LYNEN LECTURE - MIAMI 1974

When Bill Whelan invited me to give the Lynen Lecture which is of an autobiographical nature I accepted with reluctance because I don't think that anything in my life is worth telling and much less, entertaining.

My life covers the period of biochemistry in which most of the enzymes and coenzymes were discovered and in which most of the reactions of intermediary metabolism were unrevealed. I was born in 1906, the same year in which Harden and Young published their paper on the coenzyme of yeast fermentation and the role which initiated the studies on cofactors in which we became involved many years later.

After fairly normal studies in the primary and secondary school, I studied medicine, in Buenos Aires, and did not stand out as a good student.

I was working in the hospital and I heard that Dr. Houssay was doing very interesting studies on carbohydrate metabolism. They described as revolutionary findings and turned out to be in fact of great interest and eventually led to the award of the Nobel Prize to Houssay in 1947. At that time I had no idea of what I wanted to do nor which was the field I was more apt for. In relation to this I remember discussing this problem with some of my colleagues at the hospital and that one of them told me "you are not very intelligent but maybe you can be successful because you are persevering". About 2 years elapsed before I was introduced to Dr. Houssay and started working at the Institute of Physiology. It was there where I did my thesis work on the role of the adrenals in carbohydrate metabolism. Houssay helped me a lot, not only did he do the thinking but he also carried out the adrenalectomies on the dogs. During this work I realized that it would be interesting to understand physiology more deeply and this is what led me to study Biochemistry. This was similar to the previous change when I wanted to understand medicine more deeply and began studying physiology. Perhaps it was

fortunate that I did not continue trying to understand ~~xxxx~~ things more deeply because I might have ended up in nuclear physics or in phylosophy.

Cambridge After some consultations I decided to improve my knowledge on biochemistry by going to the Biochemical Laboratory of Cambridge. At the time it was one of the leading world centers in Biochemical research. The head of the Laboratory was Sir Frederich Gowland Hopkins a pioneer of studies on vitamins, the discoverer of trytophan and one of the first great British biochemists. Firstly I worked with Malcolm Dixon then with Norman L. Edson who had worked with and was a great admirer of Sir Hans Krebs. Afterwards I worked with David Green.

Cambridge was a great experience. The laboratory was different from the German Laboratories of that time where there was a Herr Professor and all the others worked directly under his directions. In Cambridge the groups worked independently and there were many ~~investigators~~ outstanding; Marjorie Stephenson, one of the pioneers in biochemical microbiology, Dorothy Needham, who worked on muscle, Robin Hill who became so well known from the Hill effect, Norman Pirie who at the time had succeeded in crystallyzing tobacco mosaic virus. This he did simultaneously with Wendel Stanley who received the Nobel Prize for this work. After one year in Cambridge I thought I had acquired some rudimentary knowledge of biochemical research and decided to return to Buenos Aires.

Fatty acids ~~and ketone~~ Those times

In ~~1938~~ it was generally believed that the oxidation of fatty acids would only take place in intact cells. This belief was based on the fact that cell homogenates were completely inactive. With J.M. Muñoz we decidéd to try to obtain a soluble preparation which would oxidize fatty acids.

We used to measure the disappearance of volatile fatty acid on incubation with liver homogenates. The analytical procedure consisted in distilling the acids and oxidizing them with chromic acid. When the acid disappeared the reaction

mixture remained brown and if there was no fatty acid consumption the final mixture was green. In most experiments nothing happened and the final mixture was green. I remember having the feeling that our faces also turned green after many of these failures. ~~After innumerable failures we obtained a cell free system which oxidized fatty acids.~~

We used to work with guinea pig liver homogenates and fractionated them in a centrifuge which was cooled by wrapping an inner automobile tyre filled with frezzing mixture around it. At that time there were no commercial refrigerated centrifuges. We had no guinea pigs nearby and had to send our assistant to fetch them from rather far away. He usually took a basket but one day he forgot to take it and used a large paper bag. The guinea pigs were clever enough to find a way of escaping; they wetted the paper so that it ~~got~~ became soft and then worked their way out. This happened in a crowded bus and resulted in such a great confusion that we lost our guinea pigs.

Finally, after innumerable failures, we overcame these and many other difficulties and obtained an enzyme preparation which oxidized fatty acids when complemented with adenylic acid or ATP, a C_4 dicarboxylic acid, dicarlixilic acid, cytochrome C and magnesium ions. I remember that one of the things that intrigued us was that activity disappeared very rapidly on leaving the enzyme mixture in water. Now that we know that the activity is in mitochondria this does not seem surprising. The separation of mitochondria by differential centrifugation in sucrose solutions was developed by Claude years after, and the mechanism of fatty acid oxidation ~~by mitochondria was studied by Lehninger and then thoroughly by Green.~~ clarified by several groups of workers, including Feodor Lynen.

The circumstances led me to change my line of research several times in my life. I doubt if this is desirable but in some cases it is inevitable.

In the Institute of Physiology of the Faculty of Medicine of Buenos Aires, Juan Carlos Fasciolo had been working on the mechanism of arterial hypertension produced by constriction of the renal artery. He had reached the conclusion that a pressor substance was involved. With J.B. Muñoz and Eduardo Braun Menendez we decided to collaborate in this project and in a rather short time we made several findings of considerable importance and furthermore we had a good time. This period as well as a few others in which I worked with pleasant and enthusiastic people were the most enjoyable experiences in my career.

It was known that kidney contains a protein, renin, which when injected into animals, produces an increase in blood pressure. ~~Our group found that renin acts indirectly on blood pressure by liberating a pressor polypeptide from a blood protein.~~ To our dismay Page and Helmer published similar findings practically simultaneously. ~~We called~~ the pressor substance hypertensin ~~while Page and Helmer~~ called it angiotonin. Each group tried to impose its name until Braun Menendez and Page Salmonically proposed the name angiotensin which is used universally.

The hypertension team lasted about one year but the amount of work it carried out is really surprising. We knew that angiotensin is a polypeptide but with the methods available it was practically impossible to obtain more information. ~~Now the aminoacid sequence is known and angiotensin may be obtained by synthesis.~~

The work on hypertension was interrupted by extraneous reasons and the team disintegrated. In one of the periodical upheavals Dr. Houssay was dismissed from his post at the University. We had to leave the Institute of Physiology and were left without a laboratory.

St. Louis and New York

In compensation for the scientific misfortune I had the luck of getting happily married and of being able to spend some time doing research in the U.S.A. I was interested in going to Dr. Cori's laboratory in St. Louis which was one of the Meccas of workers in carbohydrate metabolism. I don't recall the circumstances too well but according to Dr. Cori I presented myself unannounced and when he asked

me when I would start working I answered 'right now'. ~~I began working with Ed. Hunter on the utilization of citric acid~~. The lab was very pleasant with the two Coris, Golowick, Slein, Burger, Taylor, ~~etc~~ and others. There were few people because all this occurred during the 1939-45 war. The Coris had recently published a big paper

Arda Green

5A

5A

After some time we decided to go to New York where I again met David Green with whom I had worked ~~in~~ at Cambridge. We worked on transaminases and were able to separate two of them. David Green was always full of ideas and projects and working with him was most interesting. He was also very critical and often became ~~INEX~~ iconoclastic. It was at that time that paper chromatography was developed by Consden, Gordon and Martin. I remember very well that Green showed me the paper in the Biochemical Journal and said "have a look at this, it seems rather interesting". Since it dealt with aminoacid separation I was not interested and did not appreciate the importance the new method would acquire.

We lived fairly near the College of Physisians and Surgeons of Columbia University. My wife was still perfecting her cooking abilities and one day gave me a piece of roasted liver. It looked so repulsive that while she went out of the room I rapidly put it in an old envelope and threw it out of the door. I was happy not to have hurt her feelings. On the following morning the post arrived and my wife sorted the letters. One of them was fatter and she looked at me and said "Look at what Dr. So and So.....sends you; it must be something for the laboratory". I nearly fainted when I discovered that the roasted liver had come back with the post.

After about one year and a half in the U.S.A. we thought that it would be possible to work again in Buenos Aires.

Fundación Campomar

Dr. Houssay had been reinstated as Professor ~~of Physiology~~ so that many of us returned to the Institute of Physiology. However, this did not last long. A few months later Houssay was again dismissed from the University and the Institute

Physiology was disintegrated again. An unexpected event came to our aid. Jaime Campomar, a well known textile manufacturer, decided to create a private biochemical research center. ~~After consulting Dr. Houssay it was decided that~~ I ~~should organize the new center.~~ GA

R Ranwell Caputto who had just returned from a Fellowship in the Biochemical Laboratory in Cambridge. We also enrolled Raul Trucco who had experience in Bacteriology because at that time we were interested in fatty acid oxidation by bacteria. At first we worked in the Institute of Biology and Experimental Medicine which was a private institution where Houssay and coWorkers had taken refuge. After a short stay there we rented an old, adjoining, small house and conditions began to improve slowly. We had three small laboratories, a library where I took my private books, and a little store room. Dr. Cardini, who had been dismissed from the University of Tucumán joined us as well as A.C. Paladini who came with the first fellowship of the Fundación Campomar.

The installation of a new laboratory is always an amusing enterprise. Furthermore we were all young and enthusiastic. The Rockefeller Foundation provided us with a refrigerated centrifuge and Dr. Houssay loaned us a spectrophotometer. The first research project: fatty acid oxidation in bacteria, sank and then we went on to study lactose synthesis. As the preliminary trials were unsuccessful we thought we might get some information on the synthesis by studying lactose utilization. For this we selected a yeast that grows on lactose: saccharomyces fragilis. We grew it on milk serum which was cheaper than lactose and then dried it. For this purpose we used to extend the yeast paste on the bottom of inverted precipitation beakers. Since we had only a few we selected those that were cracked for this operation. The lady who washed the glassware come one day complaining

that we should not dry the yeast in that way because it cracked the flasks. I mention this because it is a kind of reasoning that is quite common and which even scientists use inadvertantly.

The extracts of the dried yeast contained enzymes which phosphorylated galactose to galactose 1 phosphate. The latter was then transformed to glucose 1-phosphate which gave glucose 6 phosphate.

The reaction was measured by following the increase in reducing power - with a copper reagent. We soon found that a thermostable factor increased the rate of reaction and set out to isolate it. The results were very confusing because actually we had two thermostable factors. After we realized this we could concentrate both cofactors and identify them. One was UDPG and the other glucose 1-6 diphosphate. Finding this out was not so easy. We did it all without using anion exchange columns.

Only some years later were these applied to the separation of nucleotide sugars. It was in this way that we found UDP-acetylglucosamine and guanosine diphosphate mannose.

Sometimes one forgets how primitive our knowledge on nucleotides was in 19 when UDP-G was found. The only free nucleotides known to be present in tissues were ATP, ADP, AMP and inosinic acid. UMP was only known as the 3' phosphate obtained by hydrolysis of nucleic acid. The 5' phosphate had not been isolated and of course neither UDP nor UTP was known.

At the same time when we were working with UDPG Ted Park with H. Johnson was studying the action of penicillin on staphylococcus. He found that a uridine compound and acid labile phosphate accumulated in the presence of the antibiotic. From the mixtures he isolated a compound which contained uridine and an unidentified sugar. The structure of this unidentified sugar was elucidated many years after and called acetylmuramic acid. We had more luck than Park in that our compound was made up of well known components. The muramic acid containing compound discovered by Park turned out to be of great importance

in the biosynthesis of bacterial cell walls.

The mechanism by which UDP-G catalyzes the transformation of galactose 1 phosphate into glucose 1 phosphate became more understandable when it was found ( ) that on incubation with the enzyme, part of the glucose was converted into galactose. We now know that the formation of galactose requires an oxidation at position 4 of the glucose residue of UDP-G. As to the role of sugar nucleotides as sugar donors it became apparent when Dutton and Storey ( ) found that glucuronides are formed from UDP-glucuronic acid. However I think that he did not appreciate at the time that this was a general phenomenon. The role of UDP-G as glucose donor was first suggested by Calvin, Buchanan and others to explain the formation of sucrose. The evidence they had at the time was not very convincing but the conclusion, we know now, was correct.

In our lab we were measuring the disappearance of UDP-G under different conditions. We found that glucose 6 phosphate greatly increased the disappearance of UDP-G in the presence of yeast enzymes. The changes were soon traced to the formation of trehalose 6 phosphate. Soon afterwards the synthesis of sucrose and sucrose phosphate with UDP-G and plant enzymes was obtained and a series of other transfer reactions were discovered by various workers.

The synthesis of glycogen from UDP-G, with Cardini, was a finding of some interest particularly because it was universally accepted that both synthesis and degradation were catalyzed by phosphorylase. The direction in which the reaction takes place at any moment was believed to be dictated by local concentration of glucose 1 phosphate and inorganic phosphate. Some inconsistencies of this theory were pointed out by Sutherland, Niemeyer and others but it was the finding of glycogen synthesis from UDP-G that finally settled the problem. A similar reaction was later found to be responsible for starch synthesis with the difference that ADPG is involved together with or instead of UDP-G. These were the last reactions which we studied with non radioactive substrates.

an endogenous acceptor

microsomes is transformed so that the glucose moiety is transferred to ~~another~~ yielding a compound which appears to be Dol-P-P- oligosaccharide. Incubation of this compound with microsomes and manganese ~~xx~~ ions results in a transfer of the oligosaccharide to protein. ~~The oligsaccharide appears to have starting at the reducing end,~~ two acetylglucosamine joined ^{14}- and then several mannose residues. This same type of oligosaccharide is found in thyroglobulin, aspergillius amylase, ovalbumin and several other glycoproteins in which the oligosaccharide is joined to an aspartamide residue.

Thus it has turned out that dolichyl phosphate is involved in the synthesis of one type of glycoprotein. As to the other types, that is those in which the sugars are joined to serine threonine or hydroxylysine the mechanism does not seem to be the same and up to now there is no good evidence that lipid intermediates are involved.

After working on fatty acid, then on saccharides it seems I have been driven into a corner where lipids, carbohydrates and proteins converge.

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Luis Leloir amen J

1B)

Furthermore I have already written a historical review ~~of~~ called: "Polysaccharide synthesis seen from Buenos Aires(") so that I will have to ~~do~~ ~~duplicate~~ repeat a great ~~part of many~~ part of the information +

1c

What Houssay had found was related to the role of the pituitary. ~~He observed that~~ If the pancreas is removed from an ~~the~~ animal it becomes diabetic. ~~Of course that is not surprising~~ This ~~was since it~~ had been discovered by von Mering and Minkowski. ~~The Houssay~~ The new finding was that if the pituitary was also removed then the animals did not become diabetic. The animal ~~w~~ ~~or~~ without pancreas and pituitary became known as Houssay dogs in the physiological literature.

1A

He was a really extraordinary person. As a scientist he was self made and he developed himself ~~on~~ under ~~go~~ completely adverse conditions. He had one teacher of great quality but whom he never met. This was the famous french physeologist Claud Bernard. It was by reading ~~the~~ ~~to~~ ~~the~~ the book: ~~the~~ "introduction a la medecine experimentale" that Houssay became a ~~phys~~ scientist. Besides the ~~ex~~ laboratory work, ~~and~~ ~~the direction of~~ the teaching of thousands of students and the organization of research he carried out a lasting campaign for the advancement of Science - ~~It was~~ A very great part of ~~a~~ research in Argentina developed thanks to his efforts. He was president of our National Research ~~Council~~ Council for about ~~of~~ 15 years and he did a splendid job—

see 1D

1 D

He was an ~~unb~~ untirable worker and used to say "I have no time for being ill (sick)". I has been for me a great prevledge to be in close contact for many years with such an outstanding personality as Dr Houssay

prevledge asoc PH

Actually the old theory was correct in that glycogen is formed from glucose phosphate but there were one step lacking which explains the reactions and the enzymes lacking

2A

He was awarded the Nobel Prize in 1929 together with Eijkman for "his discovery of growth stimulating vitamins"

47/ Renin is a protein and is thermolabile
On the other hand the substance which appeared
in the experiments with ~~isolated~~ isolated Kidneys was
thermolabile - This led us to do experiments in which
extracts of kidney were incubated under various
conditions and then tested for pressor
thermostable substance - The results were
always negative - One day Braun Menendez came
along and proposed to incubate partially purified
renin with blood plasma and then to test it.
I argued that I had incubated crude extracts
which certainly contained renin and it did
not work, therfore testing renin seemed
hopeless - However to make him happy I
did the experiment as he proposed - We obtained
a beautiful effect and became very excited.
We learnt afterwards that the negative results
with crude extracts was due to the presence
of an enzyme which destroyed our pressor
substance -

sigue 48

In a short time we found out that the renin acted on another protein present in blood to yield the presor substance which we named hypertensine. The blood protein we called hypertensinogen and the enzyme which inactivated the presor substance was named hypertensinogen.

4c

A lot of work has been done in the field since then. Now it is known that renin hydrolyzes off a deca peptide, Angiotensin I, from angiotensinogen. Angiotensin I is practically inactive but by the action of a converting enzyme is transformed into an active octapeptide, angiotensin II. This substance increases blood pressure and also stimulates the secretion of aldosterone from the adrenal gland—

5A

The Cori's had recently published a big paper on the crystallization of phosphorylase and on some of its kinetic properties. While I was in Washington University I worked with Ed Hunter on ~~co~~ citric ~~acid~~ acid formation.

6A)

He asked ~~consulted~~ St Hourray who could take care of organizing the new center. St Hourray ~~recommend~~ suggested my name although, I think, he was not very convinced that I could do a good job. ~~For~~ Presumably he did not find a better candidate.

One of the good things I did was to obtain the collaboration of RC et

to a product which turned out to be galactose 1 phosphate. The fate of the latter was unknown at the time but it did not take long to find out that the galactose 1 phosphate was transformed to glucose 1 phosphate and the latter to glucose 6 phosphate. The change from galactose to glucose requires an inversion of the hydroxyl group at position four. The transformation of glucose 1 phosphate to glucose 6 phosphate was known to be carried out by the enzyme phosphoglucomutase but there was no information on a cofactor requirement.

B7

We tried to purify the enzymes required in the process and soon found that addition of a heated extract (~~a bacteria~~ a Kochsaft) produced a large activation - ~~I think that we~~ We then wanted to find out what was the substance in the heated extract which produced the activation. According to my recollection Dr Caputto was testing the action on glucose 1 phosphate while I was using galactose 1 phosphate - The results were very confusing until we ~~not~~ realized that we were dealing not with one but with two thermostable substances. Once we realized this we proceeded to isolate first the cofactor of the ~~glucose → glucose 6 phosph~~ glucose 1 phosphate - glucose 6 phosphate transformation. Our job was made ~~easier~~ because we had a theory on the identity and mode of action of the active substance - The theory came from the fact that the active substance had some properties similar to

segue B7 II

B7 II

fructose diphosphate. According to the theory our substance was glucose 1-6 diphosphate and it acted by transferring its 1 phosphate to position 6 of glucose 1 phosphate. Thus the product of the reaction were glucose 6 phosphate and a new molecule a glucose 16 diphosphate. By one of those chances that rarely happen in research the theory came out to be correct. By using the classical evolution methods of that time (we were able to evolute the substance and prove that it was glucose 16 diphosphate.

Then we turned our attention to the evolution of the other substance, that is the one that accelerated the galactose 1 phosphate - glucose phosphate transformation. We used a combination of precipitation of the mercury salt and adsorption on charcoal. Our concentrates were found to

B7 III

absorb light at about 260 mμ. ~~as the it~~
and at first ~~it~~ was thought to be an
adenine nucleotide

segue p 7

C7 Finally we obtained a fairly pure compound and had no great difficulty in determining its structure as UDPG -

In the course of these studies we could detect two other compounds similar to UDPG - These were UDP-acetyl glucosamine ( ) and GDP-Mannose ( ) which are involved in reactions where acetyl glucosamine and mannose are transferred -

He reasoned that it was difficult to understand why ~~phosphorylase which was~~ the activation of phosphorylase should always produce ~~gly~~ degradation of glycogen - and that if it was also involved in synthesis it should ~~not~~ lead to glycogen formation if the equilibrium of the reaction was favorable. The suggestion that UDPG might be involved in glycogen synthesis was formulated by Niemeyer( ) but it was the ~~finding~~ detection of glycogen synthetase that finally settled the problem-

P18 The activity of the enzyme was found to be very low. Here again we tried the old trick that was used by Harden and Young in the days when I was born. We added a heated extract of liver and obtained a great increase in glycogen formation from UDPG (Labor. Carmi Ola Arrhenius). 81 508 1959 The substance responsible for the effect was found to be acid stable, ~~to be~~ ~~retained~~ alkali labile and retained by an anion exchange resin. Several known substances with these properties were then tested. One of them, glucose 6 phosphate, was found to be active and furthermore many others were without effect. That is the effect of glucose 6 phosphate was quite specific. This led to the idea that glucose 6 phosphate might have a regulatory effect on glycogen synthetase while adenylic acid would regulate phosphorylase. Some experiments were started in Baltimore on the effect of adrenalin (~~Baltimore~~ and He found that adrenalin produced a decrease of glycogen synthetase in rat diaphragm. The field was

Sigue

developed rapidly by Larner and Villar Palasi and is now a full chapter of the enzyme regulating mechanisms.

Experiments designed to inform on the ~~subcellular~~ subcellular distribution of glycogen synthetase showed that ~~it~~ on ~~factors~~ fractional centrifugation it sedimented together with the glycogen. This finding was important ~~for one~~ in connection with the biosynthesis of another reserve polysaccharide. ~~The bond~~ The bonds in glycogen and starch are α1-4 with α1-6 links in the branch points. The difference between the two resides in that starch ~~could~~ ~~and~~ ~~corect~~ consists of two components amylose and amylopetin. The first is a linear chain while the second is ramified but with less ~~th~~ longer chains than glycogen. After working on glycogen synthesis we naturally became interested in starch synthesis. It seemed obvious that the precursor was UDPG. However experiments with crude plant extracts gave negative results until it was reasoned that if glycogen synthetase activity goes with glycogen

then starch synthetase should be found in starch-
Experiments showed that bean starch
incubated with UDPG gave rise to the formation of
UDP and to transfer of the glucose (Fekete Leloir
Cardini 1960) - We had the impression that the activity was rather
low for a polisaccharide which is formed at
rather high rate in plants - Of course For this
reason it was considered worth while to test
some other substrates besides UDPG. For instance we
thought of having a sugar nucleotide of maltose
which the sugar such as UDP-maltose which would
act as donor of maltose and also of
nucleotides of glucose with different bases. At
the time Dr Eduardo Recondo who had a good
training in organic chemistry came to our
laboratory Furthermore The synthetic methods had
been developed developed by Khorana. One of
the first nucleotides that was synthesized was
ADP-glucose and it was tested

Segue III

A8 cont III

for starch formation - Surprisingly this compound turned out to act as glucose donor much more efficiently than UDPG - The reaction was actually about ten times faster - The observation ~~was~~ was interesting ~~but did not~~ and indicated that ADPG could be the glucose donor for starch synthesis. This idea was strengthened by the finding of a specific enzyme which leads to the synthesis of ADPG from ATP and glucose 1 phosphate (Espada J JBC 237 3577 1962). At present it is ~~as~~ accepted that ADPG is a natural substrate for starch biosynthesis

Sigue A8 cont IV

Many ~~other~~ ~~process~~ reactions leading to polysaccharide synthesis ~~by~~ isolated plant or animal enzymes have been studied. ~~The role of enzyme~~. Our ~~ideas~~ beliefs on the mechanism of ~~polysaccharide biosynthesis~~ these processes have been changing since ~~Levin~~ saccharide biosynthesis for many years. The study of ~~the process~~ actually started before I was born when Croft Hill in 1898 incubated concentrated glucose with yeast enzymes and obtained an α linked disaccharide. Years after Zemplen and Bourquelot also used glucose but an ~~different~~ enzyme ~~from~~ of plant origin. ~~They~~ and obtained β linked disaccharides. Bourquelot also obtained disaccharides from galactose or glucose solutions. From these facts it was deduced that reversal of hydrolysis was a possible mechanism for polysaccharide synthesis. Many years after in 1939 the Coris obtained the synthesis of glycogen from glucose 1 phosphate with the enzyme phosphorylase. After that reversal of ~~phosphorolysis~~ phosphorolysis was believed to be the mechanism of polysaccharide synthesis. Another route was discovered in 1944 by Hehre. This was the synthesis of dextran from

segue V

As cont V

with a bacterial enzyme

Sucrose. a similar process in which the fructose moiety of sucrose is transferred has also been described

The next advance in the field was the discovery of the role of nucleotide sugars. Several transfer reactions leading to the synthesis of polysaccharides such as chitin, callose, cellulose, and many others have been studied + In some cases the sugar nucleotides are not the direct donors and lipid intermediates are involved. This is one of the most important developments in the field

10A

~~The~~ Incubation of Dolichol-monophate-mannose with microsomes gives rise to a transfer of mannose to an acceptor giving a lipid-oligosaccharide - The latter can then act as donor in transfer of the oligosaccharide to protein +

segue 10A cont

10A cont I The acetyl glucosamine containing compound

~~This compound~~ ~~when~~ incubated ~~with~~ again with UDP-acetyl glucosamine gives rise to the formation of ~~and~~ a substance with two acetyl glucosamine residues joined β1-4. ~~That is it~~ This ~~was~~ a finding of considerable interest because ~~the~~ disaccharide formed from two acetyl glucosamines, that is Nacetyl chitobiose, is found as the innermost residue in ~~some~~ the oligosaccharide of several glycoproteins such as: thyroglobulin, aspergillus amylase, ovalbumin and probably many others. ~~All~~ the studies (we have) carried out up to now with Behrens Carnovsky, Parodi, and others, ~~have~~ been done ~~with~~ following the radioactivity and not by regular ~~chemical~~ analytical methods. Therefore many of the ~~same~~ conclusions remain to be confirmed. ~~We can~~ The picture which emerges from the experiments is as ~~follows~~. ~~shown in~~ Fig ~~e~~ follows.

The first step would be a reaction between dolichol monophosphate and UDP-acetyl glucosamine to give

(Segue)

dolichol diphosphate-acetyl glucosamine as follows:

$$DMP + UDP\text{-}GlcNAc \rightarrow DPP\text{-}GlcNAc + UMP$$

The reaction product, ~~dolichol~~ would then react with another UDP-acetyl glucosamine molecule ~~yielding~~ yielding a N'acetyl chitine containing lipid.

$$DPP\text{-}GlcNAc + UDP\text{-}GlcNAc \rightarrow DPP(GlcNAc)_2 + UDP$$

The product would in turn act as acceptor either directly from GDP-Man or from DMP-Man. Several mannose residues linked α or β would be thus added followed by some acetyl glucosamine so that the product would be a dolichol diphosphate linked oligosaccharide - This oligosaccharide would then be transferred to the ~~protein acceptor~~ acceptor protein and the glycoprotein would thus be completed.

For some glycoproteins glucose would be added from dolichol monophosphate-glucose before the

Segue 10A cont III

transfer to protein -
The fact that mannose is involved and some experiments in which lability to alkali was measured indicate that the glycoproteins in question are of the asparteamide type + ~~Therefore the formation of dolichol phosphate~~ Therefore it seems that the oligosaccharide is built up joined to dolichol phosphate and then transferred to an asperteamide residue in a protein - Probably other residues of

It seems that this pathway of synthesis is not general for all the glycoproteins

galactose and neuraminic acid may be added after the oligosaccharide is transferred to protein -

→ Several cases has been studied rather carefully and no evidence for the intermediate formation of lipid intermediates was found - Such is the case with collagen or ~~the~~ glomerular basement membrane which ~~has~~ a glucosyl galactosyl β residue

α1-2

segue IV

linked to dydroxylysine. ~~In so and the glycoprotein~~ where the oligosaccharide ~~is bound to~~ ~~serine~~ or Threonine. ~~The~~ ~~Sub~~ Submaxillary mucin has neuraminyl galactosaminyl residues linked to serine

Two transferases have been detected ~~(~~ one for glucose and another for galactose.

As to the glycoproteins in which the oligosaccharide is linked to a hydroxi amino acid ~~some~~ there are several which have been studied ~~from~~ in order to clarify their biosynthesis - Submaxillary mucin has a disaccharide formed by neuraminic acid and galactosamine. The linkage region of chondroitin sulfate contains glucuronic acid, two galactoses and xylose linked to serine - In none of these cases does it seem that lipid intermediates are ~~involved~~ involved -

~~The meaning of these facts is difficult to comprehend~~ ~~It might mean that the first step of Biosynthesis the glycosylation process occurs on~~

sigue V

10 A IV

~~different parts of the cell~~

~~likely~~

It seems therefore that the role of the dolichol intermediates is limited to the glycoproteins in which the oligosaccharide is joined to asparagine -

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