THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

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INTRODUCTION

When invited to present this lecture I was assured that this subject would provide a unique opportunity for perspectives. Not only was this correct, but this subject has been a unique trial for decisions on which products to exclude and include; I have excluded antibiotics, but pay tribute to their vast importance in medicine.

As a policy, contributions directly or indirectly from the chemistry of natural products are interpreted broadly, and I decided to minimize historical aspects and to maximize recent and current aspects of chemistry and medicine. Because most of us are interested in the future, I have included trends and medical objectives of great significance for new challenge.

I should like to give first priority to detailing the contribution of Swedish chemists to recognition of the impact of natural product chemistry on medicine.

CONTRIBUTIONS FROM SWEDEN

It is evident that the pharmaceutical industry bridges the basic chemistry and the medical use of drugs and makes its own vital scientific contributions. The Swedish pharmaceutical industry also fulfils this commendable role for world health. There would be few, if any, new drugs in medicine today without this industry.

Research by Jorpes of the Karolinska Institute advanced Heparin, a mucopolysaccharide, and Vitrum introduced it for medical use. Up to 25 000 patients are treated with Heparin for thromboembolic diseases in Sweden each year. After its introduction, not only in Sweden but in other countries, the mortality of patients suffering from thromboembolic diseases has dropped from 15 per cent to 0.5–1.0 per cent. Heparin has also greatly lessened postthrombotic sequela and has benefited patients in operations with the heart–lung machine.

The researches of von Euler, Nilsson-Ehle, and Erdtman on a compound, later named gramine, and found in x-ray mutants of barley, led to the synthesis of 2-(dimethylaminomethyl)-indole, which differed from gramine by being isomeric and, surprisingly having local anaesthetic activity. Löfgren and Goldberg, aided by the serendipity, continued the research, and Astra ultimately marketed Xylocaine. It is estimated that over one-half million injections of Xylocaine are given daily in approximately eighty countries of

the world. Xylocaine has been considered the world's leading local anaesthetic.

Lehmann of Sahlgren's Hospital in Gothenburg recognized that salicylic acid had an interesting effect on the metabolism of the tubercle bacillus. Then, he studied a larger number of related compounds for inhibition of the growth of the tubercle bacillus. *p*-Aminosalicylic acid, known as PAS, had promising inhibitory effects on experimental tuberculosis. With the contribution from Ferrosan, PAS became, and still is, one of the important drugs in the treatment of tuberculosis. It is estimated that the world production of PAS is about three million kilograms per year. Historically, the first successful test with PAS in clinical tuberculosis was made before the first clinical trials with streptomycin.

Dextran, which consists of polysaccharides, stemmed from the research of Grönwall and Ingelman of Uppsala and was introduced by Pharmacia into medicine. These polysaccharides were the first acceptable plasma substitutes, and their medical significance, especially in disasters and wars, is obvious. The number of patients treated with some form of Dextran approximates one million a year and usage is increasing. Many such patients could not have been saved at all before the availability of Dextran.

Kabi has succeeded in producing Streptokinase of high purity which is another step forward in the medical treatment of thrombosis. In appraising the medical potential of Streptokinase, one may estimate that a hospital of about 1000 beds might have a total of about 300 thromboembolic cases per year, including acute arterial occlusions, deep venous thrombosis, and myocardial infarctions, all of which could be eligible for Streptokinase treatment.

Biochemistry in Sweden is world renowned and will contribute to future medical therapy, and with the participation of the Swedish pharmaceutical industry. One can observe new possibilities, including the prostaglandins of Bergstrom and his colleagues.

TRADITIONAL ALKALOIDS

Of all the natural products studied by chemists in the last century, perhaps alkaloids dominate this chemistry, particularly if one takes into consideration the companion medical aspects. Although alkaloids have made a valuable contribution to medicine over many decades, one may observe that the contribution of alkaloids to medicine could diminish in the future, and that the landmark contributions to medicine will increasingly stem from investigations of basic biochemistry. This observation about alkaloids is based upon the increasing attention to rigorous requirements for safety in governmental regulations on drugs. Also, the alkaloid chemist faces formidable competition from the synthetic chemist and biomedical investigator who have biochemical theory and greater flexibility of structural variation at their command. Nevertheless, the contributions of alkaloids have not ended and may be expected to continue. The story of the *Rauwolfia* alkaloids has been told, and I only wish to pay tribute to their contribution to medicine. The use of *Rauwolfia* extracts extended over centuries, and credit is due to chemists and pharmacologists in India for their early research on the

THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

alkaloids of *Rauwolfia serpentina*. The use of *Rauwolfia* alkaloids began in Western medicine as recently as 1953.

Reserpine (I)¹ became widely used in medicine in mental conditions, including tension and anxiety, as well as in the treatment of some forms of hypertension. The introduction of chlorpromazine took place about a year later. The importance of *Rauwolfia* alkaloids in today's medicine may be

judged by sales of pharmaceutical preparations at 55 million dollars in 1963. Psychopharmacological agents have expanded in sales to over 200 million dollars; 18 million people afflicted with certain mental conditions are treated each year in the United States.

Also, in the 1950s, the periwinkle plant, *Vinca rosea* Linn., was studied because of an interest in diabetes. However, extracts of this plant caused leukopenia rather than hypoglycemia in animals. Eli Lilly and Company found such extracts to be active against experimental leukemia in mice. The alkaloid which stemmed from these observations is now known as vinblastine (II)². Since 1960, a few hundred publications have appeared on medical studies with vinblastine, particularly in the field of cancer. While no

known drug, whether natural or synthetic, is as effective in the treatment of cancer as desired, each new oncolytic drug, which has some positive effect in the treatment of clinical neoplasms, is another step of encouraging progress.

KARL FOLKERS

It appears reasonable today to consider treatment with vinblastine sulphate (II) in those cases of cancer, which include the following conditions: (1) lymphomas, Hodgkin's disease, lymphosarcoma and reticulum cell carcinoma; (2) monocytic leukemia; and (3) carcinomas of the breast, which have not responded to other treatments.

Lower dosage and longer treatment for the more slowly responding tumours, such as carcinomas of the breast and bronchus, have been advised. Vinblastine seems to lack cross-resistance with other therapies. Leukopenia is the dose-limiting factor. Although some oncolytic drugs, including vinblastine, usually bring about only temporary remissions, such drugs can bring about tumour shrinkage, prolongation of life, and significant symptomatic relief.

MAGNITUDE OF THE CANCER CHALLENGE

Cancer is the second cause of death by a wide margin in the United States. Many more people now survive infectious diseases only to succumb to cancer, and cancer is by no means reserved for the aged. Cancer is either the first or the second cause of death in children between one and fourteen years, with acute leukemia being the most common form of cancer of children. In 1963, 45 per cent of cancer deaths were of persons younger than 65, and 9 per cent were younger than 45 years of age. Cancer of the lung accounts for about one-fourth of all cancer deaths in males.

On the basis of current trends, about one out of every four people alive in the United States today can be expected to develop cancer at some time during his or her lifetime. This means that nearly 50 million people now living in the United States will develop cancer. Also, a little over 30 million Americans now alive will die from cancer unless new natural product chemistry and other endeavours lead to the discovery of new therapeutic procedures for prevention and cure. Economically, all of the costs due to cancer were estimated in 1962 to be 8 billion dollars, or 1.4 per cent of the gross national product.

Louis F. Fieser of Harvard University has made his contributions to the chemistry of natural products. In the April 1966 issue of *Readers Digest*, one may read his own story as to how he narrowly escaped death from lung cancer. Louis states that his case teaches two important lessons: the first one is "the folly of saying it's probably too late to quit now", and the second lesson is that "contrary to my expectations I found it easy to break a long-standing habit of heavy smoking". Of course, he was strongly motivated, but he did stop smoking readily. Suffering also from emphysema, bronchitis, and poor heart function, Louis reported that, after only two weeks, both his heart and lungs showed marked improvement. I commend to you this story of an ex-smoker, and trust that my rather obvious plea will have some benefit.

AMINO ACIDS OF PRIMARY IMPORTANCE

Nutrition and medicine have benefited enormously from the chemistry of the natural products—amino acids, peptides and proteins. Advances in isolation, automated sequence determination and rapid synthesis of peptide

THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

chains are achievements which might have been viewed as nigh impossible 25 years ago.

Rather than tell any story about amino acids or peptides, I prefer to cite the story of an amino acid antagonist because of the following three reasons (i) this antagonist is extremely useful in current medicine; (ii) new antagonists may have future impacts on medicine; and (iii) because this story exemplifies the present relationship between a pharmaceutical company and the Food and Drug Administration. About fifteen years ago, Karl Pfister in the Merck Sharp and Dohme Research Laboratories became interested in amino acid antagonists as a possible approach to cancer chemotherapy. Goldenberg of Columbia University, who later died of cardiovascular disease, persuaded Pfister and his group to study the inhibition of the decarboxylation of dopa as a means of possibly controlling essential hypertension. α -Methyldopa was one of the compounds synthesized and found to be an outstanding inhibitor *in vitro* of pig kidney dopa decarboxylase. However, no drop in the concentration of catecholamine was observed *in vivo*, and, unfortunately, the technique used gave this misleading negative result. The programme on α -methyldopa all but ended for about five years, and then a paper from Heidelberg reported that α -methyldopa completely prevented the blood pressure effects produced by dopa in a variety of laboratory animals. Confirmation came from Frankfurt. Udenfriend and Sjoerdsma proposed a reawakened programme on α -methyldopa between the National Institutes of Health and Merck Sharp and Dohme.

An appreciation of what a pharmaceutical company does these days to gain approval from the Food and Drug Administration of an application for a new synthetic drug may be exemplified by the background on α -methyldopa for hypertension. In 1959, Udenfriend and Sjoerdsma gave the substance to ten human patients and it showed a reduction in blood pressure confirming the biochemical studies. The rest of 1959 was devoted to chronic toxicity studies and the preclinical phase of Merck and Co., Inc. The clinical studies of 1961 encompassed 200 physicians in the United States and 31 other countries, and nearly 2000 patients. Approval by the Food and Drug Administration was granted in 1962, or about three years from the first successful clinical trial. I have no cost data for this three-year period, but one may surmise that both the costs and the losses would be very impressive. Who else but the pharmaceutical industry would support the cost, the continuity, and provide the extensive skills necessary to carry such a new compound through three trial years so that it could ultimately be marketed to provide hypertensive patients added years of life.

α -Methyldopa [*laevo*-3-(3,4-dihydroxyphenyl)-2-methylalanine] (III) is a decarboxylase inhibitor. The site of inhibition of the biochemical reactions is represented as follows.

KARL FOLKERS

Dihydroxyphenylalanine (dopa)

5-Hydroxytryptophan (5-HTP)

Dihydroxyphenethylamine (dopamine)

5-Hydroxytryptamine (serotonin)

Norepinephrine

5-Hydroxyindoleacetic acid

Methyl dopa finds its greatest usefulness in the field of moderate to severe hypertension and in selected cases of malignant hypertension. One of its chief attributes appears to be the preservation of renal blood flow in patients who have some depression of renal function. It does not generally cause any increase in cardiac rate or output. It produces a smooth and predictable lowering of blood pressure—both the supine and the standing blood pressure.

HEART DISEASE AND STROKE—THE GREATEST CHALLENGE

Surely, new chemistry of natural products will lead to new contributions for the therapy of coronary heart disease, which remains the number one cause of death in the United States. A few decades ago, tuberculosis was the greatest single menace to American health, and pneumonia and influenza took a heavy toll. Parenthetically, may I explain that my use of statistics is for the United States only because of their ready availability to me. Perhaps the corresponding statistics for Europe would not be greatly different.

The term, heart disease, as commonly used, is a complex of diseases and may be better termed as cardiovascular and renal diseases, and these diseases can be divided into three major categories: (i) strokes, or damage to the

THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

blood vessels affecting the central nervous system; (ii) diseases of the heart and blood vessels, including arteriosclerosis, degenerative heart disease, hypertensive heart disease and other specific disease entities of the heart; and (iii) kidney diseases, including chronic nephritis and renal sclerosis.

These heart diseases accounted for 50 per cent of all deaths in the United States in 1963. Heart diseases are predominantly a cause of death among older people, and men outnumber women as victims by a factor of more than one-third. This complex of heart diseases now claims nearly a million lives each year. The economic impact is also staggering. Had all those individuals who died of heart disease in 1962 lived just one more year, the economy would have gained two billion dollars worth of output. The sum of direct costs, plus losses, amounted to 22.4 billion dollars, or 4 per cent of the gross national product in 1962.

MALARIA—NOT YET UNDER CONTROL

It is believed that malaria is perhaps the most worldwide of all human diseases, and it was recently estimated that approximately 300 million people are afflicted. Most of the really effective antimalarial drugs presently used in medicine resulted from the intensive investigations before, during, and after World War II in Europe and the U.S.A. There has been a very widespread feeling that malaria is a rapidly disappearing disease. However, the parasitologists tell us that the world control of malaria has not been realized, and that malaria will be a medical problem in the world for a long time. Consequently, those who work together in chemistry and medicine still have research goals in this field. In fact, all the parasitic diseases constitute an enormous challenge for chemistry and medicine. Schistosomiasis, the dysenteries and more than a dozen other parasitic diseases are specific goals.

Drug resistance of malaria became a significant problem about 1948 to 1950 with the discovery in Malaya of the resistance of *Plasmodium falciparum* and *Plasmodium vivax* to Proguanil. Drug resistance to other important antimalarials has been reported from many countries of the world, including Africa and South America.

The war in Vietnam has heightened the interest in drug-resistant strains of malaria and new research programmes have now been launched, including broad basic biological research on experimental malaria in animals, particularly the primate, and finally on intense medical studies of clinical malaria due to resistant strains.

The history of malaria for several thousand years until and including present medical practice demonstrates the longest lasting and most far-reaching contribution of natural products to medicine; because of this extraordinary contribution, and because of the timely focus on resistant malaria in Vietnam, I wish to present a little more of the history and some of the latest findings.

Of all the natural products explored by man to treat malaria, only two are said to have withstood the test of time as well as modern chemistry and medicine. One is the Chinese drug, Ch'ang Shan, which is known to have been used in China for several thousand years. Ch'ang Shan is made from the roots of *Dichroa febrifuga*. Febrifugin has a spectrum of antimalarial

KARL FOLKERS

activity, but its value in human malaria has been limited, because of its side effects.

The other natural product which is really the prime one of the two is, of course, quinine.

Chloroquine, primaquine, amodiaquine, proguanil and pyrimethamine have been regarded as among the best of the available antimalarials. In

Chloroquine

Primaquine

Pyrimethamine

mentioning these few synthetic antimalarials, I do so for brevity and to be illustrative, and not to disregard up to twenty other antimalarials which have also had varying degrees of medical success in countries of Africa, Asia and the U.S.S.R., and which stemmed from the world's pharmaceutical industry.

In current research, carefully controlled medical studies, which are quite definitive, have been made on a drug-resistant strain of *Plasmodium falciparum* from both Thailand and Vietnam. The Thailand (JHK) strain³ and the Vietnam (Sn) strain⁴ were both obtained from blood smears of two members of the U.S. Armed Forces in these countries. Infections with both of these strains were established in non-immune volunteers in a non-endemic area under conditions precluding reinfection, that is, a prison in Joliet, Illinois. The volunteers received during acute clinical attacks of malaria chloroquine, hydroxychloroquine, amodiaquine, mepacrine, pyrimethamine, proguanil, or other antimalarials alone and in combination. These drugs failed to effect a radical cure of the infections, both before and after passage of the strain through mosquitos. A radical cure requires complete elimination from the body of erythrocytic stages and persisting tissue stages of the parasite so that relapses cannot occur.

As if in tribute to the activity of quinine, which has served mankind so well for a few hundred years, a radical cure was achieved in these patients by the administration of about 1.5 g of quinine daily for seven days for the resistant *P. falciparum* from Thailand. In the case of the resistant strain from Vietnam, the administration of either 50 mg of pyrimethamine daily for three days or nearly 2 g of quinine daily for ten days did effect radical cures.

The drug resistance of *P. falciparum* to chloroquine is today being

THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

recognized with increasing frequency, not only in South-East Asia, but in South America. Our medical colleagues, who are very knowledgeable in this field, point out that the present-day global chemotherapy of malaria could be serious.

The prodigious research of chemists on the natural products which have antimalarial activity, and knowledge of the many thousands of organic compounds screened in experimental malaria, lead to the observation that the design of new antimalarials poses formidable new research. Nevertheless, a study of this field, in depth, will reveal new approaches, extensions of old ones, and new discoveries of medical significance could evolve. New fundamental research on parasite metabolism and the biochemistry of active compounds at various stages of the life cycle of the *Plasmodium* may also lead to new and effective drugs. It does seem that there has been little significant progress in the field of malaria chemotherapy in the last ten years as compared with the earlier era.

19-NOR-STERIODS—FERTILITY CONTROL

The ovarian and testicular hormones and those of the corpus luteum were elucidated by chemists working on natural products about 31–37 years ago, and progesterone, which I wish to speak about later, was first isolated in pure form in 1934. It was in 1936 that the alphabet-lettered adrenal steroids reached a peak of chemical elucidation. Concomitantly and independently of this chemistry on the steroid hormones, medical investigators had been extensively studying rheumatoid arthritis. It is difficult to visualize how the chemistry of these steroidal hormones and the separate medical investigations would have become bridged to result in the impact of cortisone upon diseases of medicine were it not for the indispensable role of the pharmaceutical industry. In these days of preoccupation with governmental regulations, the pharmaceutical industry of the world must maintain a balance of responsibilities. The history of drugs demonstrates that certain long range investments, which become very large and appear very risky, may turn out to be quite rewarding financially. The industry may find some guidance from the idea that the greater the impact of the chemistry upon medicine the greater will be the impact of the medicine upon economics.

I have particularly mentioned the naturally occurring steroid, progesterone, because I wish to say more about the great current interest in steroidal contraceptives. On occasion, these steroids seem to assume the distinction of being known as “the pill”. When one considers that by the year 2000 the world’s population is projected to become about 7 billion, an increase of 3.5 billion from today, the significance of fertility control is evident. Consequently, there is greatly augmented interest on more basic knowledge of the process of reproduction as well as new progress on all approaches for useful control of conception.

That progesterone inhibited ovulation during pregnancy besides its other biological actions had been known earlier. Ehrenstein synthesized crude “19-nor-progesterone” which was found to exhibit the same biological activity as progesterone. The concept of structural requirements for progesterone activity became more fascinating, and Plattner and Heuser

synthesized 14-iso-17-isoprogesterone, but it was found to be inactive. Attention was then focused on synthetic 19-nor-steroids. Although Birch's synthetic 19-nor-testosterone exhibited less androgenic activity than testosterone, a Syntex group synthesized authentic 19-nor-progesterone, because the stereochemistry of Ehrenstein's earlier material was ambiguous. Miramontes, Rosenkranz, and Djerassi reported that authentic 19-nor-progesterone is really 4 to 8 times as active as progesterone in the Clauburg assay.

Before it became established that the potency of progesterone is significantly increased by removal of the methyl group at position 10, it had been observed earlier by Inhoffen, Logemann, Hohlweg, and Serini that 17- α -ethynyltestosterone, by the oral route, was an effective progestational agent. The Syntex group, Djerassi, Miramontes, Rosenkranz, and Sondheimer, then synthesized 19-nor-17- α -ethynyltestosterone. Their anticipation of increased activity by removal of the methyl group at position 10 was realized when the biological data revealed an extremely high order of progestational potency for the compound. Also, it was active by the oral route. 19-Nor-17- α -ethynyltestosterone and its β,γ -unsaturated isomer, synthesized by Colten, were the first such steroids studied in human females for inhibition of ovulation, and they were found to be effective by Rock, Pincus, and Garcia. The time between recognition of progesterone as a natural product and 19-nor-17- α -ethynyltestosterone for inhibition of ovulation in humans was 22 years. Again, an indispensable pharmaceutical industry, Syntex, participated in the early stages. Reference to a recent summary⁵ by Djerassi on steroid oral contraceptives will provide one with an enlarged account of the chemical developments. On the medical side, the total market in 1965 for the United States for sales of oral contraceptives was estimated at 66.5 million dollars for about 6 million individuals. For 1966, the corresponding sales are estimated to increase to about 85 million dollars for up to 7.5 million individuals.

VITAMINS AND COENZYMES

The impact of all the known vitamins upon medicine can be summed up in the word *indispensable*. The last vitamin to achieve an established place in medicine is vitamin B₁₂, and this was nearly twenty years ago. Before and after B₁₂, there has been the question: are there any more vitamins left to discover? Such a question is somewhat imponderable. When Dr. Aulin-Erdtman invited me to make this presentation, she encouraged me to include an account of some of my current research. Consequently, I shall provide some background on coenzyme Q and my progress report from the Stanford Research Institute. Since this research has already been extended to medical data, its inclusion is within the scope of the subject.

Coenzyme Q is a constituent of the mitochondrion and performs its indispensable role of electron transfer in this cytoplasmic organelle. It was as recent as the early fifties that research on the biochemical mechanism of oxidative cycles, electron transport, and oxidative phosphorylation merged with research on cell biology. The mitochondrion of the cell may be visualized as an ellipsoid which is frequently about 3 microns in length and a little less than 1 micron in width. The mitochondrial structure may be represented as

THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

a membrane within a membrane, and the inner membrane has countless infoldings, known as cristae. A schematic representation is given in *Figure 1*.

Mitochondria are relatively free to move in some cells, but those of skeletal muscle appear to remain fixed near contractile macrostructures. The number of mitochondria for a cell varies over a great range. Rat hepatocytes have an average of about 800 mitochondria per cell, and renal tubule cells of mammals contain about 300. Sperm cells can contain as few as 20 mitochondria.

The mitochondrial two-layer membrane system contains two-thirds protein and a noteworthy one-third lipid. About 25 per cent of the total protein of the membrane is composed of the respiratory enzyme assembly. Of great significance, then, is the realization that the mitochondrial membrane bilayer system is not altogether a biochemically inert structure, but includes the recurring multienzyme structure for respiratory electron transfer and the biosynthesis of ATP.

About four years ago, Fernández-Morán made the initial observation that negatively stained images of the inner membrane showed the presence of spheres of about 70–90 Å in diameter which are connected by thin stalks to the surface of the membrane. The existence of these spheres and stalks has been confirmed in other laboratories. An electron micrograph (*Figure 2*), also kindly provided by Dr. Fernández-Morán of the University of Chicago, shows these spheres and stalks. Each mitochondrion appears to contain several thousand of these entities, and they appear to account for about 80 per cent of the dryweight of the mitochondrion. When it is realized that these entities contain complete electron transfer chain(s) and the capacity for coupling, it is apparent that the mitochondrion can be primarily a macrostructure for the electron transfer process of respiration and the biosynthesis of ATP.

The sequence of electron transfer and coupled phosphorylation as depicted by Ernster, Green and others, is shown in *Figure 3*. In this sequence is the expression —CoQ— symbolizing coenzyme Q. In this lecture I am using the coenzyme Q nomenclature in order to emphasize a relationship of this subject to nutrition, although the name ubiquinone is preferred on grounds of nomenclature.

Coenzyme Q₁₀ is that member of the CoQ group in the tissue of man, and it has been estimated that the entire human body contains from 1 to 2 grams of it. CoQ₆ through CoQ₁₀ were isolated in the early days of this research, and very recently coenzyme Q₅ has been found⁶ in *E. coli*.

KARL FOLKERS

As shown in *Table 1*, the biological activity of coenzyme Q has certain

Table 1. Organic structural specificity of coenzyme Q for biological activity		
Monoethoxy and diethoxy analogues have low activity		Isoprenoid chain can be changed in length and somewhat reduced with some retention of activity
Monohydroxy analogue has some inhibitory activity		
Monoamino analogue is naturally occurring rhodoquinone and has low activity		Ring methyl group is apparently not too critical for activity
Cyclization to chromenols and chromanols does inactivate		

Data given is largely for succinoxidase and partly for DPNH-oxidase system

structural specificity in the succinoxidase and NADH-oxidase systems⁷. The variations in structure which permit retention of activity are limited, but are not as rigid as those usually observed for members of the vitamin B complex. The biological activity of coenzyme Q is primarily restricted to compounds having the oxygen and lipidic functionalities of the molecule. Notably, vitamins E and K are inactive in these succinoxidase and NADH-oxidase systems. Consequently, one may consider coenzyme Q in terms of the structural specificity of vitamins for activity.

Coenzyme Q₁₀ is biosynthesized within the human body, and the sequence of this biosynthesis has challenged a number of investigators for several years. Although it was predictable that the isoprenoid side chain of CoQ would be derived from mevalonic acid and, indeed, this was shown to be true, there was little more than just ideas on how the tetrasubstituted benzoquinone nucleus is biosynthesized. An early understanding that the isoprenoid side chain could be the last step of the biosynthesis now seems to be replaced by evidence that this side chain is actually introduced very early in the sequence. Fortunately, for us who study CoQ in man, the photosynthetic organism, *Rhodospirillum rubrum*, like the human body, utilizes coenzyme Q₁₀, and this microorganism has served us well.

p-Hydroxybenzoic acid is now established as a precursor to coenzyme Q^{8,9}. It has been shown that *p*-hydroxybenzoic acid becomes alkylated and presumably by an isoprenyl pyrophosphate as indicated on the top of *Table 2*. The first two precursors¹⁰ shown within the bracket (a) were elucidated in 1965. The next two precursors¹¹ within the bracket (b) were elucidated earlier this year. A new report¹² today can now be made on the remaining three steps, all expressed as quinones, prior to the last O-methylation to give coenzyme Q. The quinones (V), (VI), and (VII) are now apparent from the skill, good judgement, and strategic examination of about 5000 fractions from *R. rubrum* by Dr. Palle Friis. Quinone (VI) has been isolated and characterized. Quinone (V) has not yet been isolated in pure form, but spectral data support its presence. The intermediacy of quinone (VII) is obvious. Characterization of quinone (V) is continuing. The intermediacy of the hydroquinones is understood. We believe that the biosynthetic sequence for coenzyme Q in *Table 2* is essentially generic for all members of the coenzyme Q group and all forms of life having CoQ, but

KARL FOLKERS

with some variations and alternative pathways, depending upon specific forms of life.

Turning now to animal studies, a pivotal discovery was the finding that hexahydrocoenzyme Q_4 has vitamin-like activity, which is both prophylactic and curative, in the rabbit which becomes dystrophic by nutritional design¹³⁻¹⁵ as shown in *Table 3*. Next, this vitamin-like activity was revealed for the anaemic and dystrophic rhesus monkey¹⁶ which is also produced by nutritional design; this activity in the primate has been confirmed and extended¹⁷, as in *Table 4*. The photograph (*Figure 4*) kindly provided by

Table 3. Vitamin-like activity of hexahydrocoenzyme Q_4 in the dystrophic rabbit

Four rabbits are cured of nutritionally induced dystrophy	}	..	..	..	(a)
Dystrophy is prevented by prophylactic therapy in three rabbits					
Seven rabbits with nutritional dystrophy responded to therapy	}	..	..	..	(b)
2,3,5-Trimethyl-6-phytylbenzoquinone showed very low activity					
Life-saving activity appraised at dose levels of 2-10 mg/kg	}	..	..	..	(c)
No evidence found for significant conversion of CoQ_4 -chromanol to CoQ_4					

Table 4. Vitamin-like activity of coenzyme Q in the anaemic and dystrophic rhesus monkey

Coenzyme Q_{10}

Evoked a reticulocytosis in four monkeys
Slight or no increase in haemoglobin concentration

Hexahydrocoenzyme Q_4

Suboptimal dose elicited only a reticulocytosis, but a higher level produced a complete haematological remission in one monkey
In five monkeys, a haematological response and decrease in creatinurea were clearly produced by therapy

Dr. Coy Fitch is of a dystrophic rhesus monkey. Since the posture is somewhat unusual, it would appear that the monkey is actually dystrophic.

It was next evident that one could forecast a haematological response of children with a unique anaemia to treatment with hexahydrocoenzyme Q_4 . Such children have protein-calorie malnutrition, and a macrocytic anaemia unresponsive to known haematological agents, including folic acid and vitamin B_{12} , can develop as first described in 1956-1960 by Amin S. Majaj, Chief of the Paediatrics Department of the Augusta Victoria Hospital, Jerusalem, Jordan¹⁸.

Figure 5 shows the picture of such a child (Ibtisam, meaning Smiling) who was recently treated with this CoQ_4 by Dr. Majaj in our continuing co-operation which began in 1963. After four to five days of treatment of this child, the entire haematological status improved very significantly, as evidenced by a complete remission. Also, the creatine excretion of this child decreased after treatment showing improvement in protein metabolism. The data¹⁹ are presented in *Table 5*. After such an encouraging clinical test with CoQ_4 , it can be easily imagined that we were all smiles and are now eagerly planning an extension of the study of refractory anaemia.

During our cooperation in 1963, Dr. Majaj treated two similarly anaemic children with coenzyme Q_{10} and observed (unpublished data) a reticulocytosis and improvement in the anaemia; substantiating statements are also

Figure 1. Model of mitochondrion

Figure 2. Electron micrograph of negatively stained mitochondrial membranes

KARL FOLKERS

Figure 3. Electron transfer chain

Figure 4. Dystrophic rhesus monkey

Figure 5. Child having protein-calorie malnutrition

KARL FOLKERS

Figure 6. Jacobus Berzelius. Portrait by Johan Way 1826. By courtesy of the library of the R. Swedish Academy of Science

THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

Table 5. Treatment of a macrocytic anaemia associated with protein-calorie malnutrition

<i>With hexahydrocoenzyme Q₄ (first child)</i>	
Data on peripheral blood	<div style="display: flex; align-items: center;"> { <div> Reticulocytosis from <i>ca.</i> 0 to 34% Three-fold increase in hematocrit Increase in haemoglobin from 5.5 to 9.5% Increase in leucocytes from 5 100 to 19 350/cm </div> </div>
Data on urine	Decrease towards normalization of creatine excretion
<i>With coenzyme Q₁₀ (two children)</i>	
Data on blood and marrow	<div style="display: flex; align-items: center;"> { <div> Positive reticulocytosis Change from aplastic to hyperplastic marrow Myeloid: erythroid ratio was 21:1 before treatment and 2:1 after treatment </div> </div>

given in *Table 5*¹⁹. The fact that these children require more protein and calories does not detract from the significance of their response to therapy with CoQ₄ and CoQ₁₀.

Turning again to muscle disease, it has been evident for years that nutritional dystrophy, whatever may be its cause, is clearly metabolically different from genetic dystrophy in mammalian species and particularly in man. Supporting this differentiation is the knowledge that vitamin E is ineffective therapeutically in the treatment of genetically dystrophic mice and muscular dystrophy in man although vitamin E is effective in nutritional dystrophy.

Genetic dystrophy in mice has been known for years, and no significant long-term therapeutic response of intrinsic biochemical significance has yet been reported although, indeed, a variety of treatments have been tried. The statements of basic knowledge which led us to open a study of treatment with CoQ of genetic dystrophy are: (1) CoQ is active in the electron transfer process and coupled biosynthesis of ATP, and vitamin E is inactive; (2) CoQ has exhibited vitamin-like activity in nutritional dystrophy.

Genetically dystrophic mice have now been treated with hexahydrocoenzyme Q₄ and the results are given in *Table 6*²⁰. Of eight control animals,

Table 6. Response of mice with genetic dystrophy to therapy with hexahydrocoenzyme Q₄

<i>8 Control animals</i>	
Dystrophic status of seven progressively deteriorated	
Dystrophic status of one remained about the same	
<i>10 Treated animals</i>	
All ten dystrophic mice improved	
Four of the ten were severely dystrophic and in poor health, but responded and were able to walk	

the dystrophic status of seven progressively deteriorated. The dystrophic status of one animal remained about the same. Of ten treated animals, all ten dystrophic mice improved. Four of the ten mice were severely dystrophic and in poor health, but even they responded to this therapy and became able to walk using all their legs.

An interpretation of this apparent vitamin-like activity of CoQ in genetic dystrophy may be stated as follows. The biosynthetic sequence for coenzyme Q in mammalian tissue is complex and requires numerous proteins, lipids, known vitamins, inorganic ions, and other cofactors. Such a complex biosynthesis appears to offer many metabolic possibilities for genetic

"blocks". From such inborn errors of metabolism, an inadequacy of functional levels of coenzyme Q could result with concomitant impairment of electron transfer and the development of dystrophy. If a deficiency of CoQ actually resulted from such a metabolic "block", then therapeutic administration might well restore CoQ in the tissue to functional levels for electron transfer and diminution of dystrophy. The apparent response of these mice to therapy with this CoQ₄ could signify that this dystrophy of hereditary origin is possibly caused by a genetic "block" in the biosynthesis of CoQ. The long-standing failure of vitamin E to exhibit therapeutic activity in the genetic dystrophy of mice and humans is understandable on the basis that vitamin E should have no effect upon the mechanism of such a genetic "block" itself, and vitamin E cannot substitute for CoQ in electron transfer.

It is now very attractive to extend these favourable data on the therapy of dystrophy of hereditary origin in experimental species and particularly to the muscular dystrophies in humans.

This progress report on CoQ may be concluded with an over-all interpretation as a working basis for new research. It appears that the so-called vitamin E deficiencies can be nonspecific antioxidant deficiencies, primarily because structurally unrelated compounds show the same activity as vitamin E. Such antioxidants could protect, on a body-wide basis, unsaturated lipids, including CoQ and its biosynthetic precursors against peroxidation and loss by a free radical mechanism. In effect, vitamin E could allow functional levels of coenzyme Q to become restored by biosynthesis. There seems to be no proof yet that vitamin E has an intrinsic and specific role in mammalian tissue, like that of a classical vitamin, although such a role may yet be elucidated.

In nutrition, one differentiates between those factors which are just stimulatory from those which are essential to life, and clearly the latter are more significant. Coenzyme Q may have exhibited an essential vitamin-like activity, at least the activity is life-saving, in these studies of the rabbit and the rhesus monkey. The activity of CoQ in these children is probably also life-saving, but one cannot carry the study of these children to such a potentially dangerous end-point. Coenzyme Q may have exhibited a vitamin-like activity in genetic dystrophy by therapeutically restoring functional levels that may be inadequate due to a genetic block of biosynthesis of CoQ.

Functional deficiencies of CoQ could exist in the human body, because of loss through nutritional peroxidation, or through impaired biosynthesis as in malnutrition or through genetic block(s) of biosynthesis.

May I say again that this overall interpretation serves as our present working basis for planning new experiments which I hope will help in the final elucidation of the mechanisms of biological activity.

Hexahydrocoenzyme Q₄ appears to substitute for CoQ₁₀ in the body.

In conclusion, I wish to quote from Jacobus Berzelius (*Figure 6*), who wrote as follows in his preface addressed to King Gustaf IV Adolf in the first Swedish edition of his *Animal Chemistry* of the year 1806:

"Of all the sciences contributing to medicine, chemistry is the primary one, and, apart from the general light it throws on the entire

THE IMPACT OF NATURAL PRODUCT CHEMISTRY ON MEDICINE

art of healing, it will soon bestow on some of its branches a perfection such as one never could have anticipated."

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