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FAR AWAY AND LONG AGO

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Biochemistry and I were born and grew at about the same time. Before the turn of the century some organic chemists and physiologists had lain the bases of Biochemistry. In 1906 two journals dealing with it appeared, the *Biochemische Zeitschrift* and the *Biochemical Journal.* The *Journal of Biological Chemistry* had started publication only one year before. In 1906 Arthur Harden and W. J. Young were able to separate "yeast juice into a residue and filtrate, each of which was itself incapable of setting up the alcoholic fermentation of glucose, whereas, when they were reunited the mixture produced almost as active fermentation as the original juice." This finding occurred only nine years after Edward Buchner had prepared a cell free yeast juice capable of fermentation. This line of work led eventually to the discovery of the multitude of enzymes, coenzymes, and intermediates of cell metabolism. In 1906 Tswett published the first description of chromatography.

Another important event (from my point of view) occurred in 1906. This was my birth in Paris, 81 Avenue Victor Hugo, just a few blocks away from the Arc de Triomphe.

The growth of Biochemistry was rapid; in a few decades most of the vitamins, hormones, enzymes, and coenzymes were discovered but at the

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time of writing this essay it is showing signs of dismemberment. Molecular Biology, Cell Biology, Chemical Genetics, etc have risen from it and surely there will be others. I reached the age of 76 thanks to some clever arterial repair work carried out by Michael Debakey in Houston.

I have borrowed the title of this essay from a delightful book by W. H. Hudson (1) that describes the wild life in the country near Buenos Aires. Hudson describes the same scenery and the same animals, flamingoes, armadillos, caranchos, vizcachas, etc that I saw in my infancy. It seems that both of us were interested in animal life and understanding nature, but while I became convinced that scientific knowledge and technology would be good for mankind, Hudson (2) had some doubts and he expressed them as follows: "Ah yes, we are all seeking after happiness in the wrong way. It was with us once, and ours, but we despised it, for it was only the old common happiness which Nature gives to all her children, and we went away from it in search of another grander kind of happiness, which some dreamer—Bacon or another—assured us we would find. We had only to conquer Nature, but how weary and sad we are getting!! The old joy in life and gaiety of heart have vanished."

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B. A. HOUSSAY'S INSTITUTE OF PHYSIOLOGY

When I was two years old my Argentine parents brought me to Buenos Aires, where, after going through the studies and examinations necessary for graduating as an M.D. at the University of Buenos Aires in 1932, I worked at the Hospital of the University (Hospital de Clinicas) for about two years. I was never satisfied with what we did for the patients. Looking back on those times, I realize how profoundly medicine has changed since then. Medical treatment was in those days only slightly better than that exemplified by the French story, in which the doctor ordered, "Today we shall bleed all those on the left side of the ward and give a purgative to all those on the right side."

When I practiced medicine, except for surgery, digitalis, and a few other active remedies, we could do little for our patients. Antibiotics, psychoactive drugs, and all the new therapeutic agents were unknown. It was therefore not strange in 1932 that a young doctor such as I should try to join efforts with those who were trying to advance medical knowledge. The most active research laboratory in town was that directed by Dr. Bernardo A. Houssay, professor of physiology. In his work on the role of the pituitary gland on carbohydrate metabolism, he obtained some very novel findings, for which he, along with Carl and Gerty Cori, was awarded the Nobel Prize in Physiology and Medicine in 1947. Dr. Houssay suggested that I could do my thesis work under his direction and proposed several topics. My choice was the role of the adrenals in carbohydrate metabolism. My first

task was to learn how to measure blood sugar with the method of Hagedorn & Jensen. It was my first experience in a research laboratory. My ignorance in chemistry was unfathomable; for that reason I decided to follow some courses in the Faculty of Sciences.

Houssay helped me a lot. Not only did he do the brain work but he also carried out most of the adrenalectomies on the dogs. Houssay made daily rounds in the Institute and often left messages on minute pieces of paper. It was apparently through him that I learned to be economical. Even now, I usually write manuscripts on half sheets of paper already used on one side. Young people now are spendthrifts and would scandalize Houssay as they do me. The thesis work was awarded the Annual Prize of the Faculty for the best thesis, but it was undoubtedly Houssay's merit, not mine. Our close association lasted until Dr. Houssay's death in 1970. During all those years we saw each other daily and I could appreciate his cyclopean work in favor of Argentine science.

My enthusiasm for research increased gradually and, without noticing the change, I began to put in more hours at the laboratory and less at the hospital. I could do so because I did not have to earn my living with medical work. My great-grandparents came to Argentina, some from France, others from Spain, and bought land when it was cheap but still unsafe from the incursions of the Indians. Later these lands produced the cereal and grains and the cattle that brought riches to the country and to the pioneers who worked on them. These circumstances allowed me to devote myself to research when it was very difficult or impossible to find a full-time position for research.

It was a great privilege to be associated with Houssay. He worked very hard all his life trying to modernize the teaching of medicine, as well as directing his numerous students. His interest in research was very wide. Endocrinology was his main concern but he also ventured into many other aspects of physiology and biochemistry. He made intensive efforts to promote science. He was for many years president of the Argentine Association for the Progress of Science and later president of the Argentine Research Council. At times his efforts were very successful, but at other times the government was against him because of his outspoken manner and his liberal views.

INITIATION IN BIOCHEMISTRY (3)

After I finished my thesis work, Dr. Houssay advised me to work some time abroad. In consultation, with Dr. V. Deulofeu, professor of biochemistry, and Dr. R. de Meio, I decided that a good place would be the Biochemical Laboratory of Cambridge University, directed by Sir Frederick Gowland Hopkins, who had received the Nobel Prize in 1929, together with Eijkman,

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for "his discovery of growth stimulating vitamins." Cambridge was then at the peak of its glory with Rutherford, Dirac, and other scientific giants in the Department of Physics. Biochemistry was also excellent with Hopkins, the father of English Biochemistry, at the helm of the biochemical laboratory, and David Keilin, the discoverer of the cytochromes, in the Department of Parasitology. Arriving in Cambridge thirsty for knowledge, I began immediately to work under the direction of Malcolm Dixon on the effect of cyanide and pyrophosphate on succinic acid dehydrogenase. After that I worked with Norman L. Edson on ketogenesis using liver slices. Edson had worked with Hans Krebs, whom he admired greatly. When Edson returned to his home country, New Zealand, I worked with David Green on the purification and properties of β-hydroxybutyrate dehydrogenase. The atmosphere in the biochemical laboratory was very stimulating because of the many talented people there, such as Marjorie Stephenson, one of the pioneers in bacterial biochemistry, Norman Pirie, who had crystallized tobacco mosaic virus, Robin Hill, who was well known for his work on photosynthesis (the Hill effect), Joseph Needham, who started chemical embryology and ended up as an orientalist, Dorothy Moyle Needham, an expert in muscle chemistry, and many others.

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FATTY ACIDS (3, 4)

After my year at Cambridge, I returned to the Institute of Physiology in Buenos Aires, where I became associated with J. M. Muñoz. I never enjoyed working by myself so I was glad I could work with him. He had a rather original personality, and degrees in Medicine and Chemistry. Not content with these titles, he also earned a degree in Dentistry. Actually he did so not merely to increase his knowledge, but more importantly in order to become the professor of physiology in the school of dentistry. That type of ludicrous requirement was not too rare at our university. As a way to avoid competition from other graduates, it was of course detrimental to the university and the students.

Dr. Muñoz had been estimating ethanol, and had a reliable method with a beautiful, small distillation apparatus. Therefore we decided to work on ethanol metabolism, with interesting results that were published in the *Biochemical Journal.*

After our alcoholic adventure we took advantage of the same distillation apparatus for estimating volatile fatty acids. Muñoz found that the recovery was quantitative if the boiling point of the samples was increased by the addition of sodium sulfate. The volatile fatty acids (butyric acid was generally used) were then measured by oxidation with dichromate.

After working with tissue slices, we turned our attention to tissue homogenates, a rather ambitious project since fatty acid oxidation was believed

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to occur only in intact cells. Our approach was different from previous ones because we planned to measure fatty acid disappearance while other workers had relied on measurements of oxygen uptake with or without fatty acids. We obtained some homogenates that consumed butyric acid, but in general our experiments failed. Learning from our failures, we managed to find conditions under which our liver homogenates were active. It was a question mostly of working fast and in the cold. Because we wanted to fractionate the extracts, we had to cool our centrifuge. We did this with an automobile inner tube filled with freezing mixture and wrapped around the head of an old pulley-driven centrifuge. In this way we were able to separate a cell free particulate fraction and a supernatant fluid. The particulate fraction was found to require several factors for oxidizing fatty acids. These factors were cytochrome C, a C_4 dicarboxylic acid (we usually used fumaric acid) and adenosin phosphate (ATP was active but we used adenylic acid because it was easier to obtain the pure substance). We suspected that mitochondria were involved in fatty acid oxidation but could not prove it. In those days mitochondria were known to cytologists but practically unknown to biochemists. Claude had just started centrifugal fractionation and separated tissue homogenates into fractions containing mainly nuclei, mitochondria, or smaller constituents (microsomes).

AN ADVENTURE IN HYPERTENSION (5, 6, 7)

While we were working with Dr. Muñoz on fatty acid oxidation, J. C. Fasciolo was experimenting on the mechanism of renal hypertension. Under Houssay's guidance, he followed the discovery of Harry Golblatt, who had found that if the renal artery of dogs was mechanically constricted the animals developed a permanent hypertension. This had been a milestone because it provided an experimental method of producing high blood pressure. Fasciolo's contribution consisted in grafting one of those kidneys in a normal dog and observing the changes in blood pressure. The result was an increase in blood pressure, which proved that the effect was due to some substance that the constricted kidney poured into the blood. At that stage, I became involved. Dr. J. C. Fasciolo, E. Braun Menendez, J. M. Muñoz, and I formed a team that successfully clarified the problem. Dr. A. Taquini also collaborated in many experiments.

An early observation was that from the blood of the constricted kidney a substance that produced a transient rise in blood pressure could be extracted with acetone. The effect did not last as long as that produced by grafting a constricted kidney. In 1898 Tigersted & Bergman had discovered a pressor substance they called renin, which could be extracted from kidneys. We found that the effect of renin was clearly different from that produced by acetone extracts of the venous blood of constricted kidneys.

We then carried out a variety of experiments to obtain the in vitro production of the pressor substance. Incubations of kidney extracts or slices with blood plasma under aerobic or anaerobic atmosphere yielded only inactive extracts. One day Dr. Braun Menendez suggested that I should incubate renin with blood plasma. I told him I had incubated kidney extracts that contained renin with blood with negative results. I said I had done this experiment many times but that if it made him happy I might test renin. The incubation gave rise to the formation of a pressor substance soluble in aqueous acetone; we soon found that the properties were the same as those of the pressor compound found in the constricted kidney blood. We worked feverishly and obtained in a short time much information on the new substance, which we called hypertensin. We suggested that renin acted as an enzyme and released hypertensin from a precursor found in blood plasma. We also detected an enzyme in tissues and blood that destroyed hypertensin; this explained my previous failure to detect hypertensin formation. If the incubation time was too long or the destroying enzyme too active, all the hypertensin formed would disappear. The action of renin was found to have an interesting specificity, thus dog renin acted on dog serum, human renin on human serum, and so on, but they did not cross-react.

While we were working busily in Buenos Aires, others were conducting similar experiments elsewhere. In the laboratories of Eli Lilly in Indianapolis, Irwin Page and co-workers observed that purified renin showed pressor activity when injected into the blood but was inactive as a vasoconstrictor when tested on a dog's tail perfused with Ringer solution. They suggested that renin required a blood protein as activator. In fact they found that addition of blood plasma restored vasoconstrictor activity. Page et al proposed the name angiotensin for what we used to call hypertensin. Although our paper was already published (1939) when we learned of the work of the other group we were considerably depressed because we could not claim a discovery but only a co-discovery. Looking back now after so many years I view those feelings as quite infantile. However, such incidents happen quite often in research, and they affect workers even more experienced than we were at that time.

For several years, both groups tried to impose the names they had proposed. We used the terms hypertensin, hypertensinogen and hypertensinase, while the Indianapolis group referred to angiotensin and renin activator. Finally Dr. Braun Menendez and Page agreed on a Solomonic solution and proposed the names angiotensin and angiotensinogen. These are the names generally used today.

The purification and determination of the structure of angiotensin seemed an insurmountable problem in those days. We knew it was a polypeptide because it became inactive on treatment with proteolytic enzymes but the methods were too primitive to obtain much more information. Determining

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the sequence of a polypeptide was conceived only by stretching a wild dream.

It is now known that renin splits a leucyl-leucyl bond in angiotensinogen to yield the decapeptide angiotensin I, which is inactive, but when acted upon by a converting enzyme, it loses the dipeptide histidyl-leucine to yield angiotensin II, which is very active. The latter peptide may lose an aspartic acid residue to yield angiotensin III, which is less active than II. Much was accomplished after we abandoned the problem. The great advances came possibly because of the progress of the methods for amino acid analysis and peptide synthesis.

My incursion in hypertension research lasted only about one year but it was one of the most productive years in my career. Two important factors in the success were the congenial atmosphere and the personal quality of my teammates. They had very different personalities but worked together successfully. E. Braun Menendez was full of energy, enthusiasm and entrepreneurial ability; J. Muñoz had a very original personality and many unique ideas; good-humored Juan Carlos Fasciolo always told jokes or funny stories, but he also performed his work seriously, intelligently and efficiently. All were very clever and diligent. We had quite a lot of fun with our work. After successful experiments, I often said "You see, nothing can resist systematic research." But after failed experiments, I looked tired and depressed, so Dr. Fasciolo would make fun of me, saying "You see, nobody can resist systematic research." However, we worked hard; the rate of research was limited only by the availability of dogs for measuring pressor substances. We also used toads for measuring vasoconstrictor substances but with little success. They always gave confusing results.

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A STAY IN U.S.A. (3)

Our work in the Institute of Physiology was interrupted in 1943 by unexpected and disagreeable events. Dr. Houssay never mixed up in politics but had signed an apparently innocent letter that appeared in the newspapers and also carried the signature of many of the most important persons of the country. The letter asked for "constitutional normality, effective democracy, and American solidarity." The government reacted completely out of proportion, and decreed the dismissal of all the signers who had positions in the state institutions. Many of the best university professors lost their posts. Dr. Houssay was one of them. Most of the members of the Institute of Physiology resigned in protest and disbanded. Days of confusion and preoccupation followed. It was finally decided to continue working, not in the university but in a private institution that had to be organized from scratch. Since I was always in a hurry to do experiments and not lose time, I thought it would be a good moment to work abroad for some time. This

decision coincided with an important and fortunate event in my life, the start of a happy marriage. My wife and I decided to travel to the United States. Commercial planes were still two-motored and flew only by day; thus after several stops in various parts of the American continent, we finally arrived in New York. Since I had no previous arrangements, I had to look for a place in which to work. One place that was highly regarded then was Cori's laboratory in St. Louis. The Coris had just published a thorough and careful study on the crystallization and preparation of phosphorylase.

After looking around a few days in New York we traveled to St. Louis, where Carl Cori accepted me kindly in the laboratory. There he arranged that I should collaborate with Ed Hunter on the formation of citric acid. Thus for six months I had the privilege of working in a place full of traditions, where I met daily with Carl and Gerty Cori, Sidney Colowick, Arda Green, and other outstanding scientists. To broaden my outlook, I spent time in New York, where I arranged to work again with David E. Green. He had two rooms in the College of Physicians and Surgeons of Columbia University, and a small group of collaborators including Sarah Ratner, Eugene Knox, and Paul Stumpf. For some time we worked on the purification of aminotransferases and we were able to separate the alanine from the aspartate-aminotransferase. Green was always full of ideas and sometimes came in with a list of all the processes that had to be clarified. The list was impressive but I did not know how to attack any of those problems. When I told him about the fatty acid oxidation system that needed so many co-factors and that might be localized in mitochondria, he looked at me with a rather incredulous smile. He did not suspect then that these organelles would occupy a great part of his future.

One of the important things I learned from Green was that if one could find a place to work, one should be able to form a research group by asking around for the required salaries, equipment, and chemicals. Indeed, that was just what I did upon returning to Argentina, where I formed a small research group that grew slowly and gave rise to the Fundacion Campomar.

FUNDACION CAMPOMAR (3, 8, 9, 10, 11)

After my stay in the United States, I returned to the Institute of Physiology. Dr. Houssay had been reinstated and was trying to put the Institute together again. For some time I worked by myself and endeavored to start a small research team. The first person I attracted was Dr. Ranwel Caputto of the University of Cordoba, who had just returned from the Biochemical Laboratory in Cambridge. He had worked with Malcolm Dixon, and had succeeded in crystallizing glyceraldehyde dehydrogenase. Later he found that this enzyme is more active on glyceraldehyde phosphate, which is

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probably the natural substrate. The second to join the group was microbiologist Raul Trucco. The idea in selecting him was to continue the work on fatty acid oxidation but with bacterial enzymes.

I learned in 1946 that Dr. Houssay had been approached by Jaime Campomar, one of the owners of an important textile industry. Campomar wanted to finance a research institution specializing in biochemistry. I suspect that there were few candidates for director of the new institution; thus, even though Dr. Houssay may not have been very convinced that I could be successful in the enterprise, he suggested me.

As things were being organized, we continued to work in a cellar in the faculty of medicine, but only until Dr. Houssay was again removed from his position as professor of physiology and director of the Institute of Physiology, on the pretext that he was overage. This new abuse produced a great commotion in the faculty and most of us decided to leave. If the installations and equipment were poor at the faculty, they were disastrous in the laboratory into which we moved. This was the Instituto de Biologia y Medicina Experimental, a private institution that had been created when Dr. Houssay was first dismissed from the university. Shortly afterwards we rented a small, four-room house beside the above mentioned institute and adapted it for our laboratory. Others joined the group then, including Carlos Cardini, who had been for several years professor at the University of Tucuman, N. Mittelman, who had good training in protein fractionation, and A. C. Paladini, the youngest of all, who joined us with a fellowship. Mr. Campomar's annual contribution of 100,000 Argentine pesos was equivalent to about US $25,000. It was a very generous gift. With it we installed the laboratory, acquired some equipment, and paid some salaries.

After several false starts we happened to step into a field that turned out to be rather fertile. Dr. Caputto had told us that he had done some experiments that indicated that mammary gland homogenates produced lactose when incubated with glycogen. This seemed an interesting finding because the biosynthesis of saccharides was a rather unexplored field. At that time the only disaccharide that had been synthesized in vitro was sucrose. The enzyme used was of bacterial origin and the reactants were glucose 1-phosphate and fructose. We carried out many experiments with the mammary gland extracts but they gave generally ambiguous results, mainly because we used only very primitive methods for lactose detection, such as osazone formation. Discouraged, we thought of another approach—to find an enzyme that would catalyze the reversible synthesis of lactose. For this purpose we obtained a culture of *Saccharomyces fragilis,* a yeast that utilizes lactose. With this yeast it was found that oxygen uptake was faster when lactose was the substrate than with a mixture of glucose and galactose. At first we thought we would find some direct pathway for lactose utilization but after a time we concluded that it was a question of selective

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permeability, but we never investigated the point exhaustively. We did find a very active lactase and therefore directed our attention to galactose utilization. We obtained evidence indicating that galactose was phosphorylated and therefore the corresponding enzyme was studied. The reaction product was found to be galactose 1-phosphate and the study of its utilization led us to the detection of glucose 1, 6-diphosphate and uridine diphosphate glucose. We prepared glucose 1-phosphate and galactose 1-phosphate by chemical synthesis and observed that they were utilized when we incubated them with enzymes from galactose-adapted yeast. The disappearance was increased by the addition of a heated yeast extract. At first we thought that there was only one factor in the heated extract that activated the reactions with the two esters but after more work and many confusing experiments we realized that two thermostable factors were involved. One was necessary for the conversion of galactose 1-phosphate into glucose 1-phosphate, which requires the inversion of the hydroxyl group at carbon four. The other cofactor was required for the formation of glucose 6-phosphate from glucose 1-phosphate, that is, a change of position of the phosphate group.

Quantitative methods of estimating each of the two co-factors with the appropriate enzymes could be developed, and the next step was to purify the active substances. This was not as easy as it is now with all the current methods of fractionation. The procedures then available consisted of the separation of the barium, calcium, or mercury salts.

The co-factor of the phospho-glucomutase reaction had properties similar to those of fructose 1, 6-diphosphate; in fact, our partially purified preparations were enriched in it, but it was possible to destroy the fructose ester, leaving the active substance intact. Finally the co-factor was obtained pure, which turned out to be glucose 1, 6-diphosphate.

Studies on the other co-factor were also advancing. Purification by fractionation of the mercury salts and charcoal adsorption had yielded concentrates that adsorbed light at 260 nm. The spectrum was similar to that of adenosine but had some differences. For quite some time we were unable to discover the identity of the 260 nm absorbing substance until Dr. Caputto came in one morning with the last issue of the *Journal of Biological Chemistry* and showed us a spectrum identical to ours. This spectrum was that of uridine. Things became easier because we could measure the substance with a spectrophotometer instead of the enzymatic tests.

In addition to uridine the co-factor was found to contain glucose and two phosphates. Identification of the glucose was carried out by paper chromatography, a technique that we had just started to use. We employed a very inadequate solvent, but the results were correct. Only later were good solvents for sugar separations described. The presence of uridine in a co-factor was rather novel because in other compounds (ATP, NAD, FAD)

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the nucleoside present was adenosine. The occurrence of a sugar derivative combined with a nucleoside was also novel. The mechanism of action of uridine diphosphate glucose (UDPG) began to become clear when we found that the enzyme preparations active in converting galactose 1-phosphate into glucose 1-phosphate could also convert reversibly the glucose moiety of UDPG into galactose. Later Kalckar and coworkers found that NAD was necessary in this reaction; it seemed likely that the inversion of hydroxyl four occurred by an oxidoreduction reaction.

Dr. Paladini detected a new compound similar to UDPG by applying paper chromatography to yeast extracts. When very alkaline solvents were used, the sugar moiety of UDPG became separated from the nucleotide as could be ascertained by the noncoincidence of ultraviolet absorption and the color given by reagents for sugar detection. The products of decomposition were identified as uridine 5′-phosphate and cyclic glucose1, 2-monophosphate. Besides this compound, crude preparations of UDPG gave another substance that was not decomposed in alkaline solvents. It seemed the same as UDPG but with a different sugar moiety. This substance turned out to be uridine diphosphate acetylglucosamine. Since it has no hydroxyl group at position 2 of the sugar, it cannot form a cyclic phosphate. At about the same time James Park discovered other uridine nucleotides containing acetylmuramic acid plus some amino acids that are now known to be intermediates in the formation of peptidoglycan, a component of the cell walls of bacteria.

Paper chromatography of crude UDPG preparations allowed us to detect still another sugar nucleotide. The guanosine moiety could be identified easily because of its typical ultraviolet spectrum with a peak at 260 nm and a shoulder at 280 nm. It was studied mostly by Dr. Enrico Cabib, a bright young man who had joined our team as a fellow replacing Paladini, who had gone to work with L. Craig at the Rockefeller Foundation in New York. He had a good sense of humor and was a hard worker. Except for minor episodes that occur in all human groups, the atmosphere in the laboratory was very pleasant and all our time was dedicated to research. We had no lectures to give, no committees, and no forces pulling us away from the laboratory.

In 1957, Mr. Campomar's death left our institute without resources. Before disbanding we played our last card and asked for a grant from the National Institute of Health of the United States. We had little hopes of success, but to our surprise the grant was approved, and we continued our work.

To detect any unknown reaction involving UDPG, we used the same method of estimation that we had used for developing the isolation procedure, which consisted in measuring the activating action on the galactose

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1-phosphate-glucose 1-phosphate transformation. We used this method in experiments with yeast extracts with various additions and found that glucose-6-phosphate produced an increase in UDPG disappearance. With Dr. Cabib we traced the effect to the synthesis of trehalose-6-phosphate, an ester that W. T. Morgan had isolated several years earlier as a product of the action of yeast enzymes.

A similar procedure with wheat germ enzymes allowed us, with C. Cardini and J. Chiriboga, to detect the biosynthesis of sucrose. In the course of studies on the later process, we detected another enzyme that makes sucrose phosphate.

Hexosamine metabolism was the object of considerable research. We became interested in this field because of the discovery of UDP-acetylglucosamine. We wanted to find a role for this compound. One obvious role was the formation of acetylgalactosamine made likely by H. Pontis, who isolated UDP-acetylgalactosamine from liver. Another function of UDP-acetylglucosamine that seemed obvious was the formation of chitin. In fact, years later L. Glaser obtained a transfer of acetylglucosamine to chitin. With C. Cardini we studied the formation of glucosamine phosphate from glutamine and hexosephosphate.

In 1957 we entered another field. It was found that liver extracts could catalyze the formation of glycogen from UDPG. In those days it was firmly believed that glycogen was formed from glucose 1-phosphate. However some penetrating minds had expressed doubts about this. For instance Earl Sutherland had reasoned that it was difficult to understand why epinephrine, which activated glycogen phosphorylase in vitro, should always produce glycogenolysis in vivo. Herman Niemeyer went further and suggested that glycogen was formed not from glucose-1-phosphate but from UDP-glucose. Our finding was therefore not totally unexpected but nevertheless helped considerably in the clarification of the mechanism of glycogen synthesis and degradation. Soon after, we detected the activating effect of glucose-6-phosphate, a finding which gave new impetus to the work on regulation of glycogen metabolism. The studies on glycogen synthetase were carried out with C. Cardini, H. Carminatti, S. Goldemberg and J. M. Olavarria. In 1958 the government offered us a large house which had been a girls' school, where we are still working. Funds were scarce, so we moved into the new house after minimal adaptation. A short time thereafter the National Research Council was created and we became associated with the faculty of sciences of the University of Buenos Aires. As new workers joined the laboratory, it lost the romantic flavor it used to have.

Research on glycogen biosynthesis was extended to include starch. It seemed obvious that the synthetic mechanism should be the same or very similar. After trying many plant enzyme preparations with negative results,

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we detected activity in transferring glucose from UDPG in some starch grains. The activity seemed low and we reasoned it might be because we used the wrong substrate. G. Khorana had then developed rather simple chemical methods for the synthesis of sugar nucleotides. We attracted a new collaborator, trained in organic chemistry, Dr. E. Recondo. The idea, which at times seemed quite silly, was to synthesize nucleotides of glucose and different bases, and to test them with the starch enzyme. The most available starting materials were AMP and glucose 1-phosphate, so Dr. Recondo synthesized ADPG first. The enzymatic tests showed that this sugar nucleotide was active as donor and better than UDPG. We were not sure that the result was not an artifact until Dr. J. Espada detected in plant material an enzyme that led to the synthesis of ADPG. Later other workers found that it was the substrate for glycogen synthesis in bacteria.

Another aspect of glycogen synthesis which interested us and Dr. J. Mordoh, A. J. Parodi and C. Krisman was the high molecular weight glycogen that can be extracted from tissues by avoiding treatment with acid or alkali. These preparations have molecular weights ranging from 10 to 1,000 million daltons and give a characteristic aspect on examination with the electron microscope. Similar preparations could be obtained in vitro with glycogen synthetase and UDPG but not with glycogen phosphorylase working in the synthetic direction. The make up of high molecular weight glycogen still remains rather mysterious.

DOLICHOL DERIVATIVES (12, 13, 14)

In 1965 a rather important development in the field of polysaccharide biosynthesis was the simultaneous discovery of lipid intermediates by two groups of workers: P. Robbins et al in Boston, who were investigating the biosynthesis of lipopolysaccharides by *Salmonella,* and J. Strominger in Madison, who was working on the formation of the cell wall peptidoglycan in *Staphylococcus.* Both groups detected the formation of liposoluble intermediates identified as polyprenyl phosphate-sugars. Our colleague Marcelo Dankert, who worked in Dr. Robbins' group, returned to our laboratory and told us about these compounds. He was very enthusiastic about the new field. We happened to have some crude preparations of glycogen synthetase, and radioactive UDPG was always easy to find around the laboratory. With N. Behrens we mixed these two substances, incubated them, added *n*-butanol, as Dankert taught us, separated the organic phase, and counted its radioactivity. The incubated sample had only a few counts more than the control. The experiment was repeated with the same results, but after optimizing the conditions, we obtained a considerable incorporation of glucose into a substance soluble in some organic solvents.

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The next advance was the detection of an activation of the reaction by some liver extracts. In contrast to our previous work, in which water soluble substances were involved, the activator was soluble in certain organic solvents. We purified considerably the activating substance of liver extracts, but we could not obtain the amounts necessary for analysis. Judging from properties of the substance it seemed that a polyprenol phosphate was a good bet. Therefore dolichol, a 20-isoprene unit, saturated polyprenol present in liver, was isolated and phosphorylated chemically. The reaction product was active as an acceptor of glucose and had the same properties as the activating substance of liver. It was concluded therefore that the reaction product was dolichol-phosphate-glucose.

Here we had a new compound of unknown function that might be an artifact. Finding a role for it was not easy. Quite a lot of time was spent in trying to find out if it had a role in collagen synthesis, since this is the only glycoprotein that contains glucose. The results were negative. By chance it was found that dolichol-phosphate-glucose disappeared when incubated with liver extracts and that a new substance was formed. The role of this new substance remained rather mysterious until it was observed that it could be dissolved in chloroform-methanol-water 1 : 1 : 0.3. Mixtures of the same solvents in other proportions did not solubilize it. This was a small but important finding. Once the right solvent was found, the new substance could be fractionated on anion exchange columns and thus obtained pure. It was decomposed by mild acid treatment yielding a lipidic part and another hydrophylic part. The water soluble part had the properties of an oligosaccharide, which is now known to contain 3 glucoses, 9 mannoses, and 2 acetylglucosamine molecules; the lipophylic part appeared to be dolichol-diphosphate. The sugar moiety was found to be transferred to protein. This part of the work was done in collaboration with A. Parodi, R. Staneloni, and H. Carminatti.

Since then the field has attracted many brilliant investigators and has progressed rapidly.

WHY RESEARCH

I do not know how it happened that I followed a scientific career. It was not a family tradition, because my parents and brothers were involved mainly in rural activities. My father had graduated as a lawyer but did not practice. In our home there were always a lot of books on the most varied subjects and I had the opportunity to acquire information on natural phenomena. I suppose the most important factor in determining my future was that I received a set of genes that gave me the required negative and positive abilities.

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Among the negative abilities I might mention that my musical ear was very poor so that I could not become a composer or a musician. In most sports I was mediocre so that was another activity that did not attract me too much. My lack of oratorical ability closed the door to politics and law. I was a bad practising physician because I was never sure of the diagnosis or of the treatment.

These negative conditions were accompanied presumably by others not so negative: great curiosity in understanding natural phenomena, normal or slightly subnormal capacity for work, average intelligence, and excellent capacity for teamwork. Probably the most important thing was my opportunity to spend my days in the laboratory and to do many experiments. Most failed but a few succeeded either due to pure good luck or to having made the right mistake.

Nearly 50 years have elapsed since I started to research. These have been years of fairly hard work but with many agreeable moments. Research has many aspects that make it an attractive venture. One is the intellectual pleasure of discovering previously unknown facts. There are also human aspects worth mentioning. Some of the most pleasant periods in my career were those in which I worked with enthusiastic, clever people who had a good sense of humor. The discussion of research problems with such people is always a most stimulating experience.

The less agreeable part of research, the routine work that accompanies most experiments, is more than compensated for by the interesting aspects, which include meeting and sometimes winning the friendship of people of superior intellect from different parts of the world. The balance is clearly positive.

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