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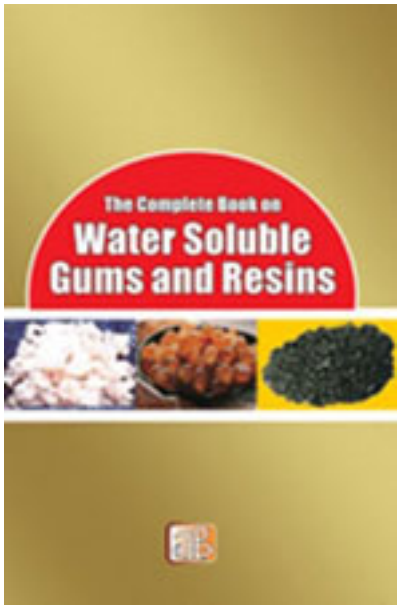
The Complete Book on Water Soluble Gums and Resins



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Resins, gums and latex are almost ubiquitous in the plant kingdom and many of them continue to play an important role in our daily lives. Numerous plants produce some kind of resin, latex or gum, but only a few are commercially important today, even though their uses and applications are truly manifold. They have been used as adhesives, emulsifiers, thickening agents, they are added to varnishes, paints and ink; they lend their aromas to perfumes and cosmetics and even play a role in pharmacy and medicine. Gums are viscous substances which are secreted by the bark of certain trees. Usually transparent (but sometimes slightly tinted) they contain a mucilage which when dissolved in water makes the latter become viscous. When this mucilage is dissolved in water it can be made to precipitate with alcohol. Resins, on the other hand, are gluey and viscous substances which may be whitish, brownish, or red and are secreted by certain trees when they are incised. Resins contain an essence and are usually not water soluble. Most commonly found types of plant exudates are chemically completely different to gums. Several acacia species are important economically. True gums are complex organic substances mostly obtained from plants, some of which are soluble in water and others of which, although insoluble in water, swell up by absorbing large quantities of it. They are used in adhesives, pharmaceuticals, inks, confections, and other products. Resins are terpene based compounds. Terpenes constitute one of the largest groups of plant chemicals and they can be very complex. They are not water soluble, but can be either oil soluble or spirit soluble, depending on their specific chemical composition. Worldwide interest and activity in gums and resins has grown dramatically in the last few years. Governments, environmentalists, research institutions and other interest groups are among those who have begun to push for stronger support for gums and resins as a way to meet a range of economic, social and environmental goals.
Some of the fundamentals of the book are photosynthesis and metabolism of carbohydrates, occurrence, properties and synthesis of the monosaccharides, nitrogen derivatives, carbohydrates in parenteral nutrition, essential carbohydrates, ethers, anhydro sugars and unsaturated
derivatives, constitution of nicotinic acid and of nicotinamide, industrial methods of preparing nicotinic acid and nicotinamide, general physiology, metabolism and mechanism of the vitamin action etc.

This book gives a complete insight of water soluble gums and resins that are used in day to day life in various Industries. It is an invaluable resource to all its readers, students, scientist, new entrepreneurs, existing industries and others.

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Sample Chapter:

Photosynthesis and Metabolism of Carbohydrates

Photosynthesis

Introduction

The essential features of the photosynthetic reaction have been known since 1845 when it was recognized that the fundamental transformation of photosynthesis was the conversion of light energy into chemical energy and that the reaction could be written as follows:

Carbon dioxide + water

organic matter + oxygen + chemical energy

Since that time plant physiologists, biochemists, biophysicists, organic chemists, physicists, microbiologists, physical chemists, and others have contributed to the literature of photosynthesis.

The early work in photosynthesis was based on the assumption that carbon dioxide fixation was a process unique in green plants, and studies were made on the effect of various external factors such as light intensity, light quality, carbon dioxide concentration, and temperature. Certain internal factors were also recognized as affecting photosynthesis. Many fundamental studies on photosynthesis were concerned with the chemistry of the chlorophyll molecule. Enzymes were studied, and attempts were made to carry out cell-free photosynthesis by conventional biochemical techniques. While these studies contributed a great deal of information regarding the photosynthetic reaction, they did little to elucidate the mechanism of the process.

During the 1930’s, however, research in the field of photosynthesis and in a number of allied sciences indicated that the concept of the nature of the problem of photosynthesis could be very profitably altered. The discovery in 1935 by Wood that propionic acid bacteria could fix carbon dioxide led to investigationsthat have shown that practically all living organisms are capable of fixing carbon dioxide. It became possible to view carbon dioxide fixation by plants as a process similar to that occurring in other organisms and to apply to the photo-synthesis problem concepts from bacterial and animal metabolism. The second discovery of major importance was the finding by Hill that iso-lated chloroplasts, when illuminated, could liberate oxygen and reduce added oxidants. This discovery freed the plant physiologist of the notion that photosynthesis could only be studied in intact living plants.

Since 1940 many new techniques and methods have been applied to photosynthesis research. Isotope studies have been especially valuable in elucidating the path of carbon in photosynthesis. Advantage has been taken of new advances in the field of enzyme chemistry. It is the purpose of this section to outline the essential features of what is presently known about photosynthesis and to review the significance of some of the recent discoveries.

Structural Aspects of the Photosynthetic Apparatus

Photosynthesis in plants is carried on by the chloroplasts of the leaves. The distribution of the chloroplasts in the leaf of a land plant is shown in Fig. 1. The surface of the leaf is covered by a layer of waxy material called cutin. This waxy layer does not completely block the passage of gases and dissolved materials to and from the interior of the leaf, but the major exchange of gas and water vapor occurs through openings in the leaf known as stomata. Gases entering the stomata diffuse into the intercellular spaces and then come into contact with the chloroplast-containing cells.

The leaves of water plants are not covered by cutin and contain no stomata. The epidermal cells very often contain chloroplasts as do the other cells in the leaf. Carbon dioxide enters the leaf in the dissolved state and comes into intimate contact with the chloroplast-containing cells.

The chloroplasts are embedded in the cytoplasm of the cell and are one of a number of cytoplasmic particles that are found in plant cells. There is no general agreement as to the nature of these various cytoplasmic par-ticles. The term “mitochondria” is commonly used to denote one of these fractions, and a

great deal of work has been carried out on their physiological role particularly with respcet to the localization of enzymes. Comparable work with mammalian tissue has shown the mitochondrial fraction to be the site of action of a number of important enzyme systems.

The chloroplasts are small green bodies that vary somewhat in size and number per cell depending upon the species of plant and the environmental conditions under which the plant is grown. They are found in the higher plants, green algae, red algae, and brown algae, but not in blue-green algae nor the photosynthetic bacteria, where the chlorophyll appears in the form of grana. The structure and morphology of the chloroplasts have been discussed thoroughly in several recent reviews. In appearance they are discs or flat ellipsoids 3-10 μacross. The chloroplasts have a membrane, and within this membrane there may be several additional structures. The chlorophyll appears to be concentrated in bodies known as “grana” which are embedded in a colorless stroma.

The grana have been investigated by means of the electron microscope, and in spinach chloroplasts approximately forty to sixty grana were found. The grana were thin discs about 6,000 Å. in diameter and 800 Å. thick. There is some evidence that the grana themselves are made up of stacks of ten to twenty thin lamallae which are 75 to 100 Å. thick. The significance of the ultra-fine structure of the chloroplast is not thoroughly understood. If all of the chlorophyll is concentrated in the chloroplast then each granum, or each lamella in a granum, must contain several chlorophyll molecules. The close packing of the grana may then enable the light energy absorbed by one chlorophyll molecule to be passed along through a number of chlorophyll molecules until the energy reaches an active center where reduction occurs. The structural properties of the chloroplast may be very important in any physical or chemical explanation of the mechanism of photosynthesis.

With the realization that the chloroplast was the site of at least part of the photosynthetic reaction, a great deal of work was done on the isolation of the chloroplasts. Such preparations are generally contaminated with nuclear and cytoplasmic material and attempts to ascribe observed results solely to the chloroplasts should be viewed with caution. Accom-panying the isolation studies have been analyses on the chemical and en-zyme composition of the chloroplasts. On a dry-weight basis, the chloro-plast contains about 45% protein and 25% lipid. The ash content is between 5 and 15% of the dry weight while the pigments account for 5 to 10%. The balance of the chloroplast is made up of carbohydrates, enzymes, and other unidentified compounds. The composition of the chloroplast material is quite variable and depends upon the plant species as well as upon the conditions of growth, i.e., nutrition, light, water, etc.

Kinetic Studies on Photosynthesis

From a consideration of the photosynthetic equation it is apparent that the rate of photosynthesis may be determined by measuring the disap-pearance of carbon dioxide, the evolution of oxygen, or the increase in dry weight (synthesis of protoplasm). Rate studies, or reaction kinetics, have been widely utilized in studying photosynthesis, and such studies have been recently reviewed by Rabinowitch who points out: “Photo-synthesis is such a complex and heterogeneous process that it is probably impossible to make a complete analysis of its mechanism merely by meas-uring the rate of the over-all process under different conditions. However, this does not mean that kinetic measurements of photosynthesis are useless, but rather that they are most useful when combined with other biochemical and biophysical methods of approach....”

Kinetic studies indicated that photosynthesis is dependent upon a num-ber of external and internal factors. Of the external factors, carbon dioxide concentration, light, and temperature are the most important, whereas the chlorophyll concentration is probably the most important internal factor. The concentrations of various enzymes as well as certain undefined “protoplasmic factors” have also been suggested as limiting photosynthesis.

a. Carbon Dioxide Concentration

The average concentration of carbon dioxide in the air is roughly 0.03% by volume. The rate of photosynthesis increases as the carbon dioxide concentration is increased until the light intensity becomes a limiting fac-tor. If the light intensity is increased, then the rate of photosynthesis is again increased by raising the CO2 concentration. Prolonged exposure of plants to high concentrations of CO2 is generally harmful.

b. Light

Two different effects of light on photosynthesis are recognized: the quan-tity or intensity of the light and the quality or spectral distribution of the light. The rate of photosynthesis increases as the light intensity is increased from zero until some other factor becomes limiting. Light-intensity curves are characterized by a linear increase in rate of photosynthesis at low light intensities. As the light intensity increases the curve flattens out, and an increase in light intensity does not bring about a corresponding increase in the rate of photosynthesis—a situation known as light saturation. At very high light intensities photosynthesis may actually be inhibited.

Plants kept in the dark do not carry out photosynthesis but do respire, i.e., evolve carbon dioxide and take in oxygen. As the darkened plants are given increasing amounts of light, photosynthesis will increase, and carbon dioxide will be utilized and oxygen evolved. At some particular light level, called the compensation point, the gas exchange due to respiration and to photosynthesis will exactly balance each other. Above the compensation point, photosynthesis exceeds respiration. In photosynthesis research, it was generally assumed that light had no effect on respiration and that “true photosynthesis” could be calculated by subtracting respiration gas-exchange values measured on a dark control. There was no direct way of measuring the photoeffect of respiration until Brown used tracer oxygen to measure the respiratory rate. He found no evidence of photo-inhibition or photoenhancement of respiration in several strains of *Chlorella* commonly used in photosynthesis research. The conditions of the experiment were similar to those that are ordinarily used in photosynthesis research. The practice of measuring photosynthesis by applying a correction for dark respiration appears to be valid, at least for *Chlorella.*

Studies on the effect of light of different wavelengths on photosynthesis have been carried out since the essential features of the process were first discovered. In their simplest form, such experiments consisted of measuring the rate of photosynthesis in plants placed in light that had passed through different coloured filters. From these and more refined measurements, it has been shown that plants carry out photosynthesis in light varying in wavelengths between 3900 Å. and 7600 Å.

The study of the wavelength dependence of photosynthesis has been extremely valuable in determining the role of the various pigments in photosynthesis. Such studies have been carried out by comparing the action spectrum of photosynthesis with the absorption spectrum of the plant. The photosynthetic action spectrum is determined by measuring the relative rates of photosynthesis in light of different wavelengths. A compari-son of the action spectrum of photosynthesis with the absorption spectrum of *Ulva taeniata* a green alga, is shown in Fig. 2. In this figure the ac-tion spectrum is calculated in terms of equal incident quanta. The effect of this correction is to raise progressively the curve toward the blue region of the spectrum. These two curves show clearly that light is absorbed by some pigment which is then active in photosynthesis.

The nature of the fat-soluble pigment system present in *Ulva taeniata* is shown in Fig. 3. Carotene absorbs light in the blue region which is utilized in photosynthesis. Chlorophyll absorbs light in both the blue and red regions of the spectrum which is utilized in photosynthesis. Such studies with a wide variety of photosynthetic organisms have revealed that a number of different pigments may contribute to the absorption of light energy. Studies with extracted pigment systems are of limited value be-cause the structural relationships of the various pigments within the chloroplast or grana are destroyed.

c. Temperature

If no other factor is limiting, the rate of photosynthesis increases with rising temperature up to the point at which permanent damage occurs to the plant. The maximum temperature tolerated by plants varies with the species as well as with the growing conditions. The decrease in photosynthetic rate at temperatures exceeding the maximum is thought to be con-nected with protoplasmic changes (enzyme denaturation, etc.). that alter the entire metabolism of the plant.

d. Interaction of Factors

From studies on the effect of temperature, carbon dioxide concentration, and light intensity, Blackman showed that at low light intensities and high carbon dioxide concentrations the rate of photosynthesis was not sensi-tive to changes in temperature. Under these conditions the rate-limiting factor is light. At high light intensities and low carbon dioxide concentra-tions, the rate of photosynthesis is extremely sensitive to changes in the temperature. For these conditions, the rate-limiting factor is the carbon dioxide concentration. Blackman concluded that there were at least two distinctly different reactions in photosynthesis: a chemical reaction, requiring carbon dioxide and sensitive to temperature, and a photochemical reaction that is insensitive to temperature. The chemical reaction has been called the “dark reaction” or “Blackman reaction.”

A number of other pigments are found in the algae and photo-synthetic bacteria. These are summarized in Table 1. Despite the fact that different chlorophylls are scattered throughout the plant kingdom, all of the organisms that carry out photosynthesis contain chlorophyll-a (bacteriochlorophyll in the bacteria). The other pigments present are believed to absorb light energy that is eventually utilized in photosynthesis. In a study involving the determination of the absorption and fluorescence spectra of photosynthetic bacteria and algae, Duysens postulated that the various pigments pass light energy by means of inductive resonance to a pig-ment which is active in photosynthesis. The transfer of light energy is unidirectional and passes the energy with high efficiency. The following scheme of energy transfer was suggested for *Chlorella,* which contains

e. Pigments

The fact that chlorophyll is essential for photosynthesis has been known for a long time, but it was not until the work of Willstatter and co-workers in the early 1900’s that the chemical nature of chlorophyll was estab-lished. These investigators showed that the chlorophyll in green plants was made up of two components, chlorophyll-a and chlorophyll-b, with the following composition:

Chlorophyll-a C85H72O5N4Mg mol. wt. 893
Chlorophyll-b C55H70O6N4Mg mol. wt. 907

The structural formula in Fig. 4 indicates the present concept of the chemi-cal structure of chlorophyll-a (the numbering system is that of Hans Fischer). Chlorophyll-b differs from chlorophyll-a with respect to the group at position 3. The methyl group in chlorophyll-a is replaced by an aldehyde group (—CHO) in chlorophyll-b.

In addition to chlorophyll-a and -b, two other pigments are generally present in leaves: carotene, an orange pigment, and xanthophyll, a yellow chlorophyll-a and -b and some carotenoids:

Light Light Light
– – –
CarotenoidsChlorophyll-bChlorophyll-a ® Photosynthesis
– –
Heat and other Fluorescence and energy losses other losses (1)

The energy absorbed by the carotenoids was transferred with 40 to 50% efficiency to chlorophyll-b, which transferred its energy with 100% effi-ciency to chlorophyll-a. In some organisms it was necessary to postulate two types of chlorophyll-a, one that could pass its energy to photosynthesis and an “inactive”

chlorophyll-a which dissipated its energy to heat. The energy that flows into chlorophyll-a is thought to be transferred by inductive resonance to a pigment “reaction center” which participates in the dark reactions of photosynthesis.

Studies on the chemical structure of chlorophyll showed that it was closely related to heme, a pigment found in mammalian tissue. Subsequent work has indicated that a common biosynthetic pathway exists for the synthesis of porphobilinogen, a precursor to these two pigments. The steps leading to the formation of porphobilinogen were worked out by Shemin and co-workers in a series of brilliant tracer experiments.

Bacterial Photosynthesis

The discovery that certain bacteria could carry out photosynthesis opened up a new field of photosynthetic research. It was found that certain green-, red-, purple-, and brown-colored bacteria could produce organic matter from carbon dioxide upon illumination. The formation of organic matter was not accompanied by oxygen evolution. As a result of work with the green sulfur bacteria, van Niel showed that their CO_2 assimilation process was in close agreement with the following equation:

$$CO_2 + 2\,H_2S \rightarrow (CH_2O) + H_2O + 2\,S \qquad (2)$$

The similarity of this process to photosynthesis in green plants is obvious:

$$CO_2 + 2\,H_2O \rightarrow (CH_2O) + H_2O + O_2 \qquad (3)$$

van Niel then suggested a generalized formulation of photosynthesis as follows:

$$CO_2 + 2\,H_2A \rightarrow (CH_2O) + H_2O + 2A \qquad (4)$$

In this equation, H_2A represents a hydrogen donor which reduces carbon dioxide with the aid of absorbed radiation, and A is the dehydrogenated donor.

Reaction is a hydrogenation-dehydrogenation reaction which con-forms to the principles of “comparative biochemistry” set forth by the Dutch microbiologist Kluyver. This concept, as stated by van Niel “postulates as a fruitful central idea that all metabolic activities are in-trinsically similar, and that each consists of a more or less extended series of inter- or intramolecular hydrogenation-dehydrogenation reactions.” The comparative biochemistry principle has been extremely valuable in develop-ing theoretical approaches to the photosynthetic reaction.

In addition to the pigmented bacteria, some colorless bacteria are able to fix carbon dioxide in the absence of light. These colorless bacteria, known as “chemosynthetic” or “chemoautotrophic” organisms, obtain energy for assimilating and reducing CO_2 by oxidizing NH_3, H_2S, and H_2.

From a consideration of the different types of photosynthesis that are carried on by green plants, photosynthetic bacteria, and adapted algae, van Niel suggested a general mechanism involving three types of actions common to all photosynthetic organisms. The first reaction is a photochemical step in which water is decomposed to produce a reducing system and an oxidizing system. The second reaction or reactions lead ultimately to the dark reduction of CO_2 by the photochemically produced reducing system. The third series of reactions leads to the eventual dark reduction of the photochemically produced oxidizing system. These reactions involve only one photochemical step, the separation of H and O or OH from water. The reduction of carbon dioxide is a dark reaction and can proceed with energy produced from a variety of sources. Photosynthetic organisms derive energy for the reduction of CO_2 from light by way of a pigment system. The chemosynthetic organisms are able to reduce CO_2 by energy derived from the oxidation of NH_3, H_2S , H_2, or other inorganic substances. Finally some organisms use energy obtained from the oxidation of organic material to reduce carbon dioxide.

A consequence of van Niel’s formulation of photosynthesis is that the evolved oxygen should originate in the water and not from the carbon dioxide. Tracer studies with O-labeled water and carbon dioxide have been consistent with this view. In a recent review, however, Brown and Frenkel have pointed out that, although the oxygen probably originates in the water molecule, the experimental evidence on this matter is not adequate to support or deny the theory that water is the sole source of photosynthetic oxygen.

The Hill Reaction

It had been known for a long time that oxygen was evolved during the illumination of ground leaves, isolated chloroplasts, or aqueous suspensions of dried leaf powders. The amounts of oxygen evolved were small, and it remained for Hill to show that illuminated isolated chloroplasts were able to produce considerable quantities of oxygen if an aqueous extract of an acetone powder of yeast or leaves was added. Subsequently it was shown that the yeast or leaf extract could be omitted if certain ferric salts were added. With ferric iron, the reaction proceeds as follows:

$$4\ Fe^{+++} + 2\ H_2O \rightarrow 4\ Fe^{++} + 4\ H^+ + O_2 \quad (5)$$

Additional work on this reaction, commonly called the “Hill reaction,” has led to the conclusion that it probably represents the photochemical phase of photosynthesis. This reaction shares with photosynthesis the con-version of light energy into chemical energy, and the appearance of molecu-lar oxygen.

Comparative studies on the Hill reaction and photosynthesis have led to the view that photosynthesis can be separated into two main groups of reactions as shown in Fig. 5. The right leg of the figure represents the photo-chemical phase which takes place in the chloroplast. The reactions include light absorption, energy transfer, and water photolysis with the concomitant production of oxygen and “active hydrogen.” The “active hydrogen drives the reaction represented on the left side of the figure, the final out-come being reduced carbon dioxide. The key reaction takes place in the chloroplast or grana where the energy from light is converted into chemical energy represented by “active hydrogen.”

The nature of the “active hydrogen” or the mechanism of its production is not known. A widely accepted theory of how light energy is transformed into chemical energy is that a “biradical” is formed from a molecule following light absorption. Such a biradical might have both oxidizing and reducing properties so that the result of such a compound XY absorbing light energy would be the formation of two radicals, XH and YOH. The mechanisms for the further reactions of these two radicals are not known. The XH reactions might be similar to other hydrogen transport reactions in metabolic systems.

The transfer of hydrogen or electrons in biological systems is relatively well understood, and it is of interest to examine some of the compounds involved in the transfer mechanism. Table 2 lists some of the compounds that might serve in the hydrogen transport system of the photosynthetic cell. Since it will be necessary to refer many times to these compounds, their structure is given at this point. The names of these compounds have not yet been systematized. Diphosphopyridine nucleotide (DPN+) contains nicotinic acid amide, D-ribose, and adenosine 5-phosphate linked through a pyrophosphate group. Triphosphopyridine nucleotide (TPN+, coenzyme II) contains a third phosphate group.

In biological oxidations and reductions the reaction can be represented as follows:

$$XH_2 + Y \rightarrow X + YH_2 \quad (6)$$

The hydrogen donor XH_2 is oxidized to X while the hydrogen acceptor Y isreduced to YH_2, the reaction being catalyzed by enzymes known as dehydrogenases. In some instances oxygen acts as the hydrogen acceptor, but in many other systems organic hydrogen acceptors or enzymes pass the hydrogen from the hydrogen donor to the terminal hydrogen acceptor. The pyridine nucleotides act in this hydrogen transport role, probably by the following mechanism:

It is likely that the hydrogen from XH_2 dissociates into a hydride ion H^- ($=H^+ + 2e^-$)and a proton, H^+. The oxidized diphosphopyridine nucleotide then combines with the hydride ion leaving the proton in solution as hydrogen ion. These reactions can be summarized as follows:

$$DPN^+ + 2\ H^+ + 2\ e^- \rightarrow DPNH + H^+ \quad (7)$$

$$TPN^+ + 2\ H^+ + 2\ e^- \rightarrow TPNH + H^+$$

In the rest of the chapter DPN+ (TPN+) will be used to denote the oxidized forms of the pyridine nucleotides and DPNH (TPNH) to designate the reduced forms.

Under physiological conditions the reduced pyridine nucleotides arereoxidized by the flavoproteins. One

such flavoprotein, flavin adenine dinucleotide, has the following structure:
The flavin component is riboflavin (vitamin B2) or 6, 7-dimethyl-9-D-ribitylisoalloxazine.
Lipothiamide pyrophosphate (LTPP) is a compound postulated by Reed to account for the biological activity of a-lipoic acid. It is not certain that such a compound does exit; however, a-lipoic acid and thiamine pyrophosphate do act in oxidation-reduction reactions.
Attempts were made to demonstrate that DPN+ or TPN+ could be reduced under conditions found in the Hill reaction. Such a reaction would occur as follows:

$$H_2O + DPN^+ (TPN^+) \rightarrow \tfrac{1}{2}O_2 + DPNH (TPNH) \quad (8)$$

These efforts were not successful until it was shown by Ochoa and Vishniae that TPNH or DPNH could be demonstrated if the reaction was coupled with a second reaction that would remove the reduced pyridine nucleotides from the system. In the presence of the “malic enzyme,” pyruvate, CO_2 , and Mn^{++}, the following reactions took place:

$$H_2O + TPN^+ \rightarrow \tfrac{1}{2}O_2 + TPNH \quad (9)$$

$$TPNH + \text{pyruvate} + CO_2 \rightarrow TPN^+ + \text{L-malate} \quad (10)$$

The reaction was carried out with a suspension of chloroplasts from spinach leaves and “malic enzyme” from a pigeon-liver preparation. The reaction was followed by measuring the production of L-malic acid. In the dark, or in the absence of either the “malic enzyme” or TPN+, no malic acid was observed. These results were confirmed by both Tolmach. The experiments of Arnon were of interest since the “malic enzyme” was obtained from the same plant material that served as the source of the chloroplasts.
Subsequent experiments have shown that illuminated chloroplasts are able to carry out a great many reactions involving DPN+ and TPN+. These studies demonstrated that the photochemical system of green leaves could be linked to the glycolytic and respiratory systems of plants, but they did not prove that either DPN+ or TPN+ was directly reduced by light or that carbon dioxide was directly reduced by DPNH or TPNH. Anderson found DPN+ and TPN+ present in green leaves in about equal amounts. The concentrations were such as would give about a 50% activation of the leaf TPN+- or DPN+-linked dehydrogenases.
The possibility that some of the energy derived from light might be stored in the form of “high-energy” phosphate bonds has been considered many times. One such compound of widespread occurrence in biological systems is adenosine triphosphate (ATP). The ATP molecule contains two “high-energy” bonds (designated by ~), each of which on hydrolysis has a DFvalue of about –12,000 cal. per mole. The energy released can be used in many different biosynthetic processes. Vishniac and Ochoa demonstrated that the photochemical reduction of DPN+ could be linked to oxidative phosphorylation and the formation of ATP. When a mitochondrial preparation from mung bean seedlings was incubated with a spinach chloroplast preparation, ATP, DPN+, and P32- labeled phosphate, the radioactivity incorporated into ATP was increased fifteenfold upon illumination.

$$H_2O + DPN^+ \rightarrow \tfrac{1}{2}O_2 + DPNH \quad (11)$$

$$DPNH + \tfrac{1}{2}O_2 + 3\,ADP + 3PO_4^{---} \rightarrow DPN^+ + H_2O + 3\,ATP \quad (12)$$

$$\text{Net reaction: } 3\,ADP + 3\,PO_4^{---} \rightarrow 3\,ATP \quad (13)$$

The ADP in the above reaction indicates adenosine diphosphate, which has the same structure as ATP but which has one less terminal phosphate group. By an entirely different procedure, Strehler has been able to show that the ATP content of illuminated *Chlorella* is markedly increased following an anaerobic period in the dark. There seems to be little doubt that the production of “high-energy” phosphate bonds is connected to the photochemical phase of photosynthesis, but the mechanism of the reactions involved is not well understood at the present time.
An entirely different sort of mechanism for the photochemical step in photosynthesis was suggested by Calvin. It had been observed that when algae in a steady state of photosynthesis were fed radio-active

carbon dioxide, the radioactivity could not be found in those products characteristic of the tricarboxylic acid cycle. If the algae were allowed to undergo photosynthesis for a short time in the pres-ence of radioactive carbon dioxide and then placed in the dark, the radio-active carbon was found to appear in the members of the tricarboxylic acid cycle. These results were interpreted in terms of the reactions known to be necessary for pyruvic acid to enter into the tricarboxylic acid cycle. The pyruvic acid is oxidatively decarboxylated to yield acetyl-coenzyme A and CO2. Acetyl-coenzyme A then enters the tricarboxylic acid cycle by con-densing with oxalacetic acid.

The decarboxylation of pyruvic acid involves the participation of at least five cofactors, thiamine pyrophosphate (TPP), thioctic acid, coen-zyme A, DPN+, and Mg ions. According to Gunsalus the formation of acetylcoenzyme A from pyruvic acid can be expressed

by the reactions. The thioctic acid is designated as R

inasmuch as the two sulfurs are the reactive sites of the molecule. Calvin and Barltrop interpreted their tracer studies as indicating

that light reduced the disulfide R to the dithiol R. In the

dithiol form, thioctic acid could not react with pyruvic acid to form acetyl-CoA. Such a reaction would prevent the pyruvic acid from entering the tricarboxylic acid cycle. In the dark, the dithiol would not be formed, and the pyruvic acid could enter the tricarboxylic acid cycle. Calvin and Barltrop also suggested that the thioctic acid might be involved in the primary quantum conversion act of photosynthesis by the following reaction:

(14)

(15)

(16)

(17)

(18)

(19)

Additional studies by Calvin have given results that are consistent with this concept.

Thioctic acid has been found in green plants but its biological form and distribution are not well established. Using S35-labeled thioctic acid it was shown that *Scenedesmus* cells rapidly incorporated the thioctic acid into some bound form. In *Chlorella,* the thioctic acid became associated with a lipid fraction of the chloroplasts. Wessels found that sulfhydryl enzyme inhibitors did not affect the Hill reaction. If thioctic acid is involved in photosynthesis, it might be expected that such inhibitors would influence the reaction. However, since little information is available re-garding the distribution and localization of thioctic acid in the plant cell, it is not possible to say much about its role in photosynthesis.

Nicotinic Acid—Nicotinamide

Nomenclature and Survey

Names

Nicotinic acid and nicotinamide.

Niacin: synonym for nicotinic acid.

Niacin amide: synonym for nicotinamide.

P.P. factor: Pellagra Preventive factor.

Pellagramine.

Niamid: Suggested abbreviation for nicotinamide.

Chemical formulas

Chemical names

Pyridine-3-carboxylic acid and acid-amide.

Pyridine-b-carboxylic acid and acid-amide.

Empirical formulas

Nicotinic acid: C6H502N.

Nicotinamide: C6H6ON2.

Nicotinic acid may possibly be identical with vitamin B5. The term vitamin B5 was given originally to a fuller's earth eluate fraction from yeast and was shown to be necessary for the growth of pigeons. Vitamin B6 was later found to be present in vitamin B5 fractions but could not fully replace the vitamin B5 requirements of pigeons. The remaining factor, designated as vitamin B5, appears to be identical with nicotinic acid.

Occurrence of Nicotinic Acid and of Nicotinamide

Nicotinic acid occurs in all living cells in small amounts. The liver, the adrenal gland and yeast and wheat germs are especially rich in nicotinic acid. The eye lenses contain a fair amount and corn meal, corn syrup, alfalfa, fatty meat and milk contain small amounts.

Free nicotinic acid apparently does not occur in the living organism but is found in the urine of animals. Nicotinic acid occurs in tissues in the form of its amide. Besides the occurrence of the free nicotinamide, there exists a number of enzyme systems in which nicotin-amide is chemically bound. Nicotinamide occurs to a much greater extent in the bound form than as the free nicotinamide. Thus, in rats, bound nicotinamide occurs, for example, in the liver, kidney and muscles, while free nicotinic acid (or its amide) has been found only in the liver.

Isolation of Nicotinic Acid and of Nicotinamide

The isolation of nicotinic acid from natural sources is a relatively simple matter. Tissue material is freed from fats by extraction with organic solvents. The remaining material is saponified, preferably with alkali. The acid fraction of the saponification mass contains the nicotinic acid, which can be separated as such, as the ester or as the Cu-salt. From this salt, the free acid is obtained by means of hydrogen sulfide.

The isolation of the total nicotinamide from animals or plants is carried out by water extraction of the material, followed by partial hydrolysis in 0.1 *N* sulfuric acid in order to split the nicotinamide from its chemical combination with the various enzymes. The water phase is then ex-tracted with butanol or chloroform. The chloroform solution is sub-jected to fractional distillation. Nicotinamide distills at 150-160°C. at 5 × 10–4 mm. Hg. The distillate may be recrystallized from chloroform and benzene.

The separation of free nicotinic acid from nicotinamide is effected by solvent extraction (ether, chloroform, butanol) of a water solution. The amide is soluble in organic solvents, while the free acid remains in the Water phase.

The separation of free nicotinamide from chemically bound nicotin-amide (coenzymes) must be carried out immediately after death, and is usually achieved by an acetone extraction of the material. Acetone dissolves nicotinamide, but does not dissolve the coenzymes. Acetone, furthermore, prevents the naturally occurring enzyme systems from splitting the nicotinamide-containing coenzymes into their components.

Properties of Nicotinic Acid and of Nicotinamide

1. Nicotinic acid crystallizes in needles from water or alcohol and melts at 235.5-236.6°C. It sublimes without decomposition. Nicotinic acid exhibits a typical absorption spectrum with a maximum at 385 mμ.

2. Nicotinamide crystallizes in needles from benzene and melts at 129°C. It distills at 150-160°C. and 5 × 10–4 mm. Hg. The absorp-tion spectrum of nicotinamide is shown in Fig. 12.

Constitution of Nicotinic Acid and of Nicotinamide

The constitution of nicotinic acid was determined when this acid was first obtained by oxidation of nicotine, from which the name of this acid originates. The acid character was established by the formation of a silver and a copper salt and by the formation of various derivatives such as esters, acid chloride, etc. The basic character was recognized by the formation of crystallized salts such as the hydrochloride, the hydrobromide, etc. By distillation of the calcium salt of the acid, the carboxylic acid group is split off and

pyridine is obtained.

The *m*- or 3-position of the carboxylic acid group in reference to the ring nitrogen was suspected by Skraup who investigated the physical constants and the decarboxylation of the three possible pyridine-mono-carboxylic acids: picolinic acid (o- or 1,2), nicotinic acid (m- or 1,3-) and g-pyridine-carboxylic acid *(p*- or 1,4-position). The m-position was proved to be the correct one upon oxidation of the synthetically prepared 3-phenyl-pyridine, the constitution of which is beyond doubt. 3-Phenyl-pyridine was prepared from b-naphtho-quinoline, which in turn was synthesized from b-naphthylamine and glycerine. b-Naphtho-quinoline yields, upon oxidation with permanganate, b-phenyl-pyridine-dicarboxylic acid, which by stepwise decarboxylation yields the mono-carboxylic acid and finally 3-phenyl-pyridine. A further proof for the 3-position of the carboxylic acid group in nicotinic acid is its formation from the synthetically prepared m-dipyridyl.

Synthesis

(a) *Nicotinic Acid*

1. By Oxidation of Nicotine. This is the method by which nicotinic acid was discovered. The oxidation may be accomplished by fuming nitric acid by chromic acid or by permanganate.

2. By Oxidation of b-Pyridines. This method is really a generaliza-tion of the method first discussed. b-Picoline, 3-ethyl-pyridine, 3-phenyl-pyridine, 3,3'-dipyridyl and similar compounds have been con-verted by this method into nicotinic acid.

3. By Decomposition of Pyridine-poly-carboxylic Acids. Any pyridine-poly-carboxylic acid, which has one carboxylic acid group in *3*-position can be converted into the 3-mono-carboxylic acid by thermal decomposition or by acidic decomposition. An exception to this ruleis the 3,4-dicarboxylic acid, which decarboxylates only under very drastic conditions, yielding pyridine-4-carboxylic acid.

4. By Synthesis from Pyridine. This is a total synthesis of nicotinic acid. Pyridine is sulfonated with fuming sulfuric acid, yielding the 3-sulfonic acid. By distillation of its sodium salt with potassium cyanide, nicotinic acid nitrile is obtained.

Considerably better yields are obtained by first brominating pyridine in the 3-position, followed by conversion into 3-cyano-pyridine by means of cuprous cyanide. Saponification of the nicotinonitrile yields nicotinic acid.

(b) Nicotinamide

1. By Amidation of Nicotinic Acid. Nicotinamide is obtained by passing ammonia gas into nicotinic acid at 230°C.

2. By Amidation of Esters of Nicotinic Acid. The methyl- or ethyl-ester of nicotinic acid yields nicotinamide on reaction with aqueous or, better, alcoholic ammonia.

Industrial Methods of Preparing Nicotinic Acid and Nicotinamide

Nicotinic acid and its amide are prepared according to the methods described before. The nitric acid oxidation of nicotine, a relatively cheap alkaloid obtained as a by-product from tobacco, is of special interest. Alternative procedures are permanganate oxidations of b-picoline or quinoline. For technical purposes the oxidation of the fraction of bases from coal tar or petroleum which distills between 135° and 142°C or the oxidation of the corresponding fraction of bone tar oil has been considered.

A number of N-substituted nicotinamide derivatives have attracted commercial interest because of their clinical use in the treatment of shock, collapse and cardiac decompensation. The most useful compound of these derivatives is the N-diethyl-nicotinamide.

Biogenesis of Nicotinic Acid

The biogenesis of nicotinic acid is not known with certainty, but is believed to be linked with the amino-acid metabolism. It has been suggested that nicotinic acid may originate from ornithine or proline. The first reaction product would be d-amino-valeric acid. This reaction is common for bacteria but has not been

proved for plants. The further course of the suggested nicotinic acid synthesis is indicated in the following scheme:

Enzyme Systems Containing Nicotinamide

All plant and animal cells contain among their enzyme systems certain dehydrogenases which transport hydrogen and take care of a number of different dehydrogenation reactions. The classical conception is that each of these enzymes or, better, holoenzymes consists of an apoenzyme and a coenzyme. The apoenzyme, which is believed to have no catalytic properties of its own, is the protein bearer of the coenzyme, which is regarded as the prosthetic group of the protein. Another view is that the protein is the enzyme itself and that the coenzyme merely acts as a specific substrate to accept hydrogen. There are two coenzymes known of this class of dehydrogenases, namely, codehydrogenases I and II, which are also called, coenzymes I and II. The number of apoenzymes which combine with these two coenzymes is considerably greater. It is believed that the two coenzymes need different apoenzymes for their action as oxidizing and as reducing agents. Specific proteins are used for each substrate and a specific protein may in special cases dehydrogenate the same substrate with different coenzymes.

Table 1 summarizes the better known dehydrogenation reactions in which the codehydrogenases participate. It is evident that these co-enzymes are involved in a wide variety of reactions. There are quite probably other such reactions, which have not been thoroughly investi-gated. It has, for example, been postulated that the oxidation of cysteine to cysteine involves the participation of a codehydrogenase.

During the course of the dehydrogenation reactions indicated in the table, the coenzymes are reduced to dihydro-compounds. The reverse reaction, the oxidation of the dihydro-codehydrogenases to the code-hydrogenases, is carried out in the presence of different apoenzymes as previously stated. Principally all dehydrogenation reactions are reversible, although in living tissues usually no such equilibrium occurs due to the fact that the reaction products do not accumulate but undergo further reaction. Practically, the equilibrium can be demonstrated in many cases, such as in the system involving alcohol and acetaldehyde and is indicated in the table above for those systems for which the reversible reaction has been experimentally demonstrated. Equilibrium constants have been determined for the coenzyme I and for many of the reactions catalyzed by the coenzymes and are usually expressed in the form of the oxidation-reduction potential. The potential E0'for the coenzyme I at 30°C. is approximately –0.29 volts.

In tissues, the oxidation of the dihydro-coenzymes may be accomplished by coenzyme-linked reactions. It is, for example possible that b-hydroxy-butyrate is oxidized to acetoacetate with the formation of dihydro-cozymase I which in turn reduces an aldehyde to an alcohol. Thus, the following compounds have been shown to act as acceptors in the presence of the corresponding apoenzymes; acetaldehyde, pyruvate, oxaloacetate, triose-phosphate, imino-glutarate (or a-keto-glutarate + NH3) and fumarate (in the presence of succinic-dehydrogenase). It has also been shown that the riboflavin-containing enzyme systems may be linked with the oxidation of the dihydro-codehydrogenases.

Coenzymes Containing Nicotinamide

(a) Codehydrogenase I

Synonyms. Codehydrogenase I, Coenzyme I, Cozymase, Coferment I, Diphosphopyridine nucleotide, Coferment of fermentation, Coreductase, Factor V.

Occurrence. Codehydrogenase I has been found in all animal and plant cells in which carbohydrates are metabolized. Yeast and red blood cells are especially rich sources, and some muscles, for example, heart muscles, contain relatively high amounts. In fresh yeast about 0.5 g. of codehydrogenase I is present per kilogram and in the heart muscle of rabbits 0.4 g. per kilogram. The same amount (0.1-0.4 g.) has been calculated to be present in muscles of man and of invertebrata. Code-hydrogenase I occurs also in microorganisms and has, for example, been obtained from *Azotobacter chroococcum.* There seems to be a fairly constant ratio of coenzyme to dihydro-coenzyme in the muscles of all animals, the reduced form being

present in about 35-45% of the total amount. An increased amount of the reduced form has been found in Jensen sarcoma.

Isolation. The isolation of codehydrogenase I is carried out by water extraction of the source, for example, of yeast or red blood cells. It is necessary to destroy some of the other enzymes present prior to the extraction, by short heating to about 80°C, since otherwise the code-hydrogenase is rapidly destroyed. After filtration or dialysis some of the protein impurities are removed by precipitation with lead acetate. The coenzyme itself may be extracted with phenol and is precipitated by mercuric acetate or nitrate, picric acid, phosphotungstic or silicotungstic acid (and decomposed by ether-amyl-alcohol-sulfuric acid), by silver salts either in ammoniacal solution or in a solution containing barium hydroxide (and freed from silver by hydrogen sulfide), by cuprous halides in the presence of hydrochloric acid (and freed from copper by hydrogen sulfide), by ethyl-acetate from an acidified methanol solution, by acetone, and by alcohol. The latter is also used for fractional precipitation of this coenzyme. Purification may also be accomplished by fractional adsorp-tion on aluminum oxide or charcoal from weakly acid solutions.

The methods used for the separation of nicotinamide from the coenzymes have been described in the section on the isolation of nicotinamide. The methods used for the separation of codehydrogenase I from codehydrogenase II will be found in the section on the isolation of code-hydrogenase II. To separate the flavin-adenine-dinucleotides which occur together with the codehydrogenases I and II in the phenol extract from certain sources such as yeast, the flavin com-pounds are precipitated in acid solution as silver salts.

Properties. Codehydrogenase I is a colorless, water-soluble sub-stance, and is insoluble in most organic solvents. It exhibits a character-istic absorption spectrum with a maximum at 260 mμ. Hydrogenation to the dihydro-codehydrogenase, which occurs during the enzyme action changes the absorption spectrum characteristically with the appearance of an additional band at 320-360 mμ with a maximum at 340 mμ. While the codehydrogenase I shows no fluorescence, the dihydro-compound exhibits a strong whitish fluorescence upon irradiation with ultra-violet light.

Codehydrogenase I is optically active, the specific rotation being ap-proximately –20° for the red cadmium line (643.9 mμ) and –70° for the green mercury line (546 mμ).

Codehydrogenase I is quite stable in acid solution at moderate temperatures, whereas the dihydro-codehydrogenase is destroyed by acids. In alkaline solution, codehydrogenase is rapidly destroyed, whereas the dihydro-codehydrogenase remains unchanged when heated for 30 minutes to 100°C. in 0.1 *N* sodium hydroxide solution. Codehydrogenase is inactivated by ultraviolet light.

Constitution. Codehydrogenase I is water-soluble and relatively stable against oxidation, for example, against hydrogen peroxide, but is attacked by this oxidizing agent in the presence of various catalysts, such as iron, etc.

Codehydrogenase I has the empirical formula C21H27N7O14P2. It is a dinucleotide which yields on hydrolysis adenine, nicotinic acid amide and two mols of d-ribose-phosphoric acid. The phosphoric acid is linked to the ribose in 5-position, since no formaldehyde is obtained by oxidation of the ribose-phosphoric acid with periodic acid. Alkaline hydrolysis of codehydrogenase I yields adenosine-diphosphoric acid, which proves the existence of a pyrophosphate linkage in the coenzyme molecule. Cozymase titrates as a monobasic acid, the naturally oc-curring dihydro-cozymase as a dibasic acid. These experimental results suggest that one of the free hydroxyl groups of the phosphoric acid parts of the molecule is linked to the pyridinium nitrogen atom. Euler and Schenk suggested the following formula for codehydrogenase I:

Synthesis. No clear-cut chemical synthesis of codehydrogenase I has been reported. It has, however, been observed that certain blood constituents are able to convert *in vitro* nicotinic acid and nicotinamide into both coenzymes I and II. It was first assumed that normal erythro-cytes accomplish this synthesis, but it was shown subsequently that nucleated cells, that is, the white cells of, for example, the lymphoid and myeloid series, must be held responsible for the synthesis of these co-enzymes.

There is also a definite indication of an enzymatic synthesis of code-hydrogenase I from codehydrogenase II.

Determination by Physical Methods. *1. Absorption spectrum:* While codehydrogenase exhibits a typical maximum at 260 mμ, which might be used for its determination, the typical band at 340 mμwhich appears only when the coenzyme is in the dihydro form, is much more useful. Codehydrogenase II gives the same absorption spectra. The spectro-photometric method therefore cannot be used to differentiate between the two coenzymes.

2. Fluorescence: The fluorescence of the dihydro-codehydrogenase has successfully been used for following the oxidation-reduction of this coenzyme. The fluorescence is acid sensitive.

Determination by Chemical Methods. Codehydrogenase can be determined by the same chemical methods which are used for the determination of nicotinic acid. These methods, however, do not differentiate the nicotinic acid from the coenzymes. The state of equilibrium of codehydrogenase with its reduced form in active enzyme systems can be analyzed by acidification which destroys the dihydro-compound but leaves the codehydrogenase itself untouched. The latter is then determined by any of the known methods. On the other hand, the dihydro-compound can be deter-mined alone by making the solution containing the reduced and the non-reduced form alkaline whereby the codehydrogenase I is destroyed. Another chemical method which has been suggested for the determination of codehydrogenase is the determination of the total hydrogen consump-tion upon catalytic hydrogenation in the presence of sodium borate.

Determination by Biochemical Methods. Codehydrogenase I and its dihydro-form are usually determined by the degree of activation which they exert on fermentation in the presence of an excess of the apodehydrogenase. The criterion is the amount of CO2 evolved under specified conditions which is proportional to the codehydrogenase I con-centration. This method is somewhat unreliable due to the fact that the purity of the apoenzyme is not standardized. This method is specific for codehydrogenase I and codehydrogenase II. Nicotinic acid and its amide do not respond to this test.

Another biochemical assay procedure for codehydrogenase I is based upon the fact that the oxidation of lactic acid by animal tissues requires the presence of codehydrogenase I.

Determination by Biological Methods. *Bacillus influenzae* can be used to measure accurately the total content of codehydrogenases in blood and of yeast (and probably of other sources), since this bacillus cannot synthesize the codehydrogenases from their constituents, but needs the coenzymes for proper development.

Standard. One unit of codehydrogenase I is defined as that quantity which produces 1 cc. of carbon dioxide in a normal fermentation under specified conditions.

(b) Codehydrogenase II

Synonyms. Triphosphopyridine nucleotide, Warburg's Coferment, Respiratory coenzyme, Growth factor V.

Occurrence. Codehydrogenase II seems, like codehydrogenase I, to occur in practically all living cells. These apparently have the power of synthesizing both codehydrogenases from nicotinic acid. It has also been postulated that the living cell is able to convert codehydrogenase I into codehydrogenase II. It seems plausible, therefore, that both coenzymes are found together. It is noteworthy that the ratio of the amounts of the two coenzymes may vary considerably in different sources. While yeast contains very little code-hydrogenase II, animal tissue contains as much as 40-80 gper gram.

Isolation. The isolation of codehydrogenase II is carried out, for example, from washed red blood cells, by destruction of the cell structure, followed by a combination of various precipitation reactions. Code-hydrogenase II is precipitated from a water solution by acetone, by ethyl-acetate, especially from a methanol-HCl solution, by mercuric acetate, by barium salts, by lead salts, etc.

Separation of Codehydrogenase I from Codehydrogenase II. The following methods have been recommended for the separation of the two coenzymes:

1. Codehydrogenase I is separated as the cuprous salt whereby code-hydrogenase II remains in solution and can be isolated separately.
2. Codehydrogenase II is precipitated by lead acetate, codehydro-genase I is not.
3. Codehydrogenase II is more strongly adsorbed on aluminum oxide than codehydrogenase I. The codehydrogenase II is eluted from the Al_2O_3 by KH_2PO_4 solutions.
4. The barium salts of the two codehydrogenases can be separated by fractional precipitation with alcohol.
Properties. The properties of codehydrogenase II resemble very closely those of codehydrogenase I. They are colorless, water-soluble compounds which are insoluble in organic solvents. Codehydrogenase II is soluble in organic solvents in the presence of hydrochloric acid, for example, in methanol-HCl. Codehydrogenase II exhibits the same characteristic absorption band at 260 mμas does codehydrogenase I. The maximum of the typical dihydro-codehydrogenase absorption is at 340 mμ. The latter compound also shows the characteristic fluorescence when irradiated with ultraviolet light. This destroys both codehydrogenases rap-idly. Codehydrogenase II is unstable in alkaline solution, but stable in acid solution. In isolated muscle tissue codehydrogenase II is rapidly inactivated. Codehydrogenase II is optically active: [a]589mμ = –24.6º, [a]546mμ = –29.4°.
Constitution. Codehydrogenase II has not yet been isolated in the pure form. The probable empirical formula is $C_{21}H_{28}N_7O_{17}P_3$. This corresponds to 1 mol adenine, 1 mol nicotinamide, 2 mols pentose (probably d-ribose) and 3 mols phosphoric acid. Codehydrogenase II seems thus to differ from codehydrogenase I only by one additional phosphoric acid group. Adenine and nicotinamide have been isolated from the break-down products of this coenzyme. Codehydrogenase II is dibasic since the results of electrophoresis determinations established two different dissociation constants: pK_1= 1.8 and pk_2 = 6.1. The following formula for codehydrogenase II has tentatively been suggested:
No definite proof for this structure has as yet been obtained. As a matter of fact, this structure seems doubtful in view of the fact that an apparent synthesis of codehydrogenase II from codehydrogenase I by two different methods has been accomplished. Since these synthetic methods consist in the addition of one mol of phosphoric acid to codehydrogenase I, it would appear that the proposed formula for the coenzyme II containing three phosphoric acid groups in one chain is rather improbable. It may be that one of the phosphoric acid radicals is attached to the molecule of codehydrogenase I as a side chain, thus forming the molecule of codehydrogenase II. It is noteworthy that code-hydrogenase II has apparently no free amino-group since it does not react with nitrite.
Synthesis. It has already been pointed out in the section on Syn-thesis of Codehydrogenase I that a partial synthesis of code-hydrogenase II can be accomplished from nicotinic acid or nicotinamide by the action of nucleated cells *in vitro.*
Codehydrogenase II can apparently be synthesized from codehydrogenase I, since the product obtained shows the same properties as does codehydrogenase II in the test for dehydrogenating Robison ester. Ultimate proof for the accomplished conversion is still lacking. This assumed conversion of codehydrogenase I into codehydrogenase II has been carried out (1) by means of phosphorus oxychloride in ether (2) by enzymatic phosphorylation.
Determination by Physical and Chemical Methods. The same Methods which were described for the determination of codehydrogenase I can also be applied to codehydrogenase II. There is no physical or chemical method of differentiating between these two coenzymes.
Determination by Biochemical Methods. 1. Codehydrogenase II and its dihydro-form are usually determined according to the Warburg technic by comparison with a standard preparation of this coenzyme in a system which dehydrogenates hexose-monophosphoric acid. In addition to the coenzyme, the specific apoenzyme and the “yellow ferment” are also necessary.
Determination by Biological Methods. Codehydrogenase II can be determined by the growth test

with *Haemophilus parainfluenzae* as described for the determination of codehydrogenase I.

Mechanism of the Nicotinamide Coenzyme Action

It has previously been stated that the action of the various nicotinamide containing enzyme systems consists in the dehydrogenation of various substrates. During this reaction, the codehydrogenase absorbs two atoms of hydrogen, thus being reduced to a dihydro-form. The dihydro codehydrogenase is in turn oxidized, and thus reconverted into the code-hydrogenase. The nicotinamide part of the molecule is responsible for the phenomenon of the reversible reduction-oxidation reaction of the codehydrogenases. *In vitro,* this reduction to the dihydro-codehydrogenases can be brought about by the action of sodium hydrosulfite, $Na_2S_2O_4$ in weakly alkaline solution. In order to study the mechanism of this reaction and the constitution of the reduced and oxidized forms of the coenzymes, the reversible reduction of a number of simple nicotinamide derivatives has been examined.It was found that only those derivative which have a pentavalent ring-nitrogen yield dihydro-compounds with the same characteristic absorption spectrum as the dihydro-coenzyme. Upon reduction, the nitrogen becomes trivalent. Nicotinamide-iodomethylate proved to be the most characteristic model substance:

The dihydro-nicotinamide compounds, but not the corresponding unreduced molecules, exhibit a typical whitish fluorescence upon irradiation with ultraviolet light. The dihydro-compounds show an additional typical absorption band in the ultraviolet with a maximum at 340 mμ.By comparison of these characteristics with known dihydro-pyridine compounds, it has been concluded that the constitution of the dihydro-compound is that of an 0-dihydro-compound. Additional evidence is furnished by the deep yellow color of the dihydro-compound, which would be improbable for a p-dihydro-compound. The results of these experiments indicate that in the natural codehydrogenases the ring-nitrogen of nicotin-amide is chemically bound in such a way that upon reduction a tertiary nitrogen is formed, The formula of the codehydrogenases is therefore written in the form of a quaternary pyridinium salt, which in the dihydro-form possesses an additional acidic group:

The exact position of the reduced double bond in relation to the caxboxylic acid-amide group in the dihydro-nicotinamide is still unknown. It is generally assumed that the double bond in a-b-position is reduced; the g,d-position, however, cannot be excluded on the basis of the present knowledge.

Specificity of Nicotinic Acid and Nicotinamide

The compounds of this group which are known to be effective when given orally are nicotinic acid, its salts, for example the sodium salt nico-tinamide, the N-methyl-amide and the N-diethyl-amide of nicotinic acid, ethyl-nicotinate, nicotinamide-glucosido-iodide and nicotinuric acid. It is assumed, as would be expected on a chemical basis, that all these compounds are converted in the organism into nicotinamide. Since a number of other N-substituted nicotinamides have been found quite effective in the treatment of heart weakness, it is suspected that these derivatives will also prove effective as vitamins.

The ring-nitrogen apparently must be unsubstituted for the exhibition of vitamin activity, since trigonelline, the nicotinic acid-methyl-betaine is inactive. This is easily understood, since in enzyme systems the ring-nitrogen is attached to the rest of the enzyme molecule.

Quinolinic acid, pyrazine-carboxylic acid and pyrazine-2,3-dicarboxylic acid are active in curing human pellagra (500-800 mg. daily *per os)* and are said not to produce the vasodilator symptoms which often follow the administration of nicotinic acid. Dysentery bacilli can use thiazole-5-carboxylic acid as a

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