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CLAUDE BERNARD AND THE DISCOVERY OF GLYCOGEN

A CENTURY OF RETROSPECT*

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On March 21, 1857, Claude Bernard communicated to the Société de Biologie in Paris a description of the isolation of glycogen from liver tissue and of the chemical and physical properties of the isolated substance. Two days later he delivered a communication on the same subject to the Académie des Sciences in Paris (Bernard, 1857).

Almost exactly a hundred years afterwards, I believe that it is appropriate to consider once again the significance of Bernard's discovery and to review briefly what has been learned about glycogen since that time. I propose also to outline the observations which led to the discovery of glycogen and the glycogenic function of the liver. I shall recall the heated controversy between Pavy and Bernard, since we can undoubtedly often learn something to-day from the mistakes of the past.

In his excellent biography of Claude Bernard the late Professor J. M. D. Olmsted (1939) states that, according to Bernard's views, carbohydrate is stored as glycogen in the liver (p. 202). This statement is not entirely correct, as I shall hope to show; and because misstatements have been made from time to time about Bernard's views on the significance of liver glycogen I shall quote in a number of instances what Bernard actually wrote. The translations from the French are my own.

When Bernard began his researches on the metabolism of carbohydrate almost nothing was known about the fate of dietary sugar in the animal body. The excretion of glucose in the urine in the disease diabetes mellitus had long been recognized, while in 1826 Tiedemann and Gmelin had found that fermentable sugar is formed from starch during the course of alimentary digestion. But what happened to this sugar was unknown. In agreement with the dominating views put forward by Dumas and others, according to which green plants alone form complex substances while animals always break them down, it was tacitly assumed that sugar was broken down to smaller molecules by the animal body.

Glycogenic Function of the Liver

In his thesis for the Doctorate of Medicine Bernard (1843) describes how cane sugar which is injected into the veins of an animal is eliminated in the urine, whereas preliminary treatment with digestive juices renders this sugar capable of complete assimilation in the animal body when it is introduced by this route. This important observation made him decide to find out what happened to the sugar which was absorbed into the blood stream during the normal course of digestion. As he said many years later, he decided to follow the metabolic fate of all absorbed foodstuffs step by step and to study the various transformations which these substances underwent. "I proposed," he wrote, "to apply these methods successively to different substances—the proteins, sugars, and fats. I began with the sugars because their study seemed to me to be the most simple. But this plan of investigation was too big; to-day, after 30 years of research which has not been wholly sterile, I am still engaged in studying the metabolism of the sugars" (Bernard, 1879, pp. 40–41). There are few engaged in original investigation who have not experienced Bernard's feeling that any research problem is too big to be completely solved.

In his thesis for the Doctorate of Science, published in 1853, Bernard wrote: "My aim was to follow closely the sugar, which was absorbed from the food, in its passage along the blood stream first to the liver, then to the lungs, and finally to all the other tissues of the body. I wanted to know if the sugar was destroyed in traversing the liver, which is the first organ through which it passes after being absorbed into the tributaries of the portal vein. For this purpose a dog which had been fed on carbohydrate food for seven days was killed during the digestion of a meal, by section of the spinal bulb. As quickly as possible I opened the thorax and abdomen in order to discover if sugar was present in the blood which had passed through the liver. Now it was easy to show quite clearly that the blood of the hepatic veins, where they joined the inferior vena cava, contained a large amount of glucose" (Bernard, 1853, pp. 10–11). This experiment seemed to indicate that the liver did not absorb or destroy the sugar passing to it in the blood flowing from the gut, but Bernard did not draw this conclusion without further experiment. He decided that he must ascertain whether or not the sugar present in the blood of the hepatic veins was similar to that absorbed from the gut. For this purpose he performed a similar experiment on a dog fed on a diet which consisted of meat only, and which was therefore apparently free from carbohydrate. To his surprise he again found that the blood of the hepatic veins

*Based on a lecture given in the University of Cambridge on March 7, 1957.

contained a large amount of sugar, although he was quite unable to detect sugar in the contents of the stomach and intestines. Moreover, he found that the blood of the portal vein contained no sugar before it entered the liver, whereas the blood leaving the liver in the hepatic veins contained much glucose. He therefore concluded that the liver possesses the power to produce sugar. He repeated this type of experiment under all sorts of different conditions but always with the same result—namely, the blood of the hepatic veins was always found to contain an abundance of sugar while that of the portal vein contained none unless the animal had received sugar in its diet.

He found, moreover, that when an animal which had fed on meat alone for two weeks was killed, an aqueous extract made of the liver tissue contained much sugar, although none could be detected in similar extracts made from other organs. By fermentation with yeast, and by means of other tests, the sugar in the extract of the liver was identified as glucose. It was clear, therefore, that sugar could be present in the body when no carbohydrate food was being absorbed and that this sugar was formed by the liver. When, as he wrote in 1853, "sugar is manufactured in the liver, which must therefore be considered as an organ which produces or secretes sugar" (Bernard, 1853, p. 54), he was introducing an important new idea—namely, that substances may be secreted directly into the blood stream. Moreover, the ideas of Dumas, Boussingault, and others that animals always destroy or decompose complex substances had to be modified, since the liver was capable of producing a substance as complex as sugar when this substance was not present in the diet.

Having discovered that glucose could be produced in the body, Bernard abandoned his original intention to investigate the site of destruction of the sugar absorbed as food from the intestine. He set out to discover the origin of the sugar newly formed in the liver.

Discovery of Glycogen

At this time Bernard's estimations of the sugar content of extract of liver tissue were made in duplicate by titration with the copper reagent of Barreswil, a modified Fehling's solution. He relates (Bernard, 1865, pp. 291–295) how one day he was pressed for time and was unable to make his duplicate determinations simultaneously. He made one estimation immediately after the death of an animal and postponed the other until the following day. The second estimation gave a value very much higher than the first, and the difference was so great that Bernard investigated the reason for this discrepancy. Hitherto he had not ascribed significance to the length of time which elapsed between the death of an animal and the determination of the sugar content of the liver tissue. He now found that time was of great importance. Immediately after the death of an animal the liver was found to contain very little sugar, but within only a few minutes the amount of sugar had substantially increased, and at the end of two hours a large quantity had usually made its appearance. By forcibly injecting a current of water through the blood vessels of a freshly excised liver he was able to free the tissues from sugar. When such a washed, sugar-free liver was allowed to remain at a moderate temperature for a few hours large quantities of sugar appeared (Bernard, 1855b). This conclusively showed that the sugar was formed, not from substances in the blood as had previously seemed possible, but from materials in the liver tissue itself.

A cold aqueous extract rapidly made from liver tissue immediately after the death of the animal contained no detectable reducing sugar, but after two days at room temperature much sugar had appeared. If, however, the fresh extract were boiled no sugar subsequently made its appearance. These results and many other similar ones showed that the liver contained an extractable substance which did not give the ordinary tests for reducing sugar but which could give rise to reducing sugar by the action of a "ferment" in the liver (Bernard, 1855a).

The fact that the liver could produce sugar from a substance within itself was published in 1855 by Claude Bernard, but it was not until 1857 that he was able to describe the isolation of the sugar-forming substance, or "la matière glycogène" as he named it.

After Bernard's announcement in 1855 of the ability of the liver to produce sugar, many investigators attempted to isolate the precursor, and it has been claimed that Hensen independently isolated glycogen. Many years later Pflüger repeated Hensen's observations, and concluded that Hensen had in fact obtained a mixture of protein and glycogen, and not pure glycogen. Nevertheless Hensen must be given some credit for his work, although Bernard makes no reference to it in his own writings.

Criticisms of Bernard's Early Experiments

For many years Bernard insisted, in spite of contradiction, that the blood of the portal vein of a dog fed on meat contained no sugar. Among those who disagreed, Figuier was so insistent that a special committee of the Académie des Sciences was set up to report on this important matter of controversy. The committee's report completely vindicated Bernard, agreeing with him that no trace of fermentable sugar was present in the blood of the portal vein of an animal fed on meat. Figuier was unconvinced, however, and he was later joined in his contentions by Chauvau, Pavy, and others. Although in 1859 Bernard was still insisting on his mistaken assertion, I have been unable to find any mention of this point thereafter until he admitted in 1877 that his earlier findings in this respect were incorrect (see Young, 1937).

The young English doctor, Frederick William Pavy, criticized Bernard's experiments on much wider grounds. In 1852 Pavy worked for a year in Bernard's laboratory in Paris, returning to become lecturer in physiology at Guy's Hospital and assistant physician. Pavy was apparently uncertain that in the experiments which he had seen performed in Bernard's laboratory the withdrawal of blood from the dying dog provided material that was characteristic of the conditions in the living animal. When he returned to England he therefore passed a catheter down the jugular vein of the living unanaesthetized dog and withdrew blood from the right heart (Pavy, 1854–5, 1860). His original intention was to investigate the disappearance of the sugar from this blood when it was perfused through the lungs of another animal. He was astonished to find, however, that there was almost no sugar at all present in the blood of the right heart obtained in this manner. He found, however, that if the animal was killed and the blood then collected from the right heart, much sugar was present therein, although sugar could not be detected in the blood obtained from a heart which had been rapidly removed from the living animal. Pavy then began to suspect that the production of sugar by the liver might be a post-mortem phenomenon, and was led to conclude that inferences about the ante-mortem state that had been drawn from post-mortem investigations of this sort must be abandoned.

In 1860 Pavy consolidated his position as a critic of Claude Bernard's views. He stressed that, on the whole, his experimental results did not disagree with those of Bernard, although in two important respects they did. First, Pavy agreed with Figuier and others in finding that portal vein blood did contain sugar. Pavy said that the amount present was indistinguishable from that found in other parts of the circulating blood of the living animal. Second, he did not agree with Bernard that blood removed from the right heart of the living animal is strongly reducing. Far from accepting the view that the liver secreted

sugar, Pavy believed that it absorbed it and stored it as glycogen in a sort of detoxication process. Only under pathological conditions did the sugar escape from the liver into the circulation, where it could produce undesirable effects, such as those found in diabetes mellitus. After death, or in the diabetic condition, the liver lost its power to form or hold glycogen and the organism was flooded with sugar. Bernard's gibe, that if Pavy considered the production of sugar by the liver to be a post-mortem phenomenon he must consider "a diabetic person to be a walking corpse—a very queer idea" (Bernard, 1877, p. 349), was really irrelevant.

In 1860 Pavy described his finding that restlessness or struggling of an unanaesthetized animal in which a catheter had been introduced into the jugular vein resulted in a substantial increase in the sugar content of the blood. He further observed that "obstruction of the breathing determines an unnatural increase of sugar in the circulation and a strongly diabetic state of the urine may be induced in this way" (Pavy, 1860). Pavy is not usually credited with these early observations of emotional hyperglycaemia and of asphyxial hyperglycaemia and glycosuria. Pavy was also aware many years before Bernard that glucose rapidly disappears from blood after it is shed (Pavy, 1854–5) and, finding that the blood became acid as the glucose vanished, made the pertinent suggestion that the disappearing sugar had been converted to lactic acid.

Although Pavy's criticisms of Bernard's investigations met with some support, the prestige of the discoverer of glycogen was such that they were comparatively ineffective. In 1877 Pavy again returned to the problem with a vigorous criticism of the methods used by Bernard for the estimation of blood sugar, at the same time describing a new method of his own. The application of his own method, he believed, demonstrated that there was no significant difference between the sugar contents of arterial and venous bloods. In his book *The Physiology of the Carbohydrates,* published in 1894,* Pavy presents evidence to show that the "blood of the general circulation contains a standard or definite amount of sugar which, under natural and ordinary conditions, may be stated to range from about 0.6 to 1.0 or a little over 1.0 per 1,000 and which presents no evidence of any essential variation in the different parts of the system spoken of," although after the ingestion of carbohydrate food the portal vein blood sugar was considerably greater than that of the hepatic veins, showing that the liver was absorbing the sugar and presumably converting it to glycogen. After a long and fruitful life, Pavy died in 1911 at the age of 82, still convinced that Claude Bernard was wrong in his belief that the liver normally secretes sugar.

Surgical removal of the liver obviously provided a crucial experiment by which a choice could be made between the views of Bernard and Pavy, and although Minkowski in 1886 and Seegen in 1890 removed the liver from geese, in which the vascular arrangement permits this without undue difficulty, Pavy (1894) had no difficulty in rejecting the relevance of this evidence to the mammal. It was indeed not until 1922, when F. C. Mann and T. B. Magath (1922) accomplished hepatectomy in the dog and observed that the blood sugar then rapidly falls, that Bernard was finally proved to be right, and Pavy wrong.

Fate and Origin of Sugar in the Liver

Bernard's early belief about the fate of the sugar liberated by the liver was that it diffuses over the entire organism by way of the circulation, the amount of sugar steadily diminishing the further the blood flows from the liver, which is the origin of the sugar (Bernard, 1885a, p. 470). By 1877 he had found, in confirmation of Chauvau and others, that arterial blood generally contains more sugar than venous blood. At the same time he corrected his previous mistaken belief that no sugar was present in the blood of the portal vein. Nevertheless, he reaffirmed, there is normally more sugar in the blood leaving the liver than in the blood entering that organ.

*The criticism, and its trenchant attempted rebuttal, evoked by Pavy's book, I have discussed elsewhere (Young, 1937).

Soon after Bernard's discovery of glycogen in the liver, Sanson found that muscular tissue also contains glycogen, while, later, Nasse and Weiss and others showed that this glycogen diminishes in amount during muscular contraction. Bernard thought it possible that this glycogen in the muscles might be transported to them from the liver by the way of the blood. He found that the glycogen in the muscles never gave rise to glucose. "Muscle glycogen always undergoes a lactic acid fermentation, and this is the only change that muscle glycogen ever undergoes, either in the living animal or after death" (Bernard, 1877, p. 428). Taking into account the fact that the muscles continuously absorb glucose from the blood, Bernard concluded that in the living animal sugar is destroyed in the muscles, and not formed there. These ideas provided the basis of what is generally known as Claude Bernard's theory of the glycogenic function of the liver, although Bernard does not define it precisely as such. According to this theory, production of sugar by the liver is closely related to the rate of utilization of sugar by the tissues in general, the glycogen in the liver being the immediate source of the secreted sugar.

According to Bernard's views, liver glycogen is formed largely from nitrogenous material in the food. He could find no evidence that the glycogen content of the liver rose after the administration of fat to an animal. The question of whether liver glycogen is ever directly formed from carbohydrate in the food was a tricky one. Although Bernard contradicts himself a number of times on this point, his view, on the whole, appears to be that the glucose which arrives at the liver from the gut is not directly converted into glycogen. Let me quote his words from *Leçons sur le Diabète* (1877, pp. 321–322). "Is the large amount of glycogen formed in the liver after the ingestion of carbohydrate, the result of a direct conversion, a recession of sugar to glycogen, or does the sugar play only the role of a powerful nutritive stimulant which increases the glycogenic function to a marked degree? I have attempted to test the hypothesis that cane sugar is directly converted to liver glycogen. If this conversion really occurs, if it has as its end the preservation of the substance until such time as it is to be utilized, it is reasonable to suppose that when this time comes the substance will resume its previous form, that is it will undergo the converse of those changes to which it had previously been subjected, and that invert sugar will be re-formed from glycogen."

Bernard found that the glycogen formed in the livers of rabbits which had been fed on sucrose did not give a laevorotatory sugar on hydrolysis. In fact, he records that the sugar formed seemed to be more dextrorotatory than glucose. As the result of these experiments Bernard wrote: "The indisputable fact is that the administration of cane sugar considerably increases the liver glycogen content; but how does the sugar act in this case—as a nutritive stimulator or as a substance which is directly converted to glycogen? I am inclined to believe, I must confess, that the first suggestion is the more correct" (Bernard, 1877, pp. 322–323). When he fed a dog on starch the liver was found to contain less glycogen than the liver of a dog fed on fibrin. "This result seems to indicate," he wrote, "that the animal body cannot form glycogen with a ternary body alone, but that it can with a more complex substance such as fibrin" (Bernard, 1877, p. 542). Apparently Bernard regarded the secretion of glycogen by the liver as a complicated process and considered that it would therefore require a complex substance for its stimulation. Since starch contains only three elements—carbon, hydrogen, and oxygen—it was not suitable, but fibrin, being the more complex substance altogether, was able to stimulate the process of sugar formation. It is clear, therefore, that the statement which is so often made to-day about Bernard's work is wrong. He did not believe that the liver absorbed sugar from the blood and converted it directly into glycogen. He was uncertain (see Young, 1937), but inclined to the view that the glycogen which appears in the liver during the absorption of carbohydrate food is not derived from exogenous sugar but secreted, newly formed, by the tissues of that organ.

Bernard's Measurement of Blood Sugar

It is of interest to know how it was that Bernard failed to detect sugar in blood—for instance, in that of the portal vein—at a time when other workers using similar methods succeeded. One reason is clear. In many of his early experiments he did not test the blood until some time after its removal from the body and so much of the sugar in the blood was lost by glycolysis. In one of his earlier publications he describes a method for the detection of sugar in "old blood." Pavy, who had worked in Bernard's laboratory, suggested that another cause of failure arose from the fact that Bernard did not allow his test mixture (Fehling or Barreswil solution plus protein-free blood filtrate) to stand for a sufficient length of time after boiling. The detection of sugar by the relatively insensitive method of fermentation was in any case not very satisfactory.

In his early experiments, carried out on a dying or dead animal, Bernard must have stimulated the breakdown of glycogen in the liver to a very substantial extent, so that the blood of the hepatic veins contained abnormally large amounts of sugar, amounts which Bernard could easily detect even with his inadequate methods. If, like Pavy, he had carried out his experiments under physiological conditions so that a stimulation of the breakdown of liver glycogen was not induced, it seems extremely probable that he would have been unable to detect sugar in the blood of the hepatic veins at all. For we know to-day that in the post-absorptive state the blood of the hepatic veins contains only about 20 mg. per 100 ml. more sugar than that of the portal vein. Since Bernard was unable, in his early experiments, to detect any sugar at all in the blood of the portal vein, the blood of the hepatic veins would almost certainly, under physiological conditions, have yielded negative results. Pavy was nearer to the truth in his statement that the blood of the portal vein and hepatic veins contained indistinguishable amounts of sugar than was Bernard in his assertion that portal vein blood contained no sugar while the blood of the hepatic veins contained an abundance. The curious paradox therefore arises that if Bernard had been more careful with his experimental conditions he might never have discovered glycogen and the glycogenic function of the liver, while if Pavy had been a little less rigid in his determination to use strictly physiological conditions he might not have been led to an incorrect theory of carbohydrate metabolism, based on very carefully determined experimental results.

Although Bernard's experimental findings were occasionally at fault and at times influenced by preconceptions, such as the importance of nervous control of secretion of sugar by the liver (see Young, 1951), his strength appears to lie in his ability to discard a theory once its experimental basis had been undermined. Even though he was apt not to state frankly that he had been wrong, he nevertheless did change his ideas. This ability Pavy did not share to such a marked degree.

Formation of Liver Glycogen from Carbohydrate

This problem, which Bernard never definitely solved, was examined critically by J. Otto, a pupil of C. Voit. On the basis of experiments by Bernard and others, Otto decided that liver glycogen could not be formed from fat. Therefore, he argued, if the glycogen content of the tissues of a sugar-fed animal rises it follows that the glycogen has been formed either from protein in the body of the animal or from ingested sugar. From the weight of the nitrogen excreted during a given time, the weight of protein carbon metabolized during this period could be calculated. If this weight of carbon from protein was less than the weight of carbon in the glycogen deposited then some part of the glycogen must have been formed from the ingested sugar. The results of experiments on these lines were published in 1891 after the early death of Otto (Otto and Voit, 1891) and demonstrated that liver glycogen could indeed be formed from ingested glucose and fructose and from any di- and poly-saccharide which could be hydrolysed in the alimentary tract to form these sugars. Once the direct conversion of ingested sugar to liver glycogen was admitted, the process of sugar secretion by the liver lost its former significance. The secretion of sugar by the liver could indicate that exogenous sugar, which had been temporarily stored as liver glycogen, was being liberated for use in other parts of the body and not that sugar was being manufactured from other sources.

In the early years of the twentieth century Pflüger denied the validity of all the evidence which had accumulated up to that time that protein could be converted to sugar in the body. He was justified in his criticism that in experiments designed to prove this the preliminary period of fasting had been too short, so that a considerable but unknown amount of glycogen was present in the body when the feeding of protein began. Nevertheless, his continued declaration that any increase in the amount of glycogen in the body which had been found as a result of feeding protein food was within the experimental error of the investigation appears to be too sweeping. Indeed, he was quite frank in his statement that his investigations on carbohydrate metabolism were begun with a prejudice. As Pflüger wrote (1907, p. 377), "My aim has been to find the origin of sugar in sugar itself and not in any other substance."

Pflüger (1905) was apparently impressed by the fact that the ingestion of certain sugars—for example, pentoses—did not result in a rise in the amount of glycogen in the liver. He considered that "as the liver cells are unable to form glycogen from such carbohydrates, it would be an extraordinary thing if they were able to do so from substances whose constitution is quite different from those of sugars," and with this argument supported his view that proteins are not converted to sugar in the body. This reasoning led him to believe that glycogen might be formed from fat, particularly since the formation of carbohydrate from fat had been rigorously proved to take place in the plant world; for instance, in the germinating castor-oil seed. Pflüger had shown experimentally that sugar could be converted to neutral fats in the animal body. Therefore, he argued, the reverse process was most likely to occur—that is, that neutral fats could give rise to sugar and so to glycogen (see Young, 1937).

Most of the evidence put forward by Pflüger to support the belief that fat is converted to sugar in the animal body was not of a positive type. It consisted mainly of arguments designed to demonstrate the improbability of the belief that sugar came from sources other than fat, and in particular that it could not be produced from protein. In his old age, however, Pflüger did become convinced that liver glycogen could arise from protein (Pflüger and Junkersdorf, 1910). The amount of glycogen in the liver and muscles of a fasting dog was reduced to a low level by the administration of phlorrhizin. The feeding of codfish to such a dog led to a substantial increase in the amount of glycogen, both in the liver and in the muscles. Since the codfish contained only a very small amount of glycogen and no appreciable amount of protein-bound carbohydrate, and since under these conditions the feeding of fat did not increase the glycogen content, Pflüger at last accepted the conclusion that liver glycogen could be formed from protein. The acceptance of this conclusion meant that most of Pflüger's work on carbohydrate metabolism had to be reinterpreted. The result of this was the general rejection of Pflüger's belief that sugar could be formed from fat; but because of all the confusion that had been brought about by the promulgation of Pflüger's ideas, Bernard's views about the formation of liver glycogen had been obscured. What is said about them to-day is often coloured by Pflüger's belief that carbohydrate must itself be the main source of liver glycogen.

Glycogen Since the Time of Pflüger

(a) The Cori Cycle

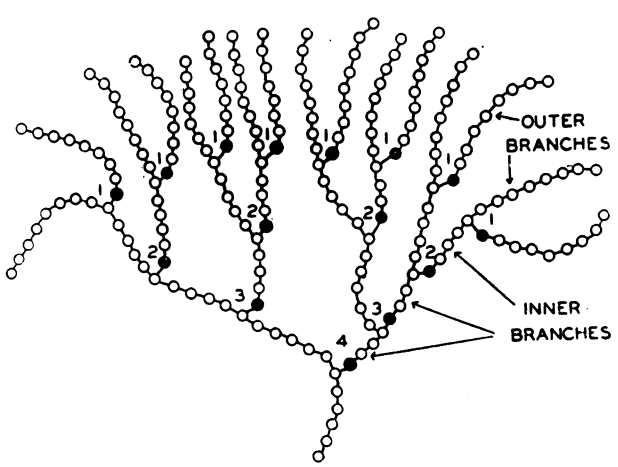
The important researches of Meyerhof and others into the role of glycogen breakdown in muscle and in yeast carried out during the inter-war period cannot be discussed

here. But what came to be known as the "Cori cycle" during the late 1920's is of particular relevance. Carl and Gerty Cori showed that lactic acid liberated by the muscles of a mammal was largely re-converted into glycogen, not in the muscles but in the liver (see Cori, 1931). Thus glucose could pass through a cyclical process in being successively converted from liver glycogen to blood sugar, to muscle glycogen, to lactic acid, and back to liver glycogen. In this process, energy was released in the muscles and absorbed in the liver.

(b) The Chemical Structure of Glycogen

That on complete hydrolysis, glycogen yields glucose alone was established by Claude Bernard. Although this finding has been challenged from time to time, its validity has never been generally doubted. The fact that maltose is formed by the hydrolysis of glycogen under the influence of amylases was demonstrated by Claude Bernard. Maltose possesses a 1 : 4-α-glucosidic linkage (Haworth, 1929), and if one postulates a repetition of such a linkage one can imagine a straight chain of n glucose molecules joined with 1 : 4-α-glucosidic linkages—an amylose structure, as it is called. In such a molecule, if n is large then the empirical formula for glycogen of $(C_6H_{10}O_5)n$, first established by Kekulé in 1858, is applicable. But this applies only if n is more than about 30, since in the formation of glycogen from a straight chain of n molecules of glucose only n-1 molecules of water are abstracted. The formula for glycogen will therefore be $(C_6H_{12}O_6)n-(n\text{-}1)\ H_2O$, which approximates to $(C_6H_{10}O_5)n$ only when n is large. The fact that glycogen is a non-reducing polysaccharide agrees with the view that n is large.

In 1932 Haworth and Percival showed that n was only 12 for the preparations of liver glycogen then examined, this figure being deduced from the amount of 2 : 3 : 4 : 6-tetramethyl glucose liberated when fully methylated glycogen was hydrolysed. This tetramethyl glucose represented the non-reducing end of the chain and therefore provided a measure of chain length. Other workers subsequently assessed n at figures between 12 and 18 for glycogen, and a figure of 14 and 15 may be regarded as representing the average (see Cori and Larner, 1951). The upper limit for glycogen may be taken as 17, a continuous series ranging between $n=12$ and $n=17$ (Cori, 1954). When n is greater than 17 the substance is not glycogen but amylopectin, the branched polysaccharide which constitutes about 80% of most natural starches. Since, for reasons we have already considered, n for the whole molecule is probably larger than this, it was likely that these short chains must be joined by lateral linkages (See Diagram).



Structure of glycogen. The segment of glycogen pictured has a molecular weight of 38,000. The open circles represent the glucose units in α-1 : 4′ linkage, the black ones those in α-1 : 6′ linkage. Reproduced, by permission, from Gerty T. Cori's "Glycogen Structure and Enzyme Deficiencies in Glycogen Storage Disease." (*The Harvey Lectures*, 1952-3, **48**, 146. Academic Press, Inc., New York.)

In their investigations on glycogen, Haworth and Percival found that fully methylated glycogen had a minimum molecular weight in molten camphor corresponding to that of a dodecasaccharide—that is, about 2,400. This indicated that any bonds joining the units of methylated dodecasaccharide were easily broken when the substance was dissolved in molten camphor, and were therefore unlikely to be co-valent bonds. I was unable myself at this time to reproduce this observation of the solubility of methylated glycogen in molten camphor, and Oakley and I (Oakley and Young, 1936) found from measurements of osmotic pressure that the molecular weight of both glycogen and fully methylated glycogen was of the order of 1,000,000.

As the result of their investigations, Haworth and his colleagues put forward a laminated structure for the molecule of glycogen, while, on the basis of physical measurments, Staudinger (1937) suggested a "comb" structure, and Meyer and Bernfeld (1940) a "tree" structure. In the early investigations the nature of the linkages between the chains of 12–17 glucose units was not specified. In 1937 D. J. Bell (1937) isolated dimethyl glucose from the products of hydrolysis of fully methylated muscle glycogen in an amount about twice that of the tetramethyl glucose found. Subsequently, Barker, Hirst, and Young (1941) identified 2:3-dimethyl glucose among the hydrolytic products of fully methylated starch. This suggested the possibility that the inter-chain link was attached to the 6 position of certain glucose residues, and since the link could easily be hydrolysed it was very likely a 1:6 linkage. With the isolation of isomaltose or brachiose (6-[α-D-glucopyranosyl]-D-glucose), which is a disaccharide with an α-1 : 6 linkage between two glucose units, from the products of the acid hydrolysis of glycogen by Wolfrom and O'Neill (1949) and with the isolation of the same disaccharide from the products of enzymic hydrolysis of starch by Montgomery *et al.* (1949), it seemed very probable indeed that the links between the chains would be 1:6 α-glucosidic bonds. Further evidence that this was so came from studies with enzymes, although the existence of other types of linkage has not been completely excluded.

(c) Enzymic Breakdown of Glycogen

In 1936 it was shown (Cori and Cori, 1936; Cori, Colowick, and Cori, 1937) that glucose-1-phosphate was the product of the breakdown of glycogen by an enzyme, present in mammalian muscle and acting in the presence of inorganic phosphate, which they named "phosphorylase." Later, they were able to demonstrate (Cori, Cori, and Schmidt, 1939) that the action of phosphorylase on glucose-1-phosphate *in vitro* could, under suitable conditions, produce a polysaccharide which resembled glycogen in many respects, though it yielded a blue colour with iodine (like starch) and not a brownish-red colour as glycogen does. Independently, in this country, Hanes (1940) showed that potato-phosphorylase would produce a starch-like polysaccharide from glucose-1-phosphate *in vitro*. Much of the subsequent enzyme researches were carried out in parallel on glycogen by Cori and Cori, and on starch by Hirst, Peat, Bourne, and others. I must confine my attention to glycogen.

In 1943 Cori and Cori showed that highly purified crystalline muscle phosphorylase gave, with glucose-1-phosphate *in vitro* under suitable conditions, only a straight chain polysaccharide (amylose) of a greater chain length than that of glycogen. But they also found that muscle extract contained another enzyme, branching enzyme, which could convert the straight-chain polysaccharide into a branched one.

Swanson (1948), in Cori's laboratory, showed that the colour with iodine yielded by these glucose polysaccharides varied with the mean chain length. With 8–12 glucose units in a chain the colour developed with iodine was red. With 30–35 it was blue. Intermediate chain lengths gave intermediate colours. I should like to recall here what Claude Bernard recorded (1877, p. 553): "I have found that if the

muscles of a rabbit are paralysed and thus forced to rest, the glycogen content rises. I have observed in this case that the glycogen gives a blue colour with iodine, just like that with starch." Presumably the outer chains of the molecule grow as the glycogen content of the muscles increases under these conditions.

In 1951 Cori and Larner showed that the complete degradation of glycogen in animal tissues required two enzymes: (*a*) phosphorylase, for the breaking of 1 : 4 linkages by the introduction of phosphoric acid (phosphorolysis) with the production of glucose-1-phosphate; and (*b*) debranching enzyme (or amylo-1:6 glucosidase) for the hydrolysate fission of the 1 : 6 linkages, with the production of free glucose. This enzyme appeared to be specific for α-1:6-glucosidic linkages. The ratio of free glucose to free + phosphorylated glucose formed when both enzymes acted upon glycogen was a measure of the degree of branching. In all instances it was found for glycogen that the outer chains of glucose residues (" the peripheral tier ") was longer than the inner branches (see Cori, 1954). In a typical glycogen with a mean chain length (*n*) of 15, the length of the outer chains is 8 or 9 glucose units, with 3–5 glucose residues in the inner branches—that is, between branching points.

In their experiments Cori and Larner (1951) found that, as might be expected from the action of debranching enzyme, the stimulation of muscle results in the release of small amounts of free glucose, as well as of lactic acid. Thus, Bernard's belief that glycogen in muscle always undergoes a lactic acid fermentation was not quite correct. According to the present formulation of glycogen, about 6% of the glucose residues can yield free glucose, and about 94% lactic acid or other molecules, when glycogen is broken down in muscle.

By applying enzymic methods of molecular analysis to the glycogen in the liver and muscles of patients with glycogen-storage disease, Illingworth and Cori (1952) and Illingworth, Cori, and Cori (1956) have distinguished several types of this disease. In the liver-kidney type, first described by von Gierke, other organs are not affected and the specific lesion appears to be a deficiency of glucose-6-phosphatase in liver and kidney cortex. In generalized glycogenosis, some cases possess glycogen with abnormally short outer chains and a deficiency of debranching enzyme (amylo-1 : 6-glucosidase), and their disease can be attributed to the deficiency of this enzyme; others do not exhibit these abnormalities, and the aetiology of their condition is still obscure.

Cori (1954) has observed that the nutritional state has a marked effect on the degree of branching of the glycogen molecule. Glycogen deposited in the liver when glucose or fructose is fed to fasting rabbits is less branched than the liver glycogen from well-fed rabbits. Glycogen from the livers of embryonic guinea-pigs is more branched than the glycogen obtained from the livers of newly born or adult animals (Cori, 1954). As Cori has pointed out, the length of the outer chains is very important in the characterization of glycogen. It varies more than the length of the inner chains and largely determines the average chain length of the entire molecule.

(d) Enzymic Synthesis of Glycogen

The debranching enzyme (amylo-1 : 6 glucosidase) is apparently purely a hydrolytic enzyme and almost certainly plays no part in the synthesis of glycogen from glucose-1-phosphate. The presence of a branching enzyme in muscle was revealed by the researches of Cori and Cori (1943), as has already been pointed out. Larner (1953), in Cori's laboratory, has observed that when this enzyme acts upon glycogen containing radioactive labelled glucose in its outer chains 1 : 6 linkages are formed between radioactive glucose units previously linked by 1 : 4 bonds. This enzyme has been described as amylo-(1:4→1:6) transglucosidase. The enzyme from the liver needs an outer chain length of more than 6 glucose units before it will induce branching. The reverse process—that is, the formation of 1 : 4 linkages at the expense of 1 : 6 linkages—has not been demonstrated.

(e) Formation of Glycogen in vivo

The enzymes that together can catalyse the conversion of glucose to glycogen are, in accordance with the studies we have considered above and other relevant investigations, hexokinase (glucose to glucose-6-phosphate), phosphoglucomutase (glucose-6-phosphate to glucose-1-phosphate), phosphorylase (glucose-1-phosphate to amylose), and branching enzyme (amylo-(1 : 4→1 : 6)-transglucosidase) (amylose to glycogen). Although these enzymes exist in liver and muscle tissues and can together catalyse the transformation of glucose to glycogen, it is not certain that the pathway thus made possible—glucose → glucose-6-phosphate → glucose-1-phosphate → glycogen—is the main one followed in intact tissue. Beloff-Chain *et al.* (1955) believe that oligosaccharides may be involved in the formation of glycogen from glucose in diaphragm muscle.

It is true that in liver tissue the increase in phosphorylase activity associated with the action of glucagon or adrenaline is always associated with a greater breakdown of glycogen and not with increased formation of this substance. But, of course, the conditions prevailing in the cell when phosphorylase activity rises under the influence of adrenaline and glucagon may be specifically appropriate for the lytic action of phosphorylase, as opposed to the reverse effect. Be that as it may, the direct conversion of glucose to glycogen in the liver, about which, as we have seen, Claude Bernard had some doubts, has been clearly demonstrated within recent years with the aid of isotopes. Using deuterium as a labelling isotope, Stetten and Boxer (1944) found that dietary glucose can indeed be converted directly to liver glycogen, although their studies showed that only a small proportion was so directly transformed, the major part of the liver glycogen being formed from smaller molecules, perhaps lactate in part, when glucose was fed to a rat.

According to Stetten and Boxer only about 3% of the dietary glucose is converted to glycogen, about 30% being transformed to fat. The rest is presumably oxidized through other routes. De Duve and his colleagues (Berthet *et al.*, 1956), using radiocarbon-labelled glucose, have recently also demonstrated the direct conversion of glucose to liver glycogen, as well as to muscle glycogen, this process being stimulated by insulin.

Stetten and Stetten (1954, 1955) have found that when glucose labelled with radiocarbon is given to normal rats, and the structure of the liver and carcass glycogens analysed by the enzymic method, the administered glucose is found first to enter the peripheral chains of glycogen and then move down the molecule. The process of the inward movement of the radioactive glucose in the molecule of glycogen is more rapid with glycogen in the liver than with muscle glycogen. Subsequent studies (Stetten, Katzen, and Stetten, 1956) have shown that the radioactive glucose preferentially enters the glycogen molecules of smaller size than average in the liver, and of larger size than average in the muscles. These observations, and the earlier ones of Cori and Cori, emphasize the metabolic inhomogeneity of the glycogen molecule.

During the past ten years the use of isotopes has confirmed that carbon atoms from certain amino-acids, contained in protein, can be transferred to liver glycogen. Although it has also been demonstrated in a similar manner that carbon can be transferred from fatty acids to liver glycogen, it is still doubtful whether a net synthesis of glycogen can occur at the expense of the breakdown of fatty acids.

Conclusion

During its first century of recognition glycogen has passed from a starch-like material of unknown constitution, the immediate precursor of the sugar secreted by the liver, to a substance whose molecule can be enzymically dissected, much as Claude Bernard dissected an animal. Already this power to effect such molecular analysis of glycogen by means of enzymes has differen-

tiated glycogen-storage disease into a number of different types, and has revealed that the peripheral part of the glycogen molecule can undergo metabolic change independently of the central part. What light these new observations will throw on medicine will become clear early in the second century of glycogen. I believe that we can confidently assume that Claude Bernard would have given his interested approval to the many investigations into the structure and biosynthesis of glycogen which have taken place during the last twenty-five years of the first century of this substance.

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Courses for the training of matrons and assistant matrons for old people's homes are organized regularly by the National Old People's Welfare Council (in association with the National Council of Social Service). Each course lasts fourteen weeks. The next course will begin on September 9 ; there will be another early next spring. Applicants, who should be women of over 30 years of age, need have no special qualifications, although a practical knowledge of nursing and housekeeping is an advantage. Intending students may apply through their local education authorities for grants towards their training from the King George VI Social Service Scheme (Old People). Further details may be obtained from the secretary, National Old People's Welfare Council, 26, Bedford Square, London, W.C.1. There are over 1,400 homes for old people, provided either by voluntary organizations or by statutory authorities, and competent staff are constantly required.

TREATMENT OF ULCERATIVE COLITIS WITH LOCAL HYDROCORTISONE HEMISUCCINATE SODIUM

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A previous article (Truelove, 1956) dealt with the treatment of mild and moderate cases of ulcerative colitis by means of a rectal drip of hydrocortisone. The result of 21 treatments was complete symptomatic relief within a few days in 14 instances, and it was concluded that this method was a useful addition to therapy. However, although clinical remission was accompanied by much-improved sigmoidoscopic appearances, it was found that the histological appearances of biopsy specimens of the colonic mucosa did not in general show a corresponding improvement.

Various possibilities which might have accounted for this discrepancy were considered. One was that the actual preparation of hydrocortisone used was not entirely harmless to the inflamed mucosa. Hydrocortisone in the form of its free alcohol is only slightly soluble in water, and it was therefore supplied by the manufacturers in solution in 50% ethyl alcohol. This concentrated solution was diluted 10 times in normal saline for actual instillation into the rectum, but nevertheless it seemed possible that even a 5% solution of ethyl alcohol might damage, or at least prevent from healing, a mucosa which was already diseased. This possible source of failure to induce mucosal healing can be eliminated if one uses a form of hydrocortisone which is freely soluble in water. Such a substance—hydrocortisone hemisuccinate sodium—has recently been synthesized, and Glaxo Laboratories kindly made supplies of it available for clinical trial.

Hydrocortisone Hemisuccinate Sodium

This is a white crystalline powder highly soluble in water, with which it forms a clear, colourless solution. There are as yet only scanty references in the literature to its use in man. The first account appears to be that of Orr *et al.* (1955), who used it intramuscularly to treat conditions such as disseminated lupus erythematosus, acute bronchial asthma, and the crisis of Addison's disease, and for replacement therapy after bilateral adrenalectomy ; the results were comparable to those obtained by corresponding doses of hydrocortisone. The same authors also studied the effect of injecting it intravenously into a human being without adrenals and found evidence of an exponential decay curve characterized by a half-life of four to five hours, which agrees well with data obtained for hydrocortisone itself by Hellman *et al.* (1954), making use of a preparation labelled with radioactive carbon. These and other facts suggest that the two compounds are metabolized by the liver and excreted by the kidney in a closely similar fashion. Orr *et al.* suggested that the hemisuccinate would be valuable in the treatment of severe emergencies in which a " shock-like " state occurs, by virtue of its