and a higher than normal precipitin reaction (G.V.) with concanavalin-A.

Treatment of various liver and muscle glycogens with salivary α-amylase resulted in the rapid disappearance of any precipitating ability with concanavalin-A solution.

The β-limit dextrin of waxy corn starch produced no turbidity when treated with concanavalin-A solution. This observation is significant in the light of the various reports that glycogen and amylopectin are very similar, the main distinction being in their chain lengths.[14,17-19,33,34] Variation of the exterior chain length is of little or no consequence in the concanavalin-A:glycogen reaction for the data in Table III show that removal of the outer branches by β-amylolysis leaves a β-dextrin showing an increase in G.V. approximately proportional to the degree of hydrolysis.

TABLE III

THE "GLYCOGEN VALUES" (G.V.) OF β-LIMIT DEXTRINS PREPARED FROM VARIOUS SAMPLES OF GLYCOGEN

Source of glycogen	G.V. Unhydrolyzed	G.V. After amylolysis	Hydrolysis,[a] %
Human liver	1.00	1.30	35
Rabbit liver	1.00	1.35	41
Rabbit hair	1.45	2.25	48
Northern pike liver	1.15	1.45	33.5
Sweet corn (*Zea mays*)	1.05	1.45	49
Baker's yeast[b]	3.0	4.5	45
Waxy corn starch	0.00	0.00	..

[a] Reducing sugars (as maltose) determined with 3,5-dinitrosalicylic acid[23] after maximum hydrolysis with β-amylase. (Incubation time, 24 hr.). [b] Purified by elution from carbon-celite column.[35]

Additional information bearing on the structural requirements for the glycogen concanavalin-A reaction is provided by the fact that methylated glycogen (from mussels, *Mytilus edulis*) had no precipitating ability, while periodate oxidation of rabbit liver and Northern pike liver glycogens, a reaction known to attack the outer before the inner branches of the molecule,[36] caused a progressive decrease in their precipitating ability.

It would appear, therefore, that the concanavalin-A complex involves not only the intact inner branches of the glycogen molecule but also the hydroxyl groups of the molecule. Further investigations into the structural requirements of the polysaccharide concanavalin-A reaction and its use in the study of the fine structure of polysaccharides is in progress.[37]

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The Reaction of Concanavalin-A with Mucopolysaccharides[1]

BY J. A. CIFONELLI, R. MONTGOMERY AND F. SMITH

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It is known that concanavalin-A, a globulin protein of jack bean meal, forms an insoluble complex with yeast mannan[2,3] and glycogen[4,5] in aqueous solution. By the use of this reaction it is possible to analyze quantitatively for these two materials[3] and also to differentiate the glycogen polyglucosans from the amylopectins,[5] a division which has been variously suggested to be chemically unsound.[6,7]

A study of the reaction has now been extended to the hexosaminehexuronic acid polymers, which include heparin, mucoitinsulfuric acid, chondroitinsulfuric acid and hyaluronic acid. The presence of such carbohydrate polymers in cartilage, connective tissue and blood group substances among products of animal origin suggested that an application of the concanavalin reaction to the qualitative and quantitative study of these compounds would be desirable.[8] Heparin has been found to give a precipitation reaction with concanavalin-A. Upon quantitative analysis[5] it was found that sodium heparinate[9] had 50% more precipitating ability than normal human liver glycogen, which throughout these investigations[5] has been taken as a

(1) Paper No. 3301, Scientific Journal Series, Minnesota Agricultural Experiment Station.

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(9) The authors wish to express their thanks to Dr. M. L. Wolfrom, for providing samples of sodium heparinate[10] and beef lung galactogen.

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standard. However, chondroitinsulfuric acid and hyaluronic acid did not exhibit a precipitation reaction with concanavalin-A.

Upon desulfation and concomitant acetylation of sodium heparinate[10] a desulfated heparin acetate is obtained which gives a precipitation reaction with concanavalin-A. This positive reaction persists when the above material is deacetylated with barium methoxide to give the N-acetyl desulfated heparin.[11] It would appear therefore that, as in the case of glycogen, the precipitation reaction reflects the over-all molecular architecture of the whole heparin molecule or part of it rather than the presence of sulfate ester groups. It is interesting to note that desulfated mucoitin acetate[10] also gives a precipitation reaction with concanavalin-A which lends support to the postulation of Jorpes[12] that heparin is a polysulfuric ester of mucoitin.

The galactogen[13] which is obtained together with heparin from beef lung does not give a precipitation reaction with concanavalin-A. It is suggested that the purity of a heparin preparation may be determined by the concanavalin-A precipitation reaction.

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Experimental

Concanavalin-A Solution.—The concanavalin-A solution was prepared by extracting jack bean meal[14] with 2% saline as described previously.[3]

Concanavalin-A–Polysaccharide Precipitation Reaction.—To an aqueous solution of the polysaccharide (1 ml. containing about 1 mg. of material) is added the concanavalin solution (9 ml.). The two solutions are well mixed and then allowed to stand for 10 minutes after which time the absorbance of the turbidity produced is determined in an Evelyn colorimeter using a No. 420 filter. A blank is prepared by using water in place of the polysaccharide solution. By interpolating the absorbance produced by the precipitation reaction on a standard curve prepared in the same way using purified human liver glycogen as a standard, the ratio of the absorbancies produced by equal weights of polysaccharide and the standard glycogen is calculated.

(14) A product of the Arlington Chemical Co., Yonkers, N. Y.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Periodate Oxidation of Cyclic 1,3-Diketones[1]

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The selective oxidizing ability of aqueous periodate ion is shown to encompass cyclic 1,3-diketones. Five- or six-membered cyclic 1,3-diketones, unsubstituted on carbon-2, reduce four molar equivalents of oxidant to yield one equivalent of carbon dioxide and one molar equivalent of a dibasic acid. Carbon-2 substituted, cyclic, six-membered 1,3-diketones reduce three molar equivalents of oxidant to yield one molar equivalent of a monobasic acid and a like amount of a dibasic acid. Postulated reaction intermediates oxidize more rapidly than the initial diketone and give the same products. Satisfactory reaction occurs between *p*H 3 and 8 with a rate maximum between *p*H 5 and 6. The kinetics were second order at low concentrations.

Oxidations of carbon compounds with aqueous periodate ion at room temperature or below can conveniently be divided into two types: (1) those producing carbon–carbon bond cleavage and (2) those where no such cleavage takes place. The second reaction type occurs when a hydrogen attached to a carbon flanked by carbonyl groups is transformed into an hydroxyl function. This gives rise to an hydroxy ketone, thus making the compound susceptible to the first type of oxidation.

The first type of periodate oxidation is the well known α-glycol cleavage reaction discovered by Malaprade[3] and initially developed by Fleury[4] and by Hudson,[5] and their associates. The same type of oxidation probably prevails in the oxidation of α-hydroxyketones,[6] α-diketones,[6] 2-aminoalcohols[7] and α-keto-acids,[8] since in each case the reduction of one equivalent of oxidant is accompanied by the cleavage of one carbon–carbon bond. The theory of this type of oxidation has been extensively studied.[9]

The second type of periodate oxidation was noted to take place in malic,[10] malonic[8,10,11] and acetoacetic acids[11] and their derivatives, and, indirectly, in malonaldehyde.[11] Other examples of this type of oxidation have appeared in the sugar series.[12] Ab-

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