoxyadenylate and deoxyguanylate in the complementary strand is designated poly dTC:dAG.

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## *IN VITRO SYNTHESIS OF PARTICULATE GLYCOGEN**

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High-molecular-weight glycogen was first extracted from liver by Lazarow,[1] who called it particulate glycogen. Since then, it has been studied by several workers,[2, 3] but it is not known whether it is composed of $\alpha$-1,4 and $\alpha$-1,6 linked glucose residues only or if, in addition, it has other types of linkages or materials.

The molecular weight of particulate glycogen has been estimated[2] to be 50–200 ×

$10^6$ that of glycogen extracted with cold trichloroacetic acid[4] 5–70 $\times$ $10^6$, and values of 1–3 $\times$ $10^6$ have been assigned to alkali-extracted glycogen.[4]

When observed with the electron microscope,[5, 6] liver particulate glycogen appears as clusters or rosettes of 60–200 m$\mu$ diameter composed of subparticles of 20–40 m$\mu$ diameter. The rosettes and the subparticles have been referred to as $\alpha$ and $\beta$ particles, respectively, by Drochmans.[5]

The mechanism of glycogen synthesis is fairly well known[7–9] but, to our knowledge no work has been done on the *in vitro* synthesis of particulate glycogen. It was reasoned that starting with a high donor/acceptor ratio it should be possible to attain very high molecular weights. In fact, by incubating glucose 1-phosphate, glycogen, and purified enzymes in the right proportions, it has been possible to prepare glycogen which appears to be the same as that obtained by cold water extraction of liver. By a similar procedure, Illingworth, Brown, and Cori[10] had prepared glycogen of a molecular weight: 25 $\times$ $10^6$.

It is generally believed that the natural donor for glycogen synthesis is uridine diphosphate D-glucose,[7, 8, 11] but it was found more convenient to use glucose 1-phosphate and phosphorylase because they can be obtained in quantity and in pure form. Since glycogen synthetase and phosphorylase have the same acceptor specificity,[8, 12] it seemed likely that the polysaccharide formed would be the same.

*Materials and Methods.*—Crystalline phosphorylase *b* from rabbit muscle and purified branching enzyme from rat liver were prepared as described by Fischer, Krebs, and Kent[13] and Krisman,[14] respectively. Glycogen and branching activity were measured according to Krisman.[14, 15]

Particulate glycogen was extracted from the livers of sucrose-fed rats. Homogenization was carried out in 3 vol of 0.1 *M* glycine buffer, pH 10.4, followed by centrifugation as described previously.[16] The glycogen pellet was resuspended in the glycine buffer, and proteins were removed with chloroform and isoamyl alcohol as described by Sevag, Lackmann, and Smolens.[17] Alkali-extracted glycogen was prepared as described by Somogyi.[18]

Electron microscopy was carried out after negative staining with phosphotungstate. The procedure was as follows: grids, covered with Formvar and coated with carbon, were immersed in a detergent solution (1% Triton X-100), washed with distilled water, and dried. A thin film of glycogen solution (about 2%) was extended over the grids with a wire loop. Excess liquid was sucked off with filter paper, and then a film of 2–4 per cent phosphotungstate was applied in a similar manner. The latter was prepared by neutralizing phosphotungstic acid with tris(hydroxymethyl)aminomethane. Observations were carried out in the electron microscope (Siemens-Elmiskop I) with a double condenser, and photographs were taken at 30,000 or 40,000 direct magnifications.

*Results.—In vitro synthesis:* The sedimentation of different glycogen samples was studied in preliminary experiments. After centrifugation at 11,300 $\times$ *g* for 10 min, it was found that the sedimentation of particulate glycogen was 78 per cent while that of alkali-extracted glycogen was only 7 per cent.

The results of incubating glucose 1-phosphate, phosphorylase, and branching enzyme are shown in Table 1. It may be observed that in sample 1, 76 per cent of the glycogen was sedimentable. In the other samples, the proportion of sedimentable glycogen formed depended on the amount of acceptor present. Thus, sample 5

in which glycogen was present only as an impurity of some of the reagents gave the highest value (93%). In contrast, sample 7 in which more glycogen was added initially gave a value of only 10 per cent. In sample 8, in which a larger amount of alkali-extracted glycogen was added but glucose 1-phosphate was omitted, no sedimentable glycogen was formed. This shows that the proteins (phosphorylase and branching enzyme) do not produce aggregation by themselves.

TABLE 1

*In vitro* FORMATION OF PARTICULATE GLYCOGEN

| | Sample no. | KOH-glycogen added (mg) | Final glycogen (mg) | Per cent sedimentable* | λ maximum with iodine† (mμ) |
|---|---|---|---|---|---|
| 1 | Complete system | 0.01 | 3.6 | 76 | 460 |
| 2 | No glucose 1-phosphate | 0.01 | <0.2 | — | — |
| 3 | No phosphorylase *b* | 0.01 | <0.2 | — | — |
| 4 | No branching enzyme | 0.01 | — | — | Blue color |
| 5 | No glycogen | — | 3.15 | 93 | 460 |
| 6 | No additional adenosine 5′-phosphate | 0.01 | 0.92 | 55 | — |
| 7 | Complete plus additional glycogen | 0.33 | 3.5 | 10 | 400–460 |
| 8 | No glucose 1-phosphate, excess glycogen | 3.3 | 3.15 | 0 | — |

The complete system contained: 35 μmoles of glucose 1-phosphate, 0.13 μmoles of adenosine 5′-phosphate, 17 μg of phosphorylase *b*, 0.1 ml of liver branching enzyme. Total volume 0.22 ml. After 30 min at 37°, 0.2 ml of 0.1 *M* glycine buffer of pH 10 was added. The branching enzyme was estimated according to Krisman.[14] Ten μl of the preparation gave a Δ absorbancy at 520 mμ of 0.47 per 30 min.

* The samples were analyzed for glycogen before and after centrifugation at 11,300 × *g* for 10 min. The difference was taken as sedimentable glycogen.

† Under the conditions described by Krisman.[15]

The absorption maximum in the presence of iodine was at 460 mμ both for the glycogen synthesized *in vitro* and for the particulate glycogen isolated from liver. In some experiments in which phosphorylase was relatively more active than the branching enzyme, the polysaccharide gave at first a purple color with iodine, similar to that given by amylopectin. On further incubation or on addition of more branching enzyme the color became brown like that of glycogen.

*Electron microscopy:* When observed with the electron microscope, the particulate glycogen isolated from rat liver (Fig. 1*A*) appeared as particles of various sizes. The diameter of most of them was 80–100 mμ, and the extreme values were 20 and 180 mμ. The subparticles had a diameter of 10–24 mμ. The results were therefore similar to those described by other workers.

The glycogen prepared *in vitro* (Fig. 1*B*) with the complete system as described in Table 1, was composed of particles of slightly more uniform size, most of them of 90–100 mμ diameter with extreme values of 40 and 160 mμ. The subparticles had a diameter of 14–25 mμ. The general appearance was identical to that of particulate glycogen isolated from liver. Figure 1*C* shows, for comparison, the appearance of alkali-extracted glycogen.

*Effect of dilution:* As shown in Table 2, dilution of the glycogen-forming system did not decrease the proportion of sedimentable glycogen formed.

*Overgrown molecules:* In order to ascertain whether there is an upper limit for the size of the particles, glycogen was prepared as described for sample 5 in Table 1. After incubation, glycogen was separated by centrifugation and treated again with enzymes and substrate. The procedure was repeated three times. This glycogen could be sedimented with a very low centrifugal force: for instance, 80 per cent of it

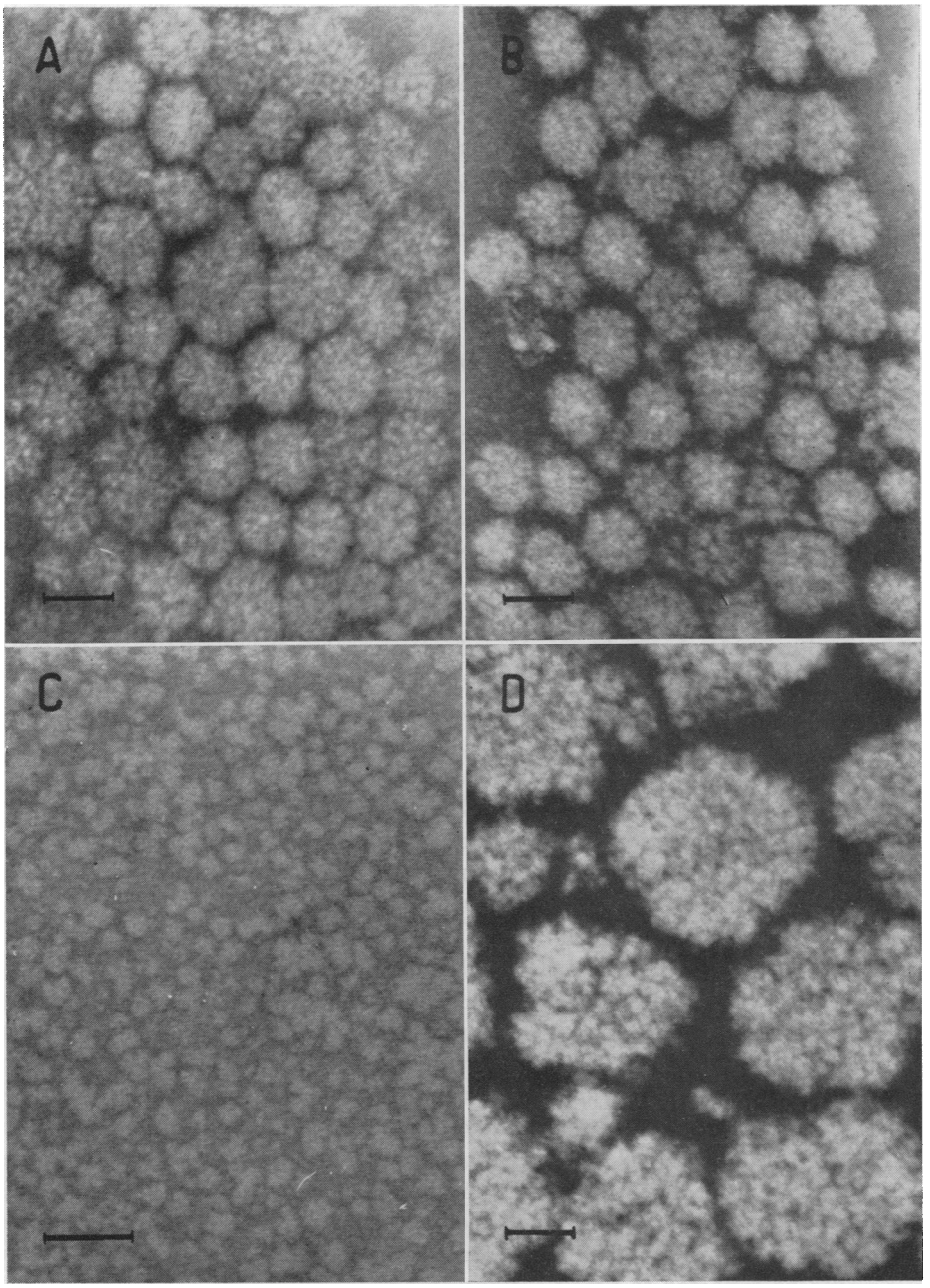


FIG. 1.—(*A*) Particulate glycogen extracted from rat liver. Negative staining with phosphotungstate, 90,000 ×. (*B*) Glycogen prepared *in vitro* as described in Table 1, 90,000 ×. (*C*) Glycogen extracted with alkali, 120,000 ×. (*D*) Overgrown particles prepared as described in text, 90,000 ×. The line in the lower left corner is 100 mμ.

TABLE 2

EFFECT OF DILUTION

| Sample no. | Final volume (ml) | Incubation time (min) | Glycogen formed (mg) | Per cent sedimentable |
|---|---|---|---|---|
| 1 | 0.13 | 45 | 2.1 | 88 |
| 2 | 0.27 | 75 | 2.0 | 90 |
| 3 | 0.41 | 120 | 1.7 | 88 |

Complete system as in Table 1 but with different total volumes.

was sedimented at 700 × $g$ for 20 min, while only 6 per cent of the liver particulate glycogen sedimented under the same conditions. Observation with the electron microscope (Fig. 1*D*) revealed enormous particles of up to 380 mμ diameter. The subparticles had a diameter of 15–25 mμ.

*Discussion.*—The glycogen formed *in vitro* from glucose 1-phosphate appears to be identical to particulate glycogen isolated from liver, as judged by sedimentation in the centrifuge and its appearance when observed with the electron microscope. This fact permits some deductions on the nature of the link which joins the subparticles. Since purified enzymes were used for the *in vitro* synthesis, it seems very unlikely that any other type of bond in addition to the α-1,4 and α-1,6 glucosidic linkages could have been formed. Low-molecular-weight glycogen did not become particulate in the absence of glucose 1-phosphate, so that none of the proteins used in the test produced aggregation *per se*.

A possibility which has to be considered is that as the subparticles grow, they may become entangled so that larger aggregates are formed. If this were the case, it would be expected that less particulate glycogen would be formed on dilution. In fact, no change was observed on increasing the volume 3-fold (Table 2).

It has been shown that liver particulate glycogen can be obtained practically free from protein and that treatment with urea, detergents, trypsin, or chymotrypsin[2] does not cause disaggregation. According to this evidence and that presented in this paper, it appears unlikely that particulate glycogen contains any other type of bond besides the glucosidic. However, it has been pointed out that this type of glycogen is degraded by acid or alkali at a rate which seems to be greater than that of alkali-extracted glycogen. This fact might be explained as follows. Assuming that glycogen has a regular structure in which the branches divide into two at regular intervals, Pollard[19] calculated that there is a maximum limit in molecular dimensions when the diameter reaches 26–40 mμ. When this size is attained, further growth becomes impossible because no more glucose residues can be packed in a sphere of that diameter. The maximum diameter calculated by Pollard corresponds to that of the subparticles observed with the electron microscope. Growth beyond that size could occur, but irregularly. A branch could grow out of the subparticle and thus start another particle; repetition of this process would give rise to particulate glycogen. The bonds between subparticles would be α-1,4 glucosidic but they would be expected to break easily under the strain produced by the thermal agitation of the subparticles. This would explain the lability of particulate glycogen.

*Summary.*—Incubation of glucose 1-phosphate with crystalline phosphorylase and purified branching enzyme leads to the formation of particles which are undistinguishable from particulate glycogen as judged by their rate of sedimentation or by their appearance when observed with the electron microscope. The structure of particulate glycogen is discussed in view of the evidence presented.

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# *THE SPECIFIC INHIBITION OF THE ENZYMATIC AMINOACYLATION OF VALYL- AND TYROSYL-sRNA BY PERIODATE-OXIDIZED sRNA*

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Chapeville *et al.*[1] observed that alanine, linked to cysteine specific sRNA, was introduced into the position normally occupied by cysteine during polypeptide synthesis. Their experiments provided direct evidence that messenger RNA recognizes only the sRNA moiety of aminoacyl-RNA. In this sense, the specificity of the interaction of sRNA with the appropriate aminoacyl-RNA synthetase plays a crucial role in determining the amino acid sequence of protein. The findings that the aminoacyl-RNA synthetases could be separated[2] and amino acid specific sRNA's could be isolated[3] are consistent with this view.