Chapter 2

The Discovery of Sugar Nucleotides

LUIS F. LELOIR and ALEJANDRO C. PALADINI*

*Instituto de Investigaciones Bioquímicas "Fundación Campomar" y Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, and *Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires (Argentina)*

The discovery of sugar nucleotides

Leloir: Partial accounts of various aspects of the research of our group in Buenos Aires have appeared in different articles [1–3]. For Dr. Semenza's collection I thought it might be a good idea to check my statements so as to correct the failures of my memory. For this purpose I asked Dr. Alejandro Paladini, who worked in our group from 1947 to 1951, to give his account at the same time as mine. Dr. Paladini joined the Foundation with a Fellowship. We had to select between several candidates, but there were two who were rather outstanding. One was Dr. Paladini who had some experience and was known to be a promising young man, the other candidate was a girl of great beauty. It was a rather painful decision but I think I made the correct one:

In 1947 the Fundación Campomar was still in its infancy – a rich textile manufacturer, Jaime Campomar, had decided to create a research laboratory and he entrusted the organization and the selection of personnel to me. The first investigator to join the Foundation was Ranwel Caputto who had recently returned from the Biochemical Laboratory of Cambridge, the director of which was Sir Frederick Gowland Hopkins, one of the founders of British biochemistry. A few years before I had also received my initial biochemical training in that same laboratory. Caputto had worked with a well known biochemist, Malcolm Dixon and had succeeded in crystallizing a glyceraldehyde dehydrogenase which turned out to be the same as glyceraldehyde phosphate dehydrogenase. At that time it was quite a feat to crystallize an enzyme. After Caputto several

Plate 2. The entrance hall of the laboratory of the Instituto de Investigaciones Bioquímicas "Fundación Campomar" during the years 1947 to 1957. Left to right: R. Caputto, A. Paladini, C. Cardini, R. Trucco and L. Leloir.

other investigators joined the Foundation: Dr. Raul E. Trucco, a microbiologist, Carlos E. Cardini, a biochemist and Naum Mittelman, a biophysical chemist. For political reasons prevailing at that time in Argentina, many of the members of the University had resigned and we were among them. We had a small private laboratory located in an old house (Julián Alvarez 1719, Buenos Aires) which only covered about 100 square meters. The equipment was poor but the place had the advantage that it was very quiet. We did not have any teaching duties nor meetings, so that we could think all day long about research problems. In later years I missed that quiet atmosphere very much.

Paladini: I would like to describe my first impressions when I joined the group. It was quite striking to me, used to the frigid atmosphere of official institutes, to become a member of a joyful lot of people where the head of the laboratory made witty, but friendly remarks all the time, and had the answer "I don't know..." to many daily questions. The equipment of the lab looked luxurious to me but even by the standards of, say, 30 years ago, it was very modest. The most sophisticated piece was a Beckman DU spectrophotometer, run by dry cells which were very difficult to get. We also had a Klett-Summerson photocolormeter, a microscope and a home-made Warburg respirometer. No refrigerated centrifuge was available for a long time and of course all the initial basic discoveries on sugar nucleotides were made without the help of radioactive materials.

1948-1949 Glucose-diphosphate

Leloir: Our first project was to continue the studies on cell-free acid oxidation which I had initiated with Dr. Juan M. Muñoz when we worked in the Faculty of Medicine in Buenos Aires University. Our studies had been carried out on liver homogenates and now the idea was to study the process in bacteria. I do not remember why, but the project did not progress and we abandoned it. Looking back now it seems that it was a rather good project.

Dr. Caputto had done his thesis work on the mammary gland phosphatase and told us that he had done some preliminary experiments according to which it seemed that gland homogenates would produce lactose from glycogen. We tried to repeat those experiments with the crude methods of that time, one of which consisted in preparing the osazones of the sugars and looking at the crystals under the microscope. What was

obtained from glycogen was probably a mixture of glucose, maltose and maltotriose which are the products formed by the action of α -amylase.

In those days it was known from the work of the Coris that glycogen could be formed from glucose-1-phosphate with the enzyme phosphorylase and it had been shown also by Hassid, Doudoroff and coworkers that sucrose could be formed from glucose-1-phosphate and fructose with an enzyme of bacterial origin (from *Pseudomonas*). Consequently the idea that lactose originated from glucose-1-phosphate and galactose was floating in the air. With the idea of looking for a lactose phosphorylase we started studies with a lactose-utilizing yeast (*Saccharomyces fragilis*) which we grew on whey in large milk cans. The search for an enzyme that would reversibly yield glucose-1-phosphate and galactose from lactose resulted only in the finding of a lactase [4]. This led us to the study of galactose utilization. Evidence for the presence of an enzyme catalyzing galactose phosphorylation was obtained and the name galactose kinase was proposed. The reaction product was found to be galactose-1-phosphate [5]. This ester had previously been isolated from the liver of galactose-fed rabbits [6]. The next obvious step was to find out the fate of galactose-1-phosphate. It was then that we stepped into a fertile field of study which compensated our previous series of failures.

Incubation of galactose-1-phosphate with our enzyme preparation led to the formation of glucose-6-phosphate. This reaction could be easily measured by making use of the fact that the latter but not the former reduces alkaline copper solutions. Besides using galactose-1-phosphate as substrate we sometimes used glucose-1-phosphate, both of which were obtained by chemical synthesis. When the enzyme preparations appeared inactive we often tried reactivation with heated yeast extracts which we called by the German expression Kochsaft. We often obtained activations with the Kochsaft but the results were very confusing because we used galactose-1-phosphate in some experiments and glucose-1-phosphate in others. However, after many discussions and apparently contradictory experiments we realized that we were dealing with two thermostable factors, one for each of the following reactions:

At that time the enzyme corresponding to the second step, phosphoglucosyltransferase was well known. It had been discovered by Cori et al. [7, 8] and obtained in crystalline state by Najjar [9]. However, no cofactor for

phosphoglucumutase was known to be required. By measuring the activation of yeast phosphoglucumutase we could estimate fairly well the concentration of our thermostable cofactor. The substance was purified by a procedure which consisted mainly in the precipitation with metals. At this point we sent a preliminary letter to *Archives of Biochemistry* [10], titled: *A coenzyme for phosphoglucumutase*. At the end of the report we had written

"Kendall and Stickland [11] obtained an activation of phosphoglucumutase by adding hexose diphosphate but Cori et al. [8] were unable to obtain any effect. The hexose diphosphate preparation of Kendall and Stickland may have been contaminated with the new co-enzyme reported in this paper."

After sending the letter we happened to test fructose-1,6-diphosphate again although it had already given negative results in previous experiments. This time, however, it proved to be active and still worse, it was found that the purified preparations of our cofactor contained as much fructose diphosphate as commercial preparations of the latter! We decided immediately to ask the editors to send our letter back or throw it in the waste-paper basket, but in the meantime we were thinking desperately. Our desperate thinking generated the idea that our cofactor might not be fructose-1,6-diphosphate but glucose-1,6-diphosphate, acting as shown in Fig. 1. The idea was that a transfer of phosphate occurred so that the diphosphate became regenerated at each step.

It was also reasoned that the two di-esters could be distinguished by treatment with hot alkali which would destroy the fructose ester because of the presence of a free-reducing group while the non-reducing glucose ester would remain intact. Most of the time one's hypothesis proves to be false but this time it was correct and thus glucose-1,6-diphosphate was firmly established as a new cofactor [12, 13].

Fig. 1. The mechanism of action of glucose-1,6-diphosphate as cofactor of phosphoglucumutase.

The scheme was confirmed by experiments of Sutherland et al. [14] who studied the reaction with ^{32}P -labeled compounds. The structure of glucose-1,6-diphosphate was confirmed by chemical synthesis [15] and the biosynthesis from glucose-1-phosphate and ATP [16] was described as well as by a transphosphorylation between two molecules of glucose-1-phosphate catalyzed by a bacterial enzyme [17].

1950 UDPG

Once we knew the structure and function of one of our cofactors we could channel our efforts to the study of the other one. It could be estimated by using galactose-1-phosphate as substrate, excess phosphoglucomutase and determining the glucose-6-phosphate formed [18]. After a few purification steps such as precipitation of the mercury salt, adsorption on charcoal etc. it seemed that cofactor activity was related to a substance absorbing at 260 nm. The substance was at first thought to be an adenosine derivative but later was found to contain uridine.

Paladini: Although the spectrum of the cofactor was similar to that of adenosine the changes observed at different pH values could not be accounted for on that basis. The only paper available, at that time, reporting the spectra of purine and some pyrimidine bases and nucleosides was that of Hotchkiss [19] but it did not include uridine. Fortunately, Dr. Caputto while idly looking through a newly arrived volume of the *Journal of Biological Chemistry* found a spectrum identical to that of our compound. It was that of uridine [20]. There was little doubt, thereafter, that the cofactor was a derivative of uracil. This was confirmed in several ways but one of them was accidental: the paper chromatograms in which we attempted to identify uridine in the hydrolysates of the cofactor were dried in a corner of the laboratory, very close to a fluorescent tube. The spectra obtained with the material eluted from the papers gave clear indications of a photochemical modification which, nevertheless, was identical to that obtained with authentic samples of uridine.

Leloir: This was rather exciting, because at that time uridine was known only as a nucleic acid constituent. Uridine-3'-phosphate had been described but uridine-5'-phosphate, UTP and UDP were only detected years later. Besides uridine, our cofactor was found to contain glucose and two phosphates.

In February 1950 we published a note in *Nature* [21] in which the correct structure was proposed but the position at which the phosphate was linked to the uridine was left open. The glucose was erroneously shown as the β anomer. Soon afterwards we published a full paper on the isolation and structure of UDPG. It was authored by Caputto et al. [22]. At that time we used to rotate the names in each paper so that the last one would become the first in the next publication.

A titration curve of the substance showed only two primary phosphoric acid groups per uridine. Other properties of the substance were as follows: (a) mild acid hydrolysis (15 min at pH 2 at 100°C) liberated a sugar which was identified as glucose by paper chromatography in several solvents, by color reactions and by its fermentability by yeast. Splitting off the glucose unmasked its reducing power and liberated a secondary acid group of phosphoric acid; (b) further acid hydrolysis (15 min in 1 N acid at 100°C) liberated 1 mol of inorganic phosphate and another secondary acid group of phosphoric acid appeared; (c) the substance remaining after splitting off the glucose and one phosphate was identified as uridine-5'-phosphate by comparison with a synthetic specimen kindly supplied by Professor Todd.

Paladini: The first determination of the molecular weight of the cofactor was laboriously made using an excellent Kuhlman microbalance which Leloir had bought many years before the Institute was born. To attain an accuracy of about 0.01 mg I had to apply a very time-consuming estimation of the amplitude of the oscillations. Finally, the acceptable value 630 was obtained instead of the theoretical 566 by measuring the dry weight of a volume of a solution of known absorbance.

When UDPG was run on paper with an alkaline solvent (ethanol—ammonia) it was found to decompose into uridine-5'-phosphate and an unknown substance that had a higher mobility than hexose phosphates. This substance was not easy to identify but after quite a lot of work and thinking we were able to present evidence showing that the decomposition product was the 1,2 cyclic monophosphate of glucose. On acid treatment the cyclic ester gave glucose-2-phosphate and the 1-phosphate which the acid broke down to free glucose [23].

The formation of the cyclic phosphate indicated that the glucose in UDPG is the α anomer because the β has the first two hydroxyl groups in *trans* position thus making difficult the formation of a five-membered ring.

Leloir: Around 1950 research was not as competitive as it is nowadays but

nevertheless one study related to ours appeared. Park and Johnson [24] who were investigating the action of penicillin in *Staphylococcus aureus* observed that this substance produced an increase in labile phosphate which turned out to belong to a uridine containing compound.

The state of the field in 1951 was reported in the Symposium on Phosphorus Metabolism organized by W.D. McElroy which took place in Baltimore [25]. Park reported the isolation from penicillin-treated *Staphylococcus* of three uridine containing compounds. The simplest of them had the structure of UDPG but with a sugar which was unknown at that time and that we now know as acetylmuramic acid. Another compound had one alanine residue in addition and the third compound contained one lysine, one glutamic acid and three DL-alanine residues joined to the nucleotide sugar. When my turn came to report our findings I mentioned the discovery of guanosine diphosphate mannose and the alkaline decomposition of UDPG.

1951 UDP-galactose

Another thing I reported at the Phosphorus Symposium was the reversible formation of UDP-galactose from UDP-glucose on incubation of the latter with our yeast extracts. This reaction could be studied thanks to a procedure for the separation of glucose from galactose by paper chromatography developed by Jermyn and Isherwood [26]. The reaction reached an equilibrium when the ratio UDP-glucose/UDP-galactose was about 3. These results were published in a five page paper [27]. It was then assumed that the transformation of galactose-1-phosphate catalyzed by what we used to call galacto-waldenase occurred as follows:

The first reaction: a transfer of uridylic acid, was hypothetical but the formulation was later proved to be correct by Kalckar and coworkers. The equilibrium of the sum of the reactions is reached when the ratio glucose-P to galactose 1-P is also 3 [28].

The difference between galactose and glucose consists only in the stereochemical position of the hydroxyl at position four. The mechanism by which this change occurs was quite mysterious and there were several unproved hypotheses. The mystery was clarified when Kalckar and coworkers discovered that DPN(NAD) was required for the transformation. This was a rather exciting finding. I remember very well that one morning I received a telegram from Denmark. It read: "UDP-galactose epimerase requires DPN" – signed Herman. This finding was a strong indication that the process occurs by an oxidoreduction reaction and further work is consistent with the idea that hydrogen is removed from the carbon at position 4 and a keto intermediate is formed.

Several important contributions from Kalckar and coworkers [29] to the field have been mentioned. When he was asked in what he was working he sometimes answered: I am interested in Place Pigalle and operate along la rue du Dr. Leloir. Not everybody understood the joke right away. When he said Pigalle he meant P-gal (phosphogalactose) and not the well known district of Paris which houses painters' ateliers, writers' cafes, night clubs and other attractions. As to the rue du Dr. Leloir he meant some of the pathways of galactose metabolism and sugar nucleotides which we also studied.

Although our laboratories worked on similar lines there never was any rivalry or resentment among us as often happens. We exchanged information quite freely.

1952 UDP-acetylglucosamine

Paladini: Another member of the family of sugar nucleotides was discovered in our laboratory by sheer serendipity. I was very much involved in finding good solvents for paper chromatography of UDPG and related compounds. It should be remembered, by the way, that the use of ion exchange columns was, at that time, still in its infancy in the world. I assayed many different solvents but obtained the best results with various combinations containing ethanol. As mentioned before ethanol-ammonia decomposed UDPG but we could detect another substance, which was not affected by the alkaline reaction and which was provisionally referred to as UDPX and later identified as UDP-acetylglucosamine [30].

Leloir: The main difficulty in our study of UDPX was that at the time we did not have a sample of acetylglucosamine to compare with our unknown

sugar. Knowing how simple it is to *N*-acetylate aminosugars this seems incredible. The procedure consists simply in adding acetic anhydride to the aminosugar at neutral reaction. Once we had identified the sugar moiety it was easy to establish the structure because everything was the same as with UDPG.

This substance was later isolated in larger amounts by ion exchange column chromatography and studied more carefully [30, 31].

Paladini: The use of ion exchange resins to isolate UDPG and other nucleotides was a great advance but to obtain sizeable amounts of these it was necessary to handle big columns and hundreds of liters of eluents. For the collection of the samples an unusual fraction collector was devised. The flasks were flat bottles originally used to store water in refrigerators. Many dozens of them were placed side by side on a shelf two meters high from the floor. Along the shelf, in front of the bottles, ran a sort of toy railroad and the wagon carried the effluent tube from the column. It was placed so as to discharge in the flasks through a basculating siphon. The wagon was pulled by a string attached to a heavy counterpoise and the change of flask was ingeniously achieved by a sort of ratchet mechanism activated by the weight of the siphon operating on nails placed all along the edge of the shelf. The column was suspended high in the air and the eluting solvent in a 50-liter bottle, was handled by rope and pulley, to attain its proper position near the ceiling of the room. Unbelievably, this contraption worked perfectly well for several years.

Charcoal absorption proved to be a good step in the purification of sugar nucleotides. The difficult step was the elution. In connection with this I can lighten this recollection by mentioning that we used to have around the lab all kinds of fancy bottles, that were originally containers of perfumes, shampoos or prescriptions. Many came from Dr. Leloir's home and he insisted in storing reagents in them with the idea that the non-uniformity of the shapes and colors helped to avoid mistakes.

The best eluent from charcoal for the sugar nucleotides landed in a bottle of perfume named Lotus Flower. For years we never used any other name for it. This gave rise to many amusing incidents when explaining our research to some visitor and we inadvertently described the experiment made with the Lotus Flower solution.

GDP-mannose

Leloir: Another compound which like UDP-acetylglucosamine was first

detected by paper chromatography was studied with E. Cabib [32]. It was prepared from a yeast extract by precipitation of the charcoal and electrophoresis on starch. The guanosine moiety was easily recognizable because it gives a very typical spectrum with a peak at 260 μm and a shoulder at about 280 μm in neutral or acid solution.

The biosynthesis of UDPG. Experiments had been carried out by Dr. Trucco in 1951 [33] in which he incubated glucose-1-phosphate, UDP and ATP with yeast enzymes. He found that addition of the three substances was required for maximal UDPG formation. The latter was measured as activator of the galactose-1-P $\rightarrow$ glucose-1-P transformation. Trucco believed that UDPG was formed as follows:

Clearer results were later obtained by Munch-Petersen et al. [34] by measuring the reaction from right to left, that is starting from UDPG and inorganic pyrophosphate.

1953-55 UDP-glucuronic acid

Dutton and Storey [35] were studying the reaction by which glucuronosyl residues become attached to phenols and various other compounds in liver, a process often called detoxication [36].

Liver enzymes were found to add glucuronosyl residues to *O*-aminophenol or menthol, faster when a heated extract of liver was added. The active substance was purified and it turned out to be identical to UDPG except that the $-\text{CH}_2\text{OH}$ of position 6 of the glucose was replaced by COOH (carboxyl). This was the first case in which the donor function of a sugar nucleotide was obtained experimentally and was therefore a very important finding.

The mechanism of formation of UDP-glucuronic acid was elucidated by Strominger et al. [37]. They used an enzyme from liver and DPN (NAD) as oxidant.

1953 Biosynthesis of disaccharides

A donor role of UDPG was suggested by Buchanan et al. [38] and Buchanan [39] to explain some results on the formation of sucrose. The experiments consisted in identifying the products formed by photosynthesizing cells from labeled inorganic phosphate and CO_2 . Two-dimensional paper chromatography showed that the UDPG spot decreased with time while sucrose increased. Buchanan [39] suggested that glucose was transferred to fructose phosphate to give sucrose phosphate.

A few years later we were able to obtain an enzyme that catalyzed this reaction. However, we first detected another enzyme which leads to the biosynthesis of trehalose (a disaccharide consisting of two glucoses joined at position 1).

Early in our studies on UDP-glucose we began to suspect that it might have some other function besides its role in the galactose-1-phosphate $\rightleftharpoons$ glucose-1-phosphate transformation. In the paper where we reported the isolation of UDP-glucose [22] we commented that

"The yeast used (for the isolation) was not adapted to galactose and hardly fermented this sugar. This raises the question as to whether UDPG may not have some other function besides accelerating the galactose-glucose transformation."

We often asked one another the question "What is the use of UDPG?" and I think we even thought of instituting a prize for the person who would solve the riddle.

Since we had a method for estimating UDPG we tried measuring its disappearance under different conditions and adding various substances. We found that the addition of glucose-6-phosphate to yeast enzymes increased the disappearance. The change was traced to the formation of trehalose phosphate as follows:

The results were reported in a preliminary note in the *Journal of the American Chemical Society* [40] and later in a full paper in the *Journal of Biological Chemistry* [41]. Trehalose phosphate may be acted upon by a specific phosphatase to yield trehalose which is a reserve carbohydrate found in yeasts and insects. A similar procedure was then applied to a wheat-germ extract and it was found that UDPG acted as glucose donor to fructose [42] to yield sucrose. Only free sucrose was detected as the

reaction product so that we thought that there might be a mistake in the suggestion of Buchanan et al. [38] that sucrose phosphate was the precursor of the free sucrose. However further research [43, 44] proved that the suggestion was probably right. After some steps of purification it became evident that we were dealing with not one but with two enzymes one which yielded free sucrose and another sucrose-phosphate.

These enzymes were then found in other plant tissues and are now believed to be responsible for sucrose formation in the plant kingdom.

Hexosamine metabolism. For some time we wondered which was the role of UDP-acetylglucosamine in tissues and this led us to study various processes in which hexosamines are involved.

An enzyme was detected in extracts of *Neurospora crassa* [45], which catalyzed the reaction.

The paper also mentioned some other reactions such as the acetylation of glucosamine-6-phosphate and a reversible transfer of phosphate from position 1 to 6, a reaction similar to that catalyzed by phosphoglucomutase. The mutase was studied in detail by Reissig [46] who also collaborated with Strominger and myself [47] in improving the method of estimation of acetylhexosamines.

An enzyme present in liver which catalyzes the phosphorylation of galactosamine by ATP was also described [48]. This enzyme which was also found in brain and in *Saccharomyces fragilis* was believed to be the same as galactokinase. Some time afterwards enzymes catalyzing the phosphorylation of acetylglucosamine and acetylgalactosamine phosphate were detected [49].

In 1955 Pontis ran an extract of bovine liver through an anion exchange column and separated the UDP-acetylglucosamine fraction. The sugar moiety was found to be a mixture of acetylglucosamine and acetylgalactosamine [50]. With Cardini [51] I tried to detect an enzyme that would interconvert the above mentioned sugar nucleotides. This led us to make our greatest published mistake. What we did was to incubate UDP-

acetylglucosamine with a liver extract and then separated the free acetylhexosamines by chromatography on borate-treated paper. We could detect the formation of a substance which had the same mobility on paper as acetylgalactosamine and gave the same color reaction. The results were published in a paper entitled *Enzymic formation of acetylgalactosamine*. After some time Comb and Roseman [52] discovered that what was formed was not acetylgalactosamine but acetylmannosamine. The two hexosamines have the same mobility when chromatographed on borate-treated paper but could be distinguished easily after deacetylation by treatment with ninhydrin followed by paper chromatography. We had carried out this test but either we did not do it correctly or we were blinded by the conviction that the product was acetylgalactosamine.

Another enzyme of hexosamine metabolism which we found with Cardini [53] was one present in kidney and liver which catalyzes the reaction.

The curious property of this enzyme is that it is activated by acetylglucosamine-6-phosphate.

Biosynthesis of polysaccharides

Leloir: Studies on polysaccharide biosynthesis from sugar nucleotides were initiated by Glaser and Brown [54] who obtained evidence that UDP-acetylglucosamine could act as donor in the formation of hyaluronic acid (a polymer of acetylglucosamine and glucuronic acid) by Rous chicken sarcoma homogenates. Glaser and Brown [55] also obtained incorporation of radioactivity from UDP-acetylglucosamine in chitin (a polymer of acetylglucosamine) with an enzyme preparation from *Neurospora crassa*. Soon afterwards studies on the biosynthesis of various polysaccharides were published by various workers.

An important development was the finding of an enzyme which catalyzes transfer of glucose from UDPG to glycogen. This enzyme is now colloquially known as glycogen synthetase. Until then it was firmly believed that glycogen was formed from glucose-1-phosphate by the action of enzyme phosphorylase. The reaction:

is reversible so that synthesis can be achieved at low phosphate concentration. One of the important advances in biochemistry was the "in vitro" synthesis of glycogen by Cori and Cori in 1936. Since then the textbooks made phosphorylase responsible for the synthesis of glycogen. Some inconsistencies in the theory were pointed out by Sutherland [56]. According to him it was difficult to understand why the activation of phosphorylase, for instance with epinephrine, always produced glycogen degradation. The reaction shown in equation 10 will go to the right when the inorganic phosphate concentration is high and to the left when phosphate is low and glucose-1-phosphate high. In tissues the relation of inorganic phosphate/glucose-1-phosphate was always such that glycogen was broken down. This difficulty had been dismissed before by saying that cells have compartments and that at the site of phosphorylase action the concentrations might be such as to favor glycogen synthesis.

After examining the available facts Niemeyer [57] suggested that UDPG might be the donor for glycogen synthesis. Finally the point was settled by the detection of the corresponding enzyme in liver. The reaction was measured by estimating the UDP formed. No labeled compounds were used in the initial experiments [58] but further work with ^{14}C -labeled UDPG confirmed the finding [59].

The activity of the enzyme was rather low so here again we tried the addition of "Kochsaft" and found that it produced an activation. The identity of the active substance was easily established and it turned out to be glucose-6-phosphate and it was found to be very specific as an activator.

Since most of the glycogen synthetase activity was recovered in the high-molecular-weight glycogen after subcellular fractionation [60] it was reasoned that something similar might occur with starch-synthesizing enzymes. It was found that starch granules catalyze transfer of glucose from UDP-glucose to starch [61, 62]. Later Dr. Eduardo Recondo prepared ADP-glucose by chemical synthesis and it proved to act as a much more efficient donor than UDP-glucose [63].

It is now accepted that ADP-glucose is the natural donor for the synthesis of starch in plants and of glycogen in bacteria. The enzyme which leads to its synthesis was first detected in potatoes by Espada [64]. The reaction is as follows:

But these findings approach modern times and are not history any more.

Final remarks

After the first members of the sugar nucleotide family were discovered, studies were continued in various parts of the world and many new compounds were described. In the 1963 census [65] more than 40 sugar nucleotides were reported. Furthermore, many enzymatic reactions involving these substances were discovered. Transfer of sugars to many oligo and polysaccharides was detected. It seemed that direct transfer of monosaccharides would explain the biosynthesis of the multitude of polysaccharides that are found in Nature. However, in 1967 another mechanism was uncovered. In studies on the biosynthesis of *Salmonella* lipopolysaccharide by P. Robbins and coworkers [66] and of bacterial peptidoglycan by Strominger and others [67] it was found that lipid intermediates were involved. These intermediates turned out to be derivatives of a polyprenol, undecaprenol, a compound containing 55 carbon atoms and eleven double bonds. Similar studies with animal tissues showed that a related intermediate, dolichol, containing about 100 carbon atoms and 19 double bonds is involved in the glycosylation of some proteins.

The style of research has changed considerably in the years that followed World War II. At first research was carried out by a few highly motivated individuals with rather scant means. Later the research workers became more professional and had the advantage of using more sophisticated equipment and receiving generous government grants. Looking back at the years 1947–1960 they seem to have been very fertile. Those were pleasant years in which we often had the joy of doing a successful experiment and were surrounded by a very stimulating atmosphere.

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