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GEORG F. SPRINGER

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Third Conference on Polysaccharides in Biology

THE JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

DURING THE PAST fifteen years the Josiah Macy, Jr. Foundation has organized more than twenty conference groups, each group meeting for at least two days annually over a period of five or more years. Each meeting is limited to twenty-five participants (members and guests), selected to represent a multidiscipline approach to some urgent problem in the field of medicine and health. The goal of this conference program is the promotion of communication, the exchange of ideas, and the stimulation of creativity among the participants. The purpose of the publication of the Transactions of the meetings is to share, as far as possible, the conference process with a larger audience than could participate personally in the discussions.

These conferences provide an opportunity for informal give and take among the participants. To further this purpose the number of presentations planned for each day is generally restricted to one or two. The member, or guest, selected to give such a presentation is requested not to "read a paper," but rather to highlight, in an informal manner, some of the more interesting aspects of his or her research, with the expectation that there will be frequent interruptions by participants in the form of questions, criticism, or comment. Such interruptions during the course of a presentation are encouraged and form an essential part of the "group interchange."

The conference program has always been viewed by the Foundation as an experiment in communication in which there is room for improvement and need for frequent reappraisal. Sufficient experience has already been gained to justify the conclusion that this type of conference is an effective way of improving understanding among scientists in medicine and allied disciplines, of broadening perspectives, of changing attitudes and of overcoming prejudices. The further conclusion has been reached, as the result of this experiment, that the major obstructions to understanding among scientists lie in the resistance of human attitudes to change, rather than in difficulties of technical comprehension. Less extensive experience with non-scientists has indicated that the effectiveness of this type of conference is not limited to groups of scientists, but will function in any group meeting where more effective

communication is the primary goal. It is also clear that the same conference technique, with minor changes, is readily adapted to small international conferences.

The style of publication of the Transactions has aroused considerable interest and some criticism. The criticism has been directed primarily to editorial permissiveness which has allowed in the final text, in some instances, too many questions, remarks, or comments which, although perhaps useful during a heated discussion, seem out of context and interrupt the sequence of thought in the printed volume. A few have objected to the principle of publishing in this style and would prefer a depersonalized summary without interruptions.

The Foundation Staff and the Scientific Editors of these volumes welcome criticism and hope to profit thereby in increasing the usefulness of the Transactions to scientists and students of science in this country and abroad.

FRANK FREMONT-SMITH, M.D.
Medical Director

HOMOPOLYSACCHARIDES

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DeWitt Stetten: The class of homopolysaccharides that has been of particular interest to myself and my colleagues for the past 15 years contains glycogen, in which glucose is the monosaccharide involved. Later in the session, we shall discuss cellulose, in which the β configuration of glucose occurs. In the homopolysaccharides of the glycogen series, the α configuration uniquely occurs. Two well established linkages occur, the α-1$\rightarrow$4 linkage and the α-1$\rightarrow$6 linkage. This permits of the possibility of branching, and a branch point, as it occurs in the glycogen-amylopectin type, occurs where one glucose residue carries three substituents, on carbon atoms 1, 4, and 6, respectively.

Table I represents a continuous family of polysaccharides ranging from the amylose of starch, in which essentially only one linkage exists, that of the α-1$\rightarrow$4, to the dextrans, in some of which only α-1$\rightarrow$6 links occur. In between these two are amylopectin and glycogen, where the 1$\rightarrow$4 linkage predominates but the 1$\rightarrow$6 also occurs. The demarcation between the animal and the vegetable worlds is not a sharp one. It can be pointed out that compounds of branching pattern resembling amylopectin have been described by Dr. G. T. Cori (1) as occurring in von Gierke's disease—

G. T. Cori: In one type of general glycogenosis, not in von Gierke's disease.

D. Stetten: —and glycogen has also been described in corn.

Figure 1 from the Cori (2) laboratory represents a reconstruction of the branching pattern which is currently believed to account for all or most of the characteristics of the glycogen molecule. There are certain generalizations that can be made about this branching pattern. In this or any other branching pattern, provided there is not a

TABLE I

Some Homopolysaccharides of α-D-glucose

	Linkage Present* α-1→4	α-1→6	Approximate Ratio 1→4/1→6
Amylose	+	−	
Amylopectin	+	+	24 to 30
Glycogen	+	+	12 to 18
Dextran	−	+	

*Certain dextrans from Leuconostoc have been found to contain virtually no α-1→4 linkages.

Reprinted, by permission, from Stetten, D., Jr.: Certain aspects of the metabolism of glycogen. *Diabetes* 6, *391 (1957)*.

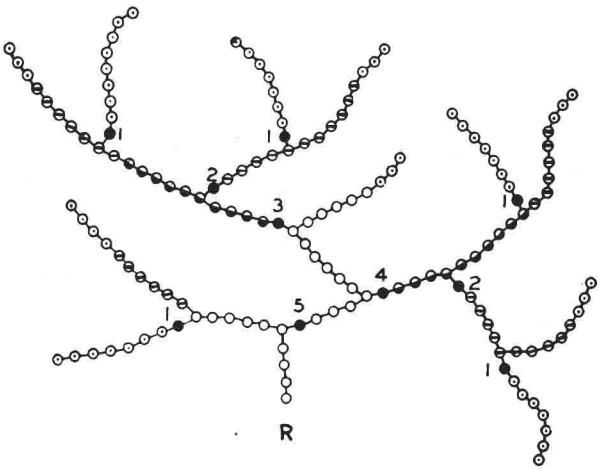

FIGURE 1. R—reducing end of molecule. ⊙ ⊖ ◒—glucose units removed by first, second, and third digestions with phosphorylase, respectively. ●—glucose unit split off as free glucose from α-1→6 linkage by amylo-1,6-glucosidase. Reprinted, by permission, from Larner, J., Illingworth, B., Cori, G. T., and Cori, C. F.: Structure of glycogens and amylopectins. II. Analysis by stepwise enzymatic degradation. *J. Biol. Chem.* 199, *641 (1952)*; also by permission from Stetten, D., Jr.: Certain aspects of the metabolism of glycogen. *Diabetes* 6, *391 (1957)*.

closed cycle, there must be and there can be only one reducing end to the molecule, which we are designating as R. The number of nonreducing termini is necessarily greater by one in this branching pattern than the number of branch points.

Larner: This holds only if the α-1$\rightarrow$6 linked glucose residues are present as branch points, that is, linked to α-1$\rightarrow$4 linked residues within a chain. If the α-1$\rightarrow$6 residues are linked at the nonreducing ends, this relationship would not hold. Such a situation might arise from the transfer of a linear segment by the branching enzyme to the nonreducing terminal glucose unit as acceptor.

D. Stetten: And the number of glucose residues between branch points and in terminal chains represents mean values. As far as I am aware, there is no information available as to the magnitude of the standard deviations, although there is a suggestion in the literature that this may be considerable. This suggestion relates to the publication from Wolfrom's laboratory (3) of the isolation of isomaltotriose from a partial hydrolysis of glycogen, which suggests that branch points may actually occur in adjacent glucose residues. If they can occur close to each other, they must also occur further apart from each other than these mean values at other parts of the molecule.

It should also be pointed out that this diagram represents not a glycogen molecule but a fragment, or a picture of the pattern of the glycogen molecule. The molecular size of glycogen is considerably larger than is represented by this figure, and is subject to a wide variation within a given population of molecules.

Some years ago, before isotopic carbon was available, we entered this field using deuterium, and one of the things that became apparent was that it was possible to introduce deuterium into glycogen in the living animal by adding deuterium oxide to the body fluid (Figure 2).

One of the problems which Mrs. Stetten explored was the distribution of this isotope within a biologically labeled molecule. The preparation was urinary glucose, secured from a rabbit whose body fluids were enriched with deuterium oxide. This material was isolated initially from the pentaacetate, the derivatives studied being the benzimidazole derivative, the phenylosazone, the saccharic acid salt, the diamide of trimethoxyglutaric acid, and the diamide of dimethyltartaric acid, as shown.

From a comparison of these compounds and the deuterium content of each one, it was possible to compute the deuterium from each of the six labeled positions, or each of the seven hydrogen atoms, of the stably bound variety (4). This could also be explored by determining the balance at the end. We found good agreement between the sum of the

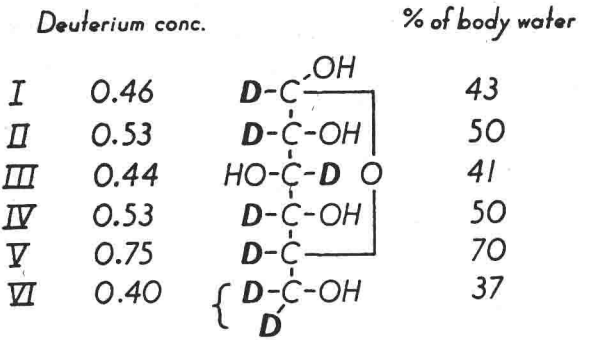

FIGURE 2. Distribution of deuterium in the stable hydrogen positions of a sample of glucose synthesized by a diabetic rabbit whose body water had been enriched with deuterium oxide. Reprinted, by permission, from Stetten, D., Jr., and Stetten, M. R.: Studies in carbohydrate metabolism. VII. Distribution of deuterium in sample of deuterio glucose excreted by diabetic rabbit. *J. Biol. Chem.* **165**, *147 (1946)*.

computed deuterium concentrations in each position and the known quantity of deuterium in the molecule.

We performed this experiment only once. It was a rather tedious one, involving the preparation of a number of derivatives and a considerable number of deuterium analyses. A point that interested us was that whereas deuterium could be demonstrated in each of the stable positions, there appeared to be more deuterium attached to carbon atom 5 of glucose than in the other position. Glucose could thus be labeled. We then proceeded to explore a number of experimental variables.

Pollard: D_2O may introduce a difference, because some symmetry is not there, at least for DOH. It is not inconceivable that quite a significant difference could occur between H_2O and D_2O.

D. Stetten: When a number of materials were compared as glycogen precursors, different values were discovered (5). These experiments were all conducted in the same fashion. Various materials were fed, various treatments rendered, and glycogen was isolated. It should be pointed out that when ordinary nonisotopic glucose was given, glycogen isolated 3 hours later from the liver contained 38 per cent as much deuterium as did the body water. On the other hand, when lactate was given, this figure was considerably higher, of the order of approximately 60 per cent, and essentially the same figure was secured with respect to liver glycogen when epinephrine was injected and nothing was fed (6).

This was in accord with the hypothesis, expressed by Dr. Cori (7)

many years earlier, that the glycogen which accumulates in the liver of the fasted animal, after epinephrine is given, arises from the blood lactate which has, in large part, in turn, stemmed from muscle glycogen, which is mobilized as a result of this treatment.

When isotopic glucose, in this case deuterium-labeled biosynthetic glucose, was fed to a fasted animal, about 31 per cent of all the hydrogen in the glycogen deposited arose from carbon-bound hydrogen of the fed glucose. Some 38 per cent of the hydrogen in this glycogen arose from the body water, whereas some 34 per cent could be shown to be exchangeable hydrogen of glycogen simply by dissolving ordinary glycogen in heavy water, evaporating to dryness and measuring the quantity of deuterium in the residue.

Thirty-four per cent is in reasonable agreement with the structure of glycogen, from which it may be predicted that of the ten hydrogen atoms per glucose residue, three are of the hydroxyl variety and seven are carbon-bound. The balance (103 per cent) we consider to be entirely satisfactory. With this kind of information, we next explored the kinetics of this labeling process.

First-order kinetics appeared to fit this situation. If deuterium oxide is introduced into the body fluid, as new glycogen is synthesized, it will incorporate a certain amount of deuterium. When all the preexisting molecules of glycogen shall have been replaced by newly synthesized molecules, a maximum value shall be achieved in deuterium concentration. This will be the deuterium concentration of the newly synthesized molecule. If the further assumption is made that the glycogen of an organ is metabolically homogeneous, that the portion of material which is destroyed at any time has the same isotope abundance as the bulk of material at the time, then one can readily derive the first-order equation relating turnover to deuterium concentration and time.

$kt = \ln \frac{i_{max}}{i_{max} - i}$ The values secured are shown in Table II and the results are plotted as first-order functions in Figure 3. The rate constants and the more familiar half-lives of these materials are shown in Table III. It will be seen that the glycogen of liver has a half-life of approximately a day in the rat under these circumstances; the half-life of the carcass glycogen is approximately 4 days.

This kind of study could be extended to other situations. One of the situations which interested us and which was studied by Dr. Goldwater and myself (8) related to the glycogen of the fetus. The fetus of the mammal is extraordinarily rich in glycogen and, as term is approached, is far richer in glycogen than is the adult animal. One of the assump-

TABLE II

Incorporation of Deuterium from Body Water into Glycogen and Fatty Acids by Normal Rats

Days	Per Cent of Deuterium in		
	Liver Fatty Acids	Liver Glycogen	Carcass Glycogen
0.125	5.4	0.6	1.7
1	15.5	19.6	3.0
2	21.9	16.4	6.1
4	27.8	23.6	9.6
8	35.8	28.8	19.9
16	37.8	28.9	24.3
∞	37.9	28.9	25.5

Reprinted, by permission, from Stetten, D., Jr.: The alterations in metabolism incident to administration of insulin, adrenalin and thyroid substances studied with the aid of isotopes. *Recent Progr. Hormone Research* **4**, *189 (1949).*

tions on which the previous mathematical treatment was based was that the quantity in the pool remained constant throughout the time of study. Obviously, this assumption was not valid in the case of the fetus, whose most striking characteristic is that it is growing rapidly.

We studied the rat fetus for the interval from 18 to 20 days, that is, up to immediately before term. The glycogen content of the fetus as a whole, the fetus wet weight, is of the order of 500 mg./100 gm. of wet weight—far more than the glycogen content of representative adult tissue (Figure 4). A graphic solution was found by means of which it was possible to compute that from the change in deuterium concentration in the glycogen of the fetus, the fetus is manufacturing each day, on the 19th day of fetal life in the rat, a quantity of glycogen approximately equal to that which it contains; that is, it is generating *de novo* approximately ½ gm. of glycogen per 100 gm. of body weight. Since

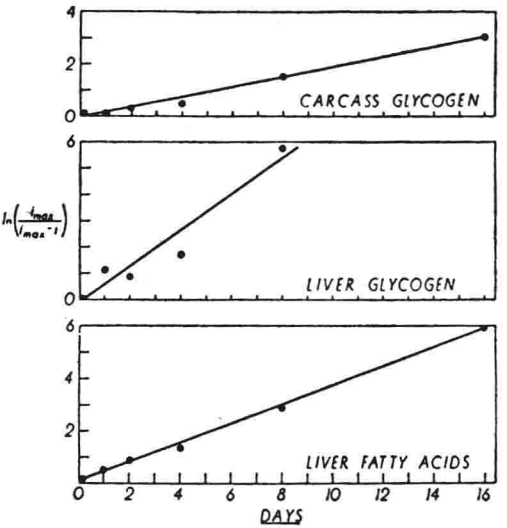

FIGURE 3. Uptake of deuterium from body water. Reprinted, by permission, from Stetten, D., Jr., and Boxer, G. E.: Studies in carbohydrate metabolism. I. Rate of turnover of liver and and carcass glycogen, studied with aid of deuterium. *J. Biol. Chem.* **155**, *231 (1944)*.

the glycogen content of the fetus is not doubling at this time, even though it is increasing, it is also apparent that glycogen is being destroyed in the fetus, and the rate of such destruction could be estimated from these data.

All these studies and, it may be mentioned, many other studies in which isotope rate data have been used for the computation of biological kinetics, contain an unfortunate and difficult assumption to prove, which has already been mentioned, namely, that the metabolite under consideration is homogeneous metabolically. Glycogen offered at least two modes of test of this hypothesis. We have taken advantage of the growing knowledge of glycogen chemistry, particularly of the enzymology which pertains to glycogen, developed in the laboratories of Meyer, of Cori, and by a number of other workers.

The studies (9) which I am about to describe relate to the use of the enzyme, β-amylase, from barley malt, whose mode of attack has been precisely defined. It attacks the branched polysaccharides of the amylopectin-glycogen variety at their nonreducing terminals, eliminating maltose successively, until a branch point is reached. When a branch point is reached, the resultant product is insusceptible to further attack by this enzyme. The black circles in Figure 5 represent the maltose that

TABLE III

Rate of Turnover of Glycogen and Fatty Acids in Rats

	k Days^{-1}	$t1/2$ Days
Liver fatty acids	0.37	1.9
Liver glycogen	0.68	1.0
Carcass glycogen	0.19	3.6

k is the fraction of each constituent replaced per day.
$t1/2$ is the half-life of each constituent.

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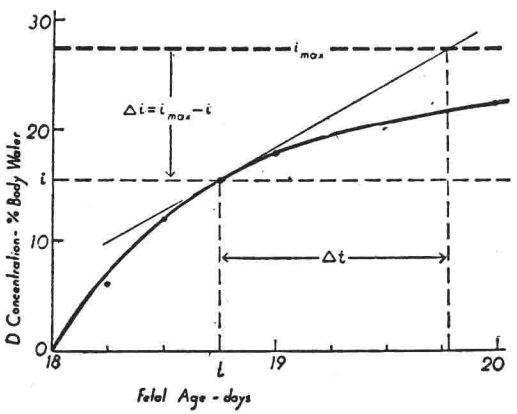

FIGURE 4. Uptake of deuterium into glycogen of the fetuses of pregnant rats whose body fluids were enriched with D_2O. Reprinted, by permission, from Goldwater, W. H., and Stetten, D., Jr.: Studies in fetal metabolism. *J. Biol. Chem.* 169, *723 (1947).*

might be anticipated to be liberated, the open circles, the component glucoside residues of the limit dextrin.

Figure 6 is a confirmation of the studies of a number of investigators that indicate the limit nature of the dextrin. Here, a sample of glycogen was subjected to digestion by β-amylase and, as will be seen, 50 per cent