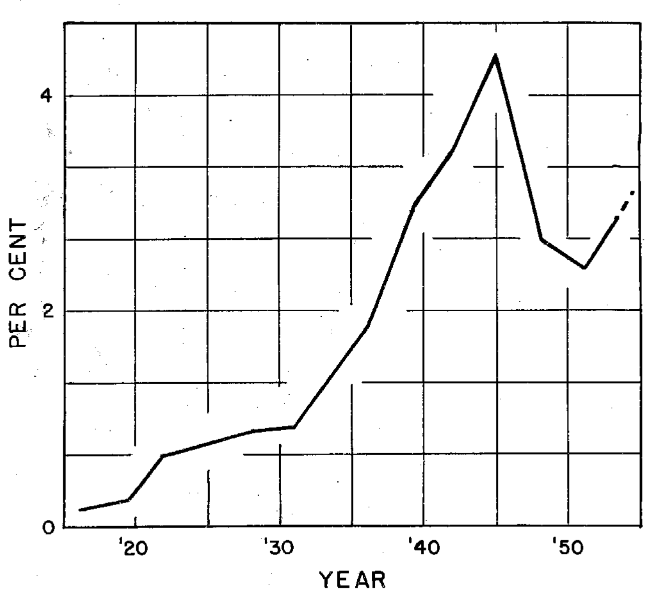
VITAMINS ARE HERE TO STAY

NEARLY EVERYONE now knows something about vitamins but it was only a few years ago that we really came to know very much about them. An approximate index of relative numbers of scientific investigations and discoveries concerning vitamins is given in the graph below. From this graph it is clear that, for some reason, in the early 1930's, vitamins quite suddenly became a focus of scientific interest. The reason for this is remarkably simple, but, like other scientific advances, this too was dependent on the painstaking accumulation of seemingly unimportant and unrelated facts over a period of many years.

Fossil bones give good evidence that prehistoric man suffered from rickets and scurvy—at least. Other vitamin deficiencies that do not leave such a record are by no means new inventions either. For example, written history of the first thousand years A.D. contains numerous references to the administration of goat liver for the cure of night-blindness—a practice by Greek, Roman, and Arab physicians that was quite sound.

In a similar category, the prevention and cure of scurvy was well known at least by the 16th century, and the Dutch navy practiced the art by providing oranges, lemons and fresh vegetables on long sea voyages. In 1665, a Dutch investigator recommended horseradish



An approximate index of relative numbers of scientific investigations and discoveries concerning vitamins.

pickled in French brandy—a therapy now reminiscent of the tonic era in this country. It is of some interest that in vitaminology, as in many other fields, communication was poor and the records show that scurvy therapy was rediscovered by an Austrian, J. G. Henrici Kramer, in 1720 and again by an Englishman, James Lind, in 1757. Subsequently, as is common knowledge, British mariners acquired the nickname "limeys" from their use of lime juice as a scurvy preventative.

Even today it is sometimes difficult to determine who really discovered what in science—but to the best of our knowledge, the Dutch physicians, Christiaan Eijkman in 1897, and G. Grijuns in 1901, are responsible for the beginnings of experimental nutrition with small animals. In the East Indies, where they worked, beriberi was a prevalent disease and the Japanese Navy had already made use of fresh vegetables as a preventative measure.

The Dutch investigators demonstrated that a beriberi-like disease was produced in birds fed a diet of polished rice. They established, furthermore, that small amounts of rice polishings would prevent and cure the condition, but there still remained an important question that was not resolved for many years to come: Did the polishings contain a substance that destroyed a beriberi-producing agent or did they contain a substance that is required for normal body functions?

Perhaps this was, or should have been, the seed from which the idea of antibiotics grew, but the nutritional explanation was soon proven and the other went into obscurity. In 1907, the small animal experimental approach moved ahead with an attempt by Axel Holst and Theodor Frölich to produce beriberi in guinea pigs. A deficiency was indeed produced but it turned out to be cured by lemon juice instead of rice polishings, and thus scurvy as well as beriberi became subject to the experimental approach.

Shortly thereafter, in reviewing nutritional problems, in 1912 Casimir Funk proposed the name vitamine (from *vita*, meaning life, and amine, which is a class of chemical substances) for nutritionally necessary materials needed in small amounts, but not for energy or structure building. The *e* was dropped in later years when many of the vitamins were found not to belong to the class of compounds called amines.

During the ensuing two decades, the vitamins were given increased attention with such developments as:

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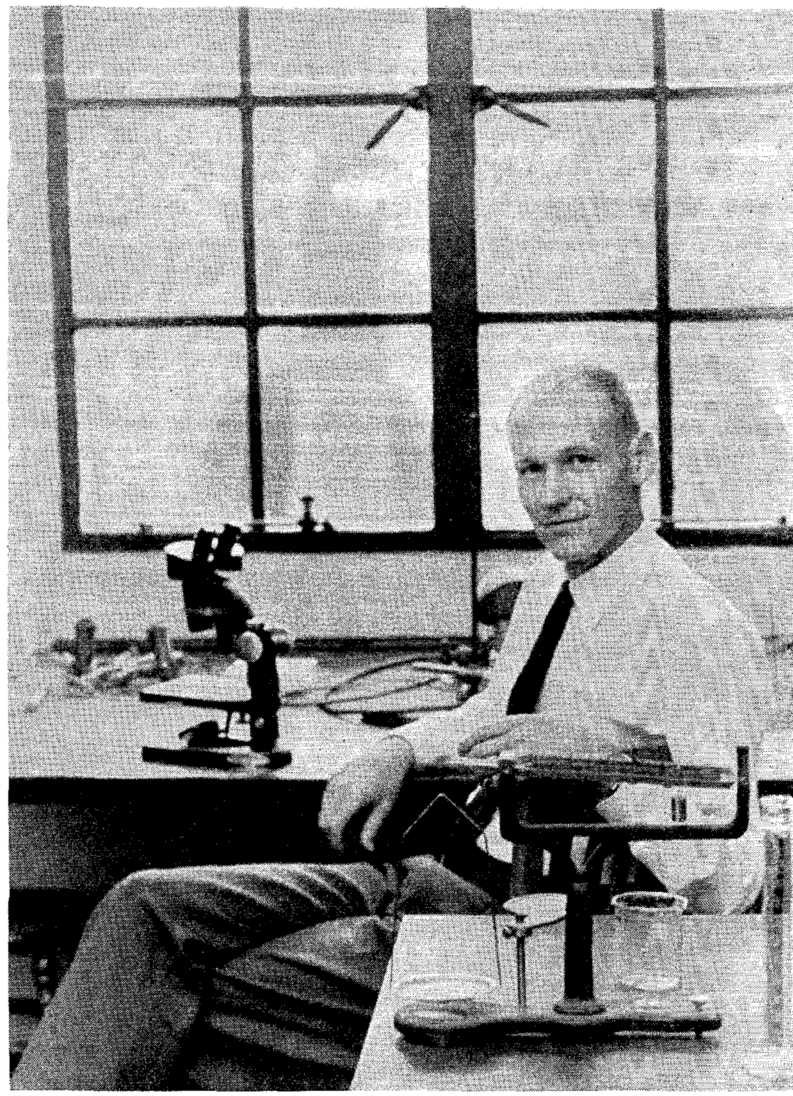


purified diets for animals by Sir Frederick Hopkins in 1912; the distinction between "fat soluble vitamin A" and "water soluble vitamin B" by Elmer V. McCollum in 1915; and the production of experimental rickets in rats by May Mellamby in 1918. During this period also there occurred some confusion between vitamin needs and "trace element" requirements (such as iron, copper, cobalt and iodine) but gradually it developed that all are important.

And then too, little by little, the evidence accumulated that the vitamin picture was more complex than it seemed at first. "Fat soluble A" clearly contained more than one active substance and even "water soluble B" was suspected of being multiple in nature. This is how things stood in the late 1920's. There was progress in vitamin nutrition but it was slow. Animal assays to detect vitamins in general took from weeks to months to perform, and progress toward the isolation of vitamins as pure chemical substances was limited by the biological methods. But then the explosion occurred, and to see what set it off it is necessary to go back again to some other matters in history.

For micro-organisms, we usually go back in history to Louis Pasteur, and indeed that is where this part of the story began. Following his demonstration of the origin of microbes from other microbes, in 1871 Pasteur described a procedure for cultivating yeast in a medium containing only purified chemical substances. He reported if such a medium was inoculated with a pinhead *(tête d' épingle)* of yeast, the organism would multiply and flourish.

This experiment led to a heated controversy when Justus Liebig, a prominent biochemist of that time, tried to repeat it and failed. It seems incredible now but the argument was not resolved satisfactorily until 30 years later, in 1901, when Eugene Wildiers brought forth a new principle, with experiments, to explain the discrepancy. He concluded, in effect, that Pasteur's pinhead was larger than Liebig's, and that the larger inoculum (containing probably several million yeast cells) carried along with it traces of a material that was required for continued growth even in the presence of sugar and minerals. This material, which he called "bios," became the subject of investigation by a number of scientists.

Still, a link to vitamins was missing, and there was little reason to think that the lowly yeast might need in its diet the same kinds of substances as animals. Such a link came in 1919, however, when Roger J. Williams demonstrated that the pure anti-beriberi factor (vitamin B_1) was required for the growth of a strain of yeast. But relatively few scientists got excited over this observation. Its significance was not immediately obvious, since it depended on the idea that, if animal vitamins were growth factors for micro-organisms, then perhaps compounds required by micro-organisms would be vitamins for animals.

This idea alone is not sufficient to explain the great outburst of activity in vitamin research that occurred shortly after 1930. There is an additional simple fact of great importance. The rate of growth of micro-organisms can be determined in hours, whereas weeks are usually needed for an equivalent determination using experimental animals. Now, in order to isolate a vitamin in a pure form, it is necessary to try innumerable procedures for purification, and equally necessary to evaluate each step on a quantitative basis by means of a biological assay. The rate of progress on such a problem, though not directly proportional to the speed of bio-assays is certainly strongly dependent on them.

In retrospect, the idea (that growth factors for micro-organisms would also be vitamins for animals) was an excellent one, and few, if any, exceptions have been found. It is a significant fact, however, that the common ground includes only the water soluble vitamins. Requirements for the fat soluble group are peculiar to animals, even though micro-organisms produce some of them. In the great vitamin outburst of 1931 to 1945, animal assays and biological determinations using yeast and bacteria went hand in hand, but there can be little doubt that much of the explosion of activity and discovery was due to the use of micro-organisms.

By 1930, a good many people had the idea that microbial growth factors might be vitamins and a feverish competitive period began. Basic science temporarily ran away with itself in competition with industrial research and development. There appeared to be economic gain in vitamins, and indeed, the business adds up to several hundred million dollars a year today.

Along with the vitamins, the science of microbiology grew by leaps and bounds as investigators made use of all sorts of yeasts, bacteria, and molds in an effort to find new growth factors. Once found, the substances had to be isolated as pure chemicals, and a new methodology for isolations grew also. This isolation was no mean task, since raw materials usually contain vitamins in amounts in the order of one part per million. The easiest way to illustrate this phase—the isolation—is from personal experience.

Vitamins and microbiology

Following the observation that vitamin B_1 (thiamine) was required for yeast, Roger Williams proceeded to examine tissue extract for other yeast growth substances, and, in 1933, published a paper describing some of the properties of a new substance which he called pantothenic acid. In 1937 I had the good fortune to join the Williams group in the later stages of the work on isolation and synthesis of this vitamin. Following its completion, I began at the beginning for the first time, with a liver extract that seemed to contain a new growth factor for a strain of lactic acid bacteria. This is a nerve-racking stage for the tyro, since it is necessary to test repeatedly all the possible known compounds in order to be sure the growth factor is new. Insomnia is not unknown under such circumstances.

We started with liver—a few pounds at a time—and applied all sorts of extraction, adsorption and precipitating procedures, in an effort to separate the growth factor from the million parts of impurity it contained. Each possible procedure was evaluated by bio-assays with the bacteria. There is some logical science in it, but not as much as one would hope for. Pig liver was a satisfactory source of the factor, and from 100 lbs. we obtained several milligrams of highly purified material—but it looked like a ton or more of liver would become necessary. The war years were on the way then, and for some reason I don't understand, the pig liver supply failed.

I put down the phone after learning this and looked out of the window into the middle distance where a gardener was piling grass cuttings into a heap. By the next morning we knew that grass was a good source of the growth factor, and by the end of the week we knew that a major crop of that area (Austin, Texas)—namely spinach—was an excellent source, and at least as good as pig liver.

There were some odd things about spinach, however. If picked in the early morning, the growth factor was far higher than in the afternoon, and, at any time of day, a treatment with chloroform approximately doubled the activity. Following these salient observations, I spent a good many hours at dawn, gathering spinach in a tarpaulin, with a bottle of chloroform in hand to provide for a maximum yield. The farmer, whose spinach field had been rented, didn't understand these details—nor did I at the time. But they all have a reasonable explanation now.

Some eighteen months, about 10,000 bio-assays, and four tons of spinach later, we obtained a few milligrams of crystalline growth factor. This was approximately 200,000 times as pure as the initial extract of spinach, and in order to obtain a gram or so (about ½ teaspoon) for work on chemical structure, it was estimated that about ten times as much spinach would be needed. Accordingly, about 30 more tons (two carloads), were processed with the assistance and equipment made available by one of the drug houses.

Soon after it was established that the lactic acid bacteria growth factor was a new compound, it acquired a name based on its abundance in spinach. The suggestion, folic acid (from *folium*=leaf) by Williams seemed suitable, and this name has been retained by common usage.

A crowded field

As in all cases during this period of great activity in the isolation of growth factors, we were not alone in the field for long, and probably we were not the first to recognize the existence of folic acid. A group of investigators working on substances that would prevent and cure certain types of anemia in monkeys were dealing principally with folic acid in a concentrate they called vitamin M. As subsequently established, folic acid is indeed a vitamin for animals, being effective in man for the cure of certain kinds of anemias.

I expect that this yarn about the isolation of folic acid is more or less typical of many others that could be told of vitamin work during this period of time, although each must have had its own peculiarities. The field became commercialized very rapidly, to the point where industrial research teams took it over almost entirely. By 1945, the rate of discovery of new growth factors had dropped to a low level, although the search is still in progress. Vitamin B_{12} is relatively recent, and only six months ago one of the industrial laboratories announced the isolation of a new growth substance (biopterin) for a species of protozoa. For other reasons, the same substance was isolated and synthesized here at Caltech last year by Hugh S. Forrest, a senior research fellow. Vitamin activity for higher animals remains to be established.

A further point of some interest, already touched on, concerns the quantity of vitamins in tissues. The folic acid case is somewhat representative, and tons of raw materials were needed for isolation of a small amount. Vitamin B_{12} usually occurs in an even lower concentration, while at the other extreme, only a few pounds of raw material is needed for vitamin C isolation.

At the present time, both vitamin B_{12} and riboflavin are produced commercially as a byproduct of mold fermentation. B_{12} comes from a fungus that also produces the antibiotic, streptomycin, whereas the riboflavin is produced in such fantastic quantities by a cotton parasite that it crystallizes out in the vacuoles within the mold and sometimes in the culture medium.

However, in general, there is rough parallelism between concentration in tissues and nutritional requirements. One pound of the least abundant vitamin (B_{12}) is sufficient to provide one day's requirement for about one third of the population of the United States.

A remarkable result of the great upsurge of vitaminology is that now, in this country, we have the best fed chickens and pigs in the world. There is still much to be done with people. One would think that the economic value of productivity by people would be almost as obvious as that of domestic animals, but it is more subtle, of course. In any case, chickens and pigs are the animals of economic value that were used extensively for evaluating new vitamin discoveries, and in terms of eggs and meat, vitamin supplements pay good dividends.

The human side

The human population is somewhat better off too. Through the efforts of some government agencies and private organizations, such as the Nutrition Foundation (industrial support) and the Williams-Waterman Fund (from vitamin B_1 patent royalties) there is now a widespread practice of restoration of vitamins removed during the processing of food for human consumption. This has helped, as has the prescription of vitamins by medical practitioners. Nevertheless, there is little doubt but that additional vitamin supplements would benefit some three quarters of the population of this country, and almost all of the population in many parts of the world.

A summary of some basic information on human vitamin requirements is given in the table at the right. It is well established that all of these substances are essential for human existence, but good diets supply maintenance quantities of most of them, and clear-cut deficiencies in man are not even known for all of them.

Herein lies a subtlety that is most difficult to evaluate for humans, but which has been well documented for chickens and pigs. This has to do with the fact that there is a considerable difference between maintenance and maximum productivity. This question was properly raised and evaluated in an excellent popular book, *Vitamins, What They Are and How They Can Benefit You,* written in 1941 by Henry Borsook, Caltech professor of biochemistry. The value of dietary vitamin supplements for people who live in good economic circumstances was considered in terms of the difference between just existing and existing with the zest of well being. We are appreciably better off now than we were in 1941, but there

VITAMINS
What they are—and what they do

FAT SOLUBLE VITAMINS	DEFICIENCY DISEASE IN MAN	SPECIFIC METABOLIC FUNCTION
Vitamin A	Night blindness	
	Xerophthalmia (degeneration of eye parts)	
	Skin Keratinization	
Vitamin D	Rickets (malformation of bones and teeth)	
	Osteomalacia (decalcification of bones)	
Vitamin E		Indications of involvement in oxidation.
Vitamin K	Hemorrhage	
WATER SOLUBLE VITAMINS		
Vitamin C (ascorbic acid)	Scurvy (multiple abnormalities in tissues and bones)	
Thiamine (Vitamin B_1)	Beriberi (polyneuritis, mental and physical depression, etc.)	Removal of carbon dioxide from and oxidation of keto acids.
Riboflavin (Vitamin B_2)	Mouth sores, vision impairment, dermatitis, etc.	Biochemical oxidations
Niacin (Nicotinic acid)	Pellagra (dermatitis, inflamation, psychic changes, etc.)	Removal of hydrogen atoms from a variety of biochemical compounds.
Pyridoxine (Vitamin B_6)	Mental depression, blood disturbances, etc.	Utilization of amino acids. Nitrogen metabolism.
Pantothenic acid		Metabolism of organic acids and fats. Transfer reactions.
Choline	Liver disease	
Inositol		
Biotin	Dermatitis, muscle pains, etc. (Produced in man by feeding raw egg white.)	
Folic acid and citrovorum factor (Folinic acid)	Anemia, diarrhea, etc.	Utilization of one-carbon compounds. Synthesis of nucleic acids. (Exact cofactor not known)
Vitamin B_{12}	Pernicious anemia	

is still room for improvement. In this country it is within the reach of each of us.

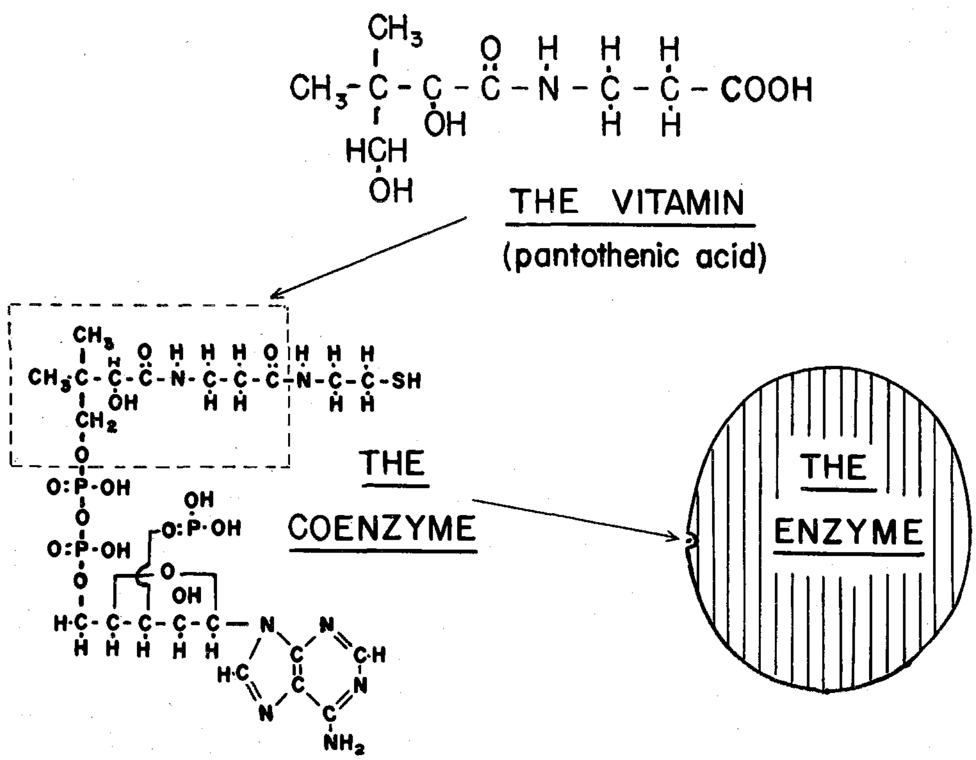
The total number of publications per year on vitamins is now at an all-time high, and even on a percentage basis there is a decided upswing. Although part of this is due to increased interest in nutrition as such, the principal reason is derived from recognition of the importance of finding out just exactly how the vitamins function. The need for understanding function is not nearly as obvious as that for obtaining vitamins in order to cure clear-cut dietary deficiencies. It rests on the fact that vitamins are absolutely essential in all living organisms, whether healthy or not, in order to build or replace tissue, and in order to burn food to obtain energy.

Diseases of all kinds influence the balance of the very complex pattern of chemical reactions that produce tissue or energy, and in order to combat such adverse influences in an understanding way, it is necessary to know what chemical reactions occur and what determines their rates. The vitamins are key substances in this picture in that they are rate-controlling materials.

We have made only a beginning in understanding exactly how the vitamins function, but it is a fair generalization at present that they go to make up part of the biological catalysts, the enzymes. An example of how this comes about is diagrammed below. The vitamin, in this example, is pantothenic acid and it has the arrangement of atoms shown. This substance cannot be made by the tissue and it must be provided in the diet in order to combine, first with other substances to produce the co-enzyme, and then with a certain kind of protein to give the enzyme. As indicated in the diagram, the enzyme is very much larger than the co-enzyme, but the most specific functional part of the enzyme is the —SH group of the co-enzyme. This actually was not even part of the vitamin, nor was it directly a dietary essential. Nevertheless, for its function, the enzyme requires all of the parts put together in this particular structure.

The body contains hundreds of specific enzymes and a great many of these carry vitamin co-enzymes. It is their function to speed up biochemical reactions, and the utilization of all foods requires enzyme action, some beginning the instant food enters the mouth. In the example given here, a combination of a variety of organic acids with the —SH group of the enzyme permits rapid chemical reactions such as those needed for the formation and combustion of fats.

The water soluble vitamins particularly are known for their co-enzyme functions and these have to be supplied daily. A considerable excess intake is harmless, and of no real value except in some pathological conditions. The specific functions of the fat soluble vitamins, in general, are not known. These can be stored in body tissue and some may have toxic effects when taken in great excess. A reasonable daily intake is probably the best procedure here too. In the immediate future much remains to be done to establish further the specific kinds of biochemical mechanisms in which the vitamins participate. Then too we need to know what contribution each potential mechanism makes to maintenance of and to well-being in the living organism.



An example of how vitamins go to make up part of the biological catalysts, the enzymes. Pantothenic acid cannot be made by the tissue, so it must be provided in the diet in order to combine, first with other substances to produce the co-enzyme, and then with a particular protein to give the enzyme.