of the oil. Should the latter be sufficiently great, it will undoubtedly be reflected in commerce and in the Pharmacopœia.

Another instance of the kind may be selected from our work. The aldehydes[1] present to a very slight extent in the natural oil are highly important in the suggested scheme of biochemical oil formation. On the other hand they are now refined away during rectification because of their objectionable properties. To study them more closely, material representing 12,000 pounds of natural oil[2] was worked up, yielding approximately 500 Gm. of isovaleric aldehyde. The mere handling of such a quantity of material suggests to the thoughtful experimenter ways of utilizing a by-product.

Although only a few of the more important developments have been sketched, we hope to have shown that a prosaically common pharmaceutical article like oil of peppermint may offer a little universe of fascinating ideas and problems to the inquisitive mind. We hope that the dispenser of this oil will share with us the sense of pleasure which these ideas bring and will regard his bottle of oil as a connecting link between himself and the living out of doors.

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## PROGRESS IN VITAMINE RESEARCH.*

BY CASIMIR FUNK AND HARRY E. DUBIN.

Vitamine research has advanced along three lines: first, the demonstration of the actual existence and the nature of specific vitamines; second, the study of the vitamine content of various foodstuffs, raw and cooked; third, investigation of the chemistry of the vitamines.

As regards the chemistry of the vitamines, little of real significance has been accomplished. A number of vitamine concentrates have been made by various investigators, while we ourselves have prepared concentrates from yeast and cod-liver oil which are approximately from five thousand to ten thousand times as active as the starting material. Nevertheless, despite this high degree of concentration, we are still far removed from the isolation of the various known vitamines and the determination of their chemical composition.

The study of the vitamine content of different foodstuffs is of practical importance in that particular dietaries may be chosen with confidence in the effects they will produce. In this direction, our knowledge of nutrition has been materially augmented.

However, it is the demonstration of the existence of specific vitamines which has spelled the greatest progress. Until very recently, only three vitamines were known:

A—The antirachitic vitamine, found in certain fats and oils and in the leafy parts of some vegetables.

B—The antiberiberi vitamine, occurring in a variety of grains, vegetables, fruits and in yeast.

C—The antiscorbutic vitamine, present in certain fruits and vegetables.

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[1] Ref. to R. E. K., *Jour. Biol. Chem.*, 50, 31, 1923.

[2] Generously furnished by the A. M. Todd Co., of Kalamazoo, Mich.

* Read before the Scientific Section, American Pharmaceutical Association meeting at Asheville, N. C., Sept. 3 to 8, 1923.

The above classification was made in 1913 by Funk, who suggested at the same time the possibility of the existence of other vitamines. That this prophecy has been amply fulfilled will be presently made evident.

The vital importance of these chemically unknown substances, the "infinitely little," as they have been called, has been established over and over again by a host of investigators. Furthermore, not only must there be sufficient vitamines present in the diet, but it is also necessary that there be a suitable relationship between the other dietary constituents. There must be a suitable balance between the proteins, carbohydrates and fats of the diet as well.

The original classification of Funk held good for a long time, but little by little, evidence accumulated which tended to show that vitamine A was not identical with the antirachitic vitamine, and that vitamine B included something else besides the antiberiberi vitamine.

Funk and Dubin proved that what had been known as vitamine B really included another vitamine, named vitamine D, necessary for the growth of microorganisms. Soon after this, McCollum was able to demonstrate that vitamine A, originally known as the antirachitic vitamine, apparently consisted of two vitamines—one the antirachitic, the other the antiophthalmic. McCollum designated the latter vitamine A, while the former is generally called vitamine E.

We have, therefore, at the present time, definite knowledge of five vitamines. We need not concern ourselves here further with the vitamines first classified as the antiberiberi, antiscurvy and antirickets vitamines. As for the antiophthalmic vitamine and vitamine D, a few words will not be amiss.

Regarding vitamine D, we do not know what part it plays in animal metabolism. That it has some function seems likely, since it is found in association with vitamine B. It is, therefore, necessary that investigators desiring to study the action of vitamine B should make sure that vitamine D has been eliminated. This may be accomplished in two ways—one, by fractional adsorption with fuller's earth or charcoal; the other, by allowing microörganisms to grow in a medium containing a mixture of these two vitamines. In the former case, vitamine B is removed, while in the latter, vitamine B is left in solution, vitamine D being utilized by the growing cells. Under suitable conditions, the separation of vitamine B from vitamine D can be made complete.

Certain microörganisms cannot multiply in the absence of vitamine D. Under such circumstances, yeast presents a spore-like shrunken appearance. All statements to the contrary are due either to the contamination of the medium, as shown by Funk and Freedman, or to the presence of a marked quantity of vitamine D in the transplants.

The present importance of vitamine D lies in the fact that without this factor, life is dormant, and no synthesis of vitamine B occurs.

It is quite generally conceded that vitamines cannot be synthesized by the body, so that we must depend upon our food for an adequate supply of these factors. On the other hand, fat-soluble vitamines, antirachitic and antiophthalmic, can be stored in the body. Steenbock, Sell and Nelson showed that the liver varies in fat-soluble vitamine content with the ration fed. Thus, an individual may continue in health on a vitamine-poor diet immediately following one rich in vitamines, when a surplus is accumulated.

Another important advance in this field of research is the recognition of the divergence of the vitamine requirements of different species. Apparently not all of the known vitamines, possibly excepting vitamine B, are essential to all species. For instance, a diet which will suffice for pigeons will not do for rats, and one that is adequate for rats will not do for guinea pigs. Rats, dogs, pigeons and chicks do not need vitamine C, while for monkeys and man it is indispensable. Again, antirachitic vitamine seems necessary alike for dogs, rats and man, while the anti-ophthalmic vitamine, so essential to animals, seems to be little needed by man, in whom the characteristic ophthalmia due to the absence of this particular vitamine is rarely seen.

In this connection, it is obvious that there is much to be learned. Conceivably, research along this line may result in the discovery of new vitamines. We may mention here the work of Evans and Bishop and of Mattill, who have found that on a synthetic diet, regarded as perfect, growth can be normal, yet a substance seems lacking that is essential for reproduction. This substance has been shown to be present in lettuce and whole cereals, but it has not yet been identified.

Further progress was marked by the work of Hess and of McCollum and his collaborators. They point out that cod-liver oil appears to contain two vitamines—one having antirachitic action; the other, antiophthalmic action. The importance of this finding is not to be underestimated, even though unquestionable proof has not been submitted that we are really dealing with two distinct vitamines and not with a single vitamine, which possibly loses one of its properties by some internal molecular change. Thus, oxidation or reduction eliminates the antiophthalmic vitamine and leaves the antirachitic vitamine intact. Cod-liver oil is rich in both of these lipo-vitamines; they are also present to a lesser degree in egg-yolk, while butter is poor in antirachitic vitamine.

That the number of specific vitamines mentioned thus far by no means excludes the likelihood that new ones will be discovered is strongly suggested by a study of pellagra. It is known that certain types of diet produce pellagra while others do not. Goldberger and his associates have come to the conclusion that the most significant difference between these two types of diet is their content of protein of animal origin. They state that the proteins in pellagra-producing diets may lack certain important amino acids; at the same time, they admit that an unknown vitamine may play a rôle in the cause of pellagra. Hindhede, the noted Danish expert on nutrition, is a staunch supporter of the latter conclusion, with which we are also in agreement.

Despite the prodigious amount of data which has accumulated on the subject of nutrition, many of the problems involved are as yet far from solution. To prove this contention, it suffices merely to take two batches of rats, place one on the best possible artificial diet, plus vitamines, while the other is fed a natural diet. The second series reproduces normally and shows much better growth and resistance. All this shows that we are but at the beginning of our understanding of the nutritive needs of animals and that much more information must be gained before we can hope to master the subject of artificial nutrition.

Another field of investigation which holds out the promise of further development is the study of the influence of diet, particularly of vitamines, on the resistance of the body to poisons and infections. Reid Hunt, experimenting with mice and

rats, has shown that deficient diets produce a lowered resistance to poisons, like acetonitrile. While the mechanism of this lowered resistance remains unknown, it is nevertheless certain that the resistance to infection is actually diminished. These studies have a practical bearing, especially in tuberculosis, in which food has for some time past been recognized as one of the important factors.

Perhaps the most fascinating aspect of vitamine research lies in the possible relationship that exists between insulin and the blood-sugar reducing substance present in yeast and in various plants and vegetables. Working with pigeons, kept on a diet of polished rice only, Funk and Schonborn found that not only was there a lack of vitamine B, but also of a substance which metabolized sugar. In the above experiments, a semidiabetic condition developed with high blood sugar and the disappearance of glycogen. On the injection of a highly concentrated vitamine B, obtained from yeast, beriberi was cured and the blood-sugar returned to normal. Naturally, this is but a beginning and further work should be done in this direction.

As the research continues and the sum-total of our knowledge increases, other vitamines will undoubtedly be found to account for a number of disturbances in normal nutrition. It is in this field that we must look for the most fruitful developments.

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## A NEW METHOD FOR OBTAINING WATER-SOLUBLE GLUCOSIDES FROM PLANTS IN A PURE STATE.

BY SUDHAMOY GHOSH.

The author was working on the problem of isolating the glucoside present in the bark of *Terminalia Arjuna*, the extract of which is used widely as a cardiac tonic by the Ayurvedic physicians of India. The aqueous extract was highly colored.

The use of litharge, which required a rather prolonged heating, partly decomposed the glucoside.

The ordinary method of using neutral or basic lead acetate gave quite clear solutions, but the later operations, *viz.*, complete removal of lead by $H_2S$ (which took a long time) and the subsequent evaporation under reduced pressure, invariably led to a highly colored viscous residue. The presence of either Na- or $NH_4$-acetate in the residue, which decomposes to a certain extent, and its solubility in alcohol made it almost impossible to free the glucoside (which was highly soluble in water) from the above and allow it to crystallize. Further, the presence of acetates, which are cardiac depressants, somewhat vitiates the pharmacological experiments.

The method which was devised by the author was as follows: A finely powdered mixture of lead chloride (about 10% of the weight of the dry bark) and litharge (about one-third of the weight of $PbCl_2$) was put into the aqueous extract of the bark and steam passed for 5 to 10 minutes, while the solution was vigorously stirred