SOME day—and the day may not be far off—a white-coated scientist is going to look up from his data books or his laboratory bench and tell himself, with no more excitement than his scientist's caution allows, "This is it!"

It will be a new vitamin or a new growth factor that he and perhaps many other laboratory workers have tried for a long time to find or identify exactly.

This new vitamin, or a food factor, will be a link between the first findings in nutrition years ago and today's research. It will push farther back the frontiers of man's knowledge. It will benefit people or animals or both. It will be as exciting as the solution of a mystery story and perhaps as outstanding as Dr. Jonas Salk's discovery of vaccine for poliomyelitis.

It may rank alongside the discovery of vitamins A, C, and D and the B-complex vitamins, which were complete mysteries before 1920 and minor mysteries between 1930 and 1955, when their chemical structures were discovered.

For there are still discoveries to be made of unidentified substances in foods.

Natural foods, such as milk, meat, eggs, vegetables, and cereals, contain at least several important nutrients and substances whose identity we still do not know. We call them unidentified factors. The list of them has been dwindling steadily as our knowledge has grown, but the ones remaining in 1959 seem to be important in nutrition.

How do we know that unidentified factors exist?

To find out, we start with a purified diet that contains all known nutrients in ample amounts in the form of a mixture of pure proteins, carbohydrates, vitamins, minerals, and fat. We feed it to an experimental animal and keep a record of the results. If we can get better results—such as improved growth or reproduction—by adding any natural food to this diet, we can be fairly certain that an unidentified factor is present in the natural food. White rats, mice, guinea pigs, chicks, and other small animals are the favorite "tool" for this work.

The next job is to identify the factor. That is not easy. We make concentrates—or extracts—of the factor from the natural food by chemical fractionation. We test the concentrates by adding them to the original purified diet.
and feeding the diet to the test animal. Thus we can follow the activity through repeated tests to the final isolation of the pure substance and to its identification. This is more difficult than it sounds and may take many months or years. There are many pitfalls and blind alleys, which can slow down the work considerably.

Often it is possible to find a 1- or 2-day test for the unknown factor if we use certain fast-growing microorganisms as the test "animals." That hastens the process. Most of the B vitamins were discovered in this way—by using bacteria.

The discovery of vitamin B\textsubscript{12} in 1948 is an example of how unidentified factors become known and identified. A number of supposedly different unidentified factors were being studied in different laboratories before 1948. Many of these turned out to be vitamin B\textsubscript{12}. As far back as 1926, Dr. George Richards Minot and Dr. William Parry Murphy, of Harvard and Boston, discovered that pernicious anemia, an incurable disease in man until then, could be treated by feeding large amounts of liver. Concentrates of the antipernicious anemia factor, as it was called, became available in the form of injectable liver extracts in a few years—but attempts to identify the active factor were unsuccessful.

Two groups of workers at the Agricultural Research Center at Beltsville, Md., had been working with unidentified factors for animals since the 1930's. Their investigations were along two different lines.

A number of investigators in the Poultry Division—Drs. Theodore C. Byerly, H. W. Titus, H. R. Bird, A. C. Groschke, N. R. Ellis, J. C. Hammond, M. Rubin, and others—studied an "animal protein factor." They learned that it occurred in protein concentrates from such sources as fishmeal and meat scraps and helped growth and reproduction of poultry. They discovered by a fortunate observation in 1946 that ordinary cow manure is an excellent source of a similar unidentified growth factor for chicks. It became known as the cow manure factor. Long and tedious attempts to purify and identify the substance were unsuccessful, but the scientists were able to prove that the factor is synthesized by micro-organisms in the rumen.

C. A. Cary, A. M. Hartman, and their coworkers in the former Bureau of Dairy Industry of the Department of Agriculture were working at the same time on what was thought to be another unidentified factor in milk. It was necessary for the growth and reproduction of laboratory rats. This factor was different from all vitamins and minerals known at that time (1943 to 1947). They called it factor X. Many time-consuming studies were made to identify the factor, but it proved to be elusive and difficult to purify. These scientists found that antipernicious anemia factor concentrates were good sources of factor X, a finding that later proved to be useful to others (even though they believed at the time that the two factors were not identical).

In another laboratory, Lois M. Zucker and T. F. Zucker of Columbia University, a husband-and-wife team, were studying growth effects obtained by adding casein (the protein of milk), liver, or fish solubles to purified diets for rats. They obtained concentrates of an unidentified factor, which they named "zoopherin."

Other workers with rats and chicks in various laboratories, including the University of Wisconsin, Cornell University, and Lederle Laboratories, confirmed these findings and were actively attempting to identify the animal protein factor, as it was most commonly called in 1946 to 1948. All found the animal tests expensive and difficult.

Still another line of attack was being made to isolate this unidentified factor. Mary Shorb, a bacteriologist, working first at Beltsville in the former Bureau of Dairy Industry and later at the University of Maryland, discovered in 1947 that a certain bac-


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Lactobacillus lactis (Dorner), would not grow unless concentrates of the antipernicious anemia factor for humans were added to their food. She called this unknown substance the LLD factor, from the initials of the name of the bacterium.

Following this discovery, Dr. Shorb and I, at the University of Maryland Poultry Department, attempted to purify the LLD factor further. We had little success, but we were able to improve the assay enough so that it could be used by ourselves and others in short-time routine tests to identify the compound.

Using the Maryland test with bacteria, research workers in the Merck & Co. laboratories in Rahway, N. J., isolated a deep-red, crystalline compound from liver and other sources in 1948. Like others, the Merck laboratory had spent thousands of dollars and many years in attempts to find the antipernicious anemia factor. The goal was reached by the use of the 2-day bacterial test, which was better than testing each new batch on hospital patients suffering from pernicious anemia. The new compound was named vitamin B\textsubscript{12}.

Thus a scientific mystery of long standing was solved in 1948, and many loose ends were brought together. After the discovery of vitamin B\textsubscript{12}, scientists proved that it was not only the LLD factor but also the antipernicious anemia factor for humans, factor X, the animal protein factor, the cow manure factor, and zoopherin for chickens, turkeys, and mice.

The recognition of selenium as a trace element in nutrition goes back to 1951 to the work of K. Schwarz and his co-workers at the National Institutes of Health in Bethesda, Md. They discovered that some foods, such as milk, brewer's yeast, meat, and certain cereals (corn, soybeans, and wheat) contain an unidentified substance, which they called factor 3. It prevented liver damage and death in rats fed special diets low in vitamin E and low in cystine, an amino acid.

After 6 years of intensive study and purification, Dr. Schwarz and his group in 1957 discovered that highly purified concentrates of factor 3 contained selenium. Certain crystalline salts of selenium fed in small amounts (0.1 part per million or less) prevented liver damage in rats. This amount is far less than the amounts necessary to produce the well-known toxic effect of selenium in feeds.

Several groups of workers discovered soon thereafter that selenium-containing compounds are also exceedingly active in preventing exudative diathesis in chicks (a condition characterized by fluid under the skin), liver damage in pigs, and heart and kidney damage in mice.

Vitamin E had to be absent from the diet before the selenium compounds were effective in all animals. Vitamin E prevented the conditions, but it was only about one five-hundredth as active as the selenium compounds. Not all the functions of vitamin E could be replaced by the selenium compounds, however.

An active search began in 1957 to find the most active selenium-containing compounds in foods and to find out why selenium-containing compounds are necessary under these conditions. As is typical of such studies, complete answers will not be found until after years of devoted work on thousands of animals and much expense.
Nobody can say how many nutritional factors remain unknown or how soon they will be discovered.

I list some of the substances that seem to be necessary for animals and that were not identified in 1958. We have little information about their importance in human nutrition.

Streptogenin is the name given to a growth factor for certain bacteria by Dr. D. W. Woolley, of the Rockefeller Institute for Medical Research in New York, in the 1940's. It is present in many natural foods and is associated closely with proteins. Highly purified diets or synthetic media must be used in studies of it.

Certain peptides—compounds containing several amino acids—are known to have streptogenin activity in bacteria. Thousands of man-hours have been spent to identify the substance, but after 15 years of work the final answer has not been found. Its discovery must await the development of new techniques in protein and amino acid chemistry.

Studies with chicks in our laboratories at the National Institutes of Health and in other laboratories have indicated that this unidentified factor may be important for animal growth.

Protein sources, such as egg yolk, liver meal, dried whey, peanut meal, and fishmeal, have been reported to contain an unidentified vitamin (or possibly more than one) for the growth and reproduction of chickens and turkeys and for the growth of swine.

Drs. Henry Menge, Robert J. Lillie, and Charles A. Denton, of the Agricultural Research Service, have conducted extensive studies at Beltsville on this factor. Much work has been done at many State agricultural experiment stations and commercial laboratories.

In order to get growth responses with these materials, levels of as high as 5 to 12 percent of the material must be used in the diet. This would indicate that the protein portion of the crude materials might be responsible for the growth effect. The growth responses cannot be duplicated with pure proteins or with amino acids, however.

Little progress had been made up to 1959 in the identification of the factor (or factors), which at various times has been termed the egg yolk factor, liver factor, whey factor, and fish factor. Much has been written about it. It promises to be important in animal nutrition and possibly for people.

The grass juice factor, or the forage juice factor, was reported in 1938 to be necessary for guinea pigs. With the discovery of several other B vitamins in 1938 to 1948, the grass juice factor was almost forgotten. Now there is renewed interest in it. In studies in our laboratory and elsewhere, evidence has been found of this growth-promoting factor in grass, alfalfa, and possibly other green forage and pasture crops. It may also be present in plant seeds, such as corn, soybean meal, and wheat.

One might suspect that the factor would be important to grass-consuming animals, like sheep and cattle. It is not safe to make too many predictions about unidentified factors, however. The unexpected is more usual than the expected.

The grass juice factor is a good example of how long studies on unidentified factors often take. It takes particularly devoted scientists to stick to a problem, for example, that goes on for more than 20 years without a final answer.

Unidentified trace minerals, with nutritional activity for poultry, swine, and ruminants, exist, according to scientists' reports in 1957 and 1958. A number of trace elements are known to have important functions in plants or in lower forms of life, but their importance to animal nutrition has never been proved. Among them are such minerals as nickel, aluminum, boron, silicon, and vanadium. These
elements could not be considered as essential trace elements for animals in 1959, but no one can predict what will be found in future years.

Experiments with trace minerals are just as difficult to perform as studies with vitamins. It is extremely difficult to remove the very last amount of a mineral from purified diets so that their role in animal nutrition can be studied. Often a study becomes completely worthless if as little as one part in a million of a mineral remains in a test diet.

Promising new techniques are being developed, however, for studies on unidentified minerals. Scientists in some laboratories use animals that are the offspring of several generations of animals fed special diets. This is done in order to remove all traces of the mineral from the animal. In other laboratories, compounds are fed that are unique in being able to tie up the last remaining traces of an element within the animal.

In still other studies, animals are given special feeds produced on soils absolutely devoid of certain trace minerals. The curiosity of a nutritionist is unlimited, and he will go to any extremes possible to discover new facts.

The unidentified antitoxic factors found in natural foods counteract the effects of toxic agents in animal nutrition.

Factors in meat and liver, for example, are known to prevent the toxic effect of large doses of the thyroid hormone and cortisone. Unidentified factors in meat overcome the toxicity of the drug thiouracil.

The study of antitoxic factors is but one pathway that may lead to the discovery of a new vitamin or growth factor. It is possible that a small amount of an antitoxic factor may be necessary by animals even in the absence of the toxic factor. The importance of these factors in animal or human nutrition was not known in 1959.

Growth factors in microbial products (such as brewer’s yeast, distiller’s solubles, bacterial and mold cultures, and fermentation residues) continue to receive attention by men who study nutrition of animals. Such products appear to contain unidentified factors for bacteria as well as for animals. It is possible that short-time tests for these factors can be developed with bacteria to replace the much longer animal tests.

No predictions can be made at this stage of research as to the actual importance of the factors in animal and human nutrition. However, even now crude sources of these factors are being used with apparent benefit in commercial poultry and swine rations: It is possible to make good use of unidentified factors in nutrition even before they are identified.

Bacteria, protozoa, insects, and other lower forms of life are known to require unidentified factors besides those I mentioned previously.

Such compounds, when they are identified, are known as growth factors, rather than vitamins, because, by definition, a compound must be shown to have a beneficial physiological function by one or more of the higher animals—vertebrates—before it can be called a vitamin. Furthermore, by definition, the compound must be an organic substance in natural foods but not a fat, carbohydrate, or amino acid.

Unidentified growth factors for lower forms of life include one or more substances in bacterial cultures for Lactobacillus casei; a growth factor in liver for Lactobacillus leichmannii; a corn-leaf factor for the European corn borer larva; and an unknown substance in spleen for Escherichia coli.

Many others have been reported. We do not yet know the importance of these growth factors in the nutrition of higher animals, including people. The answer can only be obtained by further study.

On the one hand, many of our present B vitamins were discovered to be needed by bacteria (folic acid, vitamin B₁₂, and biotin, for example) before we knew they were needed by animals.
On the other hand, many important growth factors for lower forms of life have no known role in vertebrate nutrition. These should not be considered as vitamins. Among them are the purines and pyrimidines, the bifidus factor, para-aminobenzoic acid, melvalonic acid, inositol, lipoic acid (thioctic acid), various sterols (including cholesterol), nucleosides, orotic acid, asparagine, carnitine, shikimic acid, and others.

Growth promotants are compounds that stimulate the growth of animals by indirect ways, usually by some type of action in the intestinal tract. Some may improve absorption or stimulate the synthesis of nutrients in the intestine. Some may prevent subclinical diseases—diseases that are present but do not severely affect an animal.

Such growth-promoting compounds as the antibiotics, surface-acting agents (such as ordinary detergents), organic arsenicals, several sulfa drugs, various drugs used for the prevention of coccidiosis, and other drugs act in this manner. These substances, not present in most natural foods, should not be confused with vitamins, which the body cells need for normal metabolism.

Some of the unidentified factors reported to be necessary for animals undoubtedly will fall into the class of growth promotants rather than vitamins when they eventually are isolated.

It is highly possible, for instance, that the so-called vitamin B13, found in fermentation products and distiller's solubles, is really a growth promotant. The only way one can be sure is to characterize chemically the growth-stimulating compound and study it in animals in the laboratory.

A vitamin B14 and vitamin B15 have been mentioned since 1948, but their real importance to animal or human nutrition is not known, and the terms should be dropped until such information is available.

Known nutrients have been inadvertently rediscovered many times, because of inadequate levels of minerals or vitamins in experimental diets or because of nutritional imbalances. Because of the complexities of experimental work in this field, one should regard unconfirmed announcements of new unidentified factors with caution.

Nutritional knowledge has improved so much since 1930 that today animals are being reared on synthetic diets of known composition composed of highly purified ingredients—sugar, fat, proteins, vitamins, and minerals—with no natural foods at all. In fact, white rats and chickens have been carried through several generations on such diets.

These are convincing demonstrations that the most essential vitamins for these animals have been discovered, except perhaps the substances present in protein. Similar statements cannot be made for the guinea pig, the turkey, or the pig.

Studies with synthetic diets in human nutrition over long periods are especially incomplete.

Because we know that unidentified factors do exist in foods of plant and animal origin, it obviously is wisest to eat a wide variety of foods from the many excellent food groups—milk and milk products, meats, eggs and poultry, fish, cereals and grain products, vegetables, and citrus and other fruits. One who does this routinely does not need to eat vitamin pills or the so-called health foods (like wheat germ, molasses, yoghurt) to supply known nutrients and the unidentified factors that may be necessary for optimal health except on the advice of a physician or except by personal preference.

When scientists develop further information on the present unidentified factors in food, people will benefit.

George M. Briggs has been doing research in nutrition, particularly on vitamin B complex and unidentified factors, since 1940. He took an active role in early studies on folic acid and vitamins B12, B11, and B12. In 1956 he became Executive Secretary of the Biochemistry Training Committee, Division of General Medical Sciences, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md.