### A SIXTH PATHWAY OF PROGRESS

Finally, the physiologist can frequently link his talents with those of a clinical investigator in the pursuit of collaborative research. Such interdepartmental research is particularly valuable when investigators have interests in similar problems but possess different talents required in their solution. Obvious benefits accrue from such cooperative efforts in the science of research as in the science of warfare. It is important that a feeling of mutual understanding and fellowship exist and, above all, that both parties be ready to disregard self and self-interest. The individual must be subservient to the progress of the research.

Such interdepartmental investigation must necessarily be so ordered that the primary duties of each participant to his own calling do not suffer. Each must be careful to limit his interest. For example, the physiologist who becomes so enamored of clinical problems that he neglects his interest in orderly fundamental studies may be popular with clinicians but soon loses standing among his colleagues.

Older physiologists should ever be ready to assist younger clinical investigators in technical methods, new ways of approach and, if competent to do so, even in suggesting new lines of investigation. Having started a line of investigation and assisted the clinical investigator in the technique of special methods, the physiologist should gracefully withdraw, leaving the field to his clinical associate. In this way, his time becomes free again for other duties or for helping others, but, what is more important, the clinical investigator left with a method as well as a problem is given an opportunity to test his own ability and develop his resourcefulness.

### SUMMARY

During the three decades that have passed, medical science has ascended to a high plateau of achievement. The climb has involved several pathways; among them: (1) the physiological approach toward disease as experiments which nature performs on organisms, (2) the more intelligent interpretation of the functional reactions of the body in disease in accordance with latest discoveries in physiology, (3) the supplementation of observable phenomena through use of laboratory instruments, (4) the assumption of active investigation both on patients and experimental animals by clinicians themselves, (5) the shuttling of problems between clinical and experimental laboratories and (6) correlated research in clinical and physiological departments.

As we look down from the heights we have reached, we have reason to be pleased with our progress; but when we look ahead we become aware that there are still high mountain ranges to be climbed. We realize that their ascent can not be accomplished by employing merely the methods, equipment and strategy that have proved successful so far; we must improve the application of principles that are old and well established, and evolve others that are new. Above all, we from laboratories and clinics must join hands to help each other climb; and through correlated teamwork overcome the great obstacles that jealous nature places in our way.

I have ventured to suggest a few directions which such mutual help may take. They include (1) means by which new fundamental discoveries can be utilized more quickly by clinicians and practitioners of medicine; (2) plans by which younger clinical investigators can be given approximately the same opportunity for training in research technique as their colleagues entering experimental sciences; (3) pleas that the shuttling of problems between hospitals and laboratories of fundamental science may continue in order that the ultimate significance of clinical results may be better understood and that the applicability of fundamental discoveries to nature's experiments may be tested; (4) judicious combination of talents of laboratory and clinical workers, whenever this leads to greater economy of effort and does not infringe upon the primary duties of each participant to his calling.

The spirit of correlation which is involved in all these plans of advance is a silent force which grows not only through mutual interest in each other's problems but also through frank respectful criticism of each other's trends. With such a spirit of correlated effort science marches on.

# THE SYNTHESIS OF VITAMIN $K_1$[1]

By Dr. LOUIS F. FIESER

SHELDON EMERY PROFESSOR OF ORGANIC CHEMISTRY, HARVARD UNIVERSITY

I HAVE a story to tell to-night about the naming of a cat. This was by no means a simple matter, for it involved a fully pedigreed Siamese aristocrat requiring an appropriate and dignified name. Fortunately, it took all summer to conclude the various arrangements involved in getting the cat born, for this gave time for deliberation on a suitable name for the prospective offspring of Oriental Nanki Pooh and his dam

[1] From an address before the Boylston Chemical Club, Harvard University, October 24, 1939.

Sola Bella Maria. It thus happened that by the time the kitten arrived an incident had occurred providing the inspiration for a baptismal name. In the registry our cat will be known as Sin Kai Pooh, and all that remains to the story is to explain that Sin is our attempted pseudo-Siamese equivalent of synthetic and that Kai stands for a new addition to the list of vitamins now available in a pure form for use in medicine.

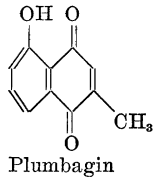
It should be made clear that the discovery of vitamin K and its biological characterization and isolation from natural sources was all the work of others. The brief excursion into the field undertaken during the past summer in our laboratory was of a purely chemical nature and consisted in an investigation of the problem by the methods of synthetic organic chemistry. Guided by an early conception of the probable nature of the vitamin, our work culminated in the establishment of the structure by a synthesis which was accomplished before other evidence was at hand and which has the merit of providing a practical method for the production of the pure material in quantity.

The discovery of the antihemorrhagic principle is due to Henrik Dam, Copenhagen. In 1929 this investigator recognized the occurrence in chicks of a disease characterized by hemorrhages and other pathological changes and by a prolonged clotting time of the blood. Dam established that the hemorrhagic syndrome is due to a dietary deficiency and in 1935 discovered that the preventive factor normally derived from foods is a fat-soluble substance, the "Koagulations-Vitamin." The conclusion was confirmed in independent work by H. J. Almquist and E. L. R. Stokstadt at the University of California. Early tests revealing the presence of vitamin K in various foods were conducted by an approximate preventive method, and Dam's collaborator, F. Schønheyder, later developed a more convenient and accurate curative method (1936). With a satisfactory method of bio-assay available it was soon ascertained that green plants are particularly rich in the vitamin and that dried alfalfa constitutes a suitable source. The initiation of active work on the chemical characterization and isolation of vitamin K was announced independently by Dam and Almquist in May, 1936, both groups reporting the preparation of concentrates by extraction with hydrocarbon solvents and enrichment by adsorption and distillation procedures. In September the problem of isolation was taken up by the research group of E. A. Doisy in St. Louis. The concentration of petroleum ether extracts of alfalfa without destruction of the vitamin proved a difficult task, for the active factor is a delicate substance highly sensitive to alkalis, light and excessive heat, and progress during the three years following the fundamental discoveries of Dam and of Almquist was slow and uncertain. Crystalline preparations believed to be the vitamin or a derivative isolated in this period by Almquist (1937, 1939) and by Doisy (1938) were later recognized as inactive companion substances or reagents.

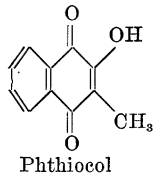
Dam, in attacking the chemical problem, enlisted the cooperation of P. Karrer, Zurich, and in March, 1939, these investigators and their associates announced the isolation from alfalfa of a yellow oil of very high antihemorrhagic activity which they regarded as pure or nearly pure vitamin K. It was difficult to be sure of the absolute purity of the preparation because it was an oil from which no crystalline derivative was obtained, and the authors reserved judgment on this point. They did characterize the substance by bio-assays, by an analysis, by the observation that it gives a beautiful transient purple-blue color with alcoholic alkali and by determination of the ultra-violet absorption spectrum. The absorption curve is of so elaborate a design that it provides an intimate characterization of the vitamin whereby it can be distinguished easily from substances of other types. In May the Doisy group reported the isolation from alfalfa of a similar active yellow oil. Their analytical characterization was more extensive than in the previous work, and a strong case for the purity of the preparation was at hand in the demonstration of a similarity to a second antihemorrhagic factor obtained from putrefied fish meal. While the alfalfa principle (vitamin $K_1$) is an oil at room temperature, the second substance ($K_2$) is a crystalline solid, and by determination of the melting point of samples treated in various ways it was possible to secure substantial evidence of purity. Doisy's vitamin $K_1$ preparation corresponded closely in absorption spectrum with that of Dam and Karrer, except for a displacement of the curve to a region of greater intensity. The intensity of absorption as measured by the extinction coefficient is an accurate index of the relative purity of different samples. Whereas Dam and Karrer had found the coefficient 280 for their preparation, the Americans observed the value 385 and concluded that the previously isolated sample had been only about 70 per cent. pure. They later (June) provided further evidence of the purity of their sample by the preparation of a crystalline diacetate derivative from which material of the original biological potency could be regenerated, and on this occasion they stated that a redetermination of the extinction coefficient had given the still higher value 540. In two further papers Karrer has affirmed the purity of the first preparation but has presented no data in explanation of the discrepancy. Repeated spectrographic determinations on both natural and synthetic samples prepared at Harvard confirm Doisy's first reported extinction coefficient but not the higher value.

In the May report the Doisy group interpreted their analytical results in terms of the formulas $C_{32}H_{46-48}O_2$ for $K_1$ and $C_{40}H_{54-56}O_2$ for $K_2$, but of course with

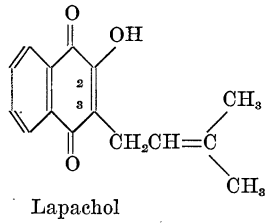
substances of such complexity formulas differing by the increment $CH_2$ can not be distinguished by ordinary analysis. Of particular moment was the suggestion that the vitamins are quinones, a conclusion based upon the evidence that they both contain two atoms of oxygen and upon the lability and the spectra of the substances and their behavior on hydrogenation. This recognition of the quinone character of the antihemorrhagic factors was of particular interest to me because of an association with the chemistry of quinones dating from work for the doctorate initiated in 1921 under Professor James B. Conant. A research on oxidation-reduction potentials was extended in later years to include a study of some 167 compounds of this special series. Several researches had been concerned with quinones of the naphthalene series, including certain naturally occurring substances. Among the yellow pigments known to be naphthoquinones are lawsone, the yellow dye of henna leaves, juglone, which occurs in the reduced form in walnut shells, plumbagin, a pigment of the Indian drug Chita, phthiocol, isolated from human tubercular bacilli, lapachol, from the grain of certain tropical woods and the related pigments lomatiol, alkannin and shikonin. The structures of a few of these substances are shown in the formulas; that of lapachol is particularly interesting because of the presence at position 3 of an unsaturated side chain with



Plumbagin



Phthiocol



Lapachol

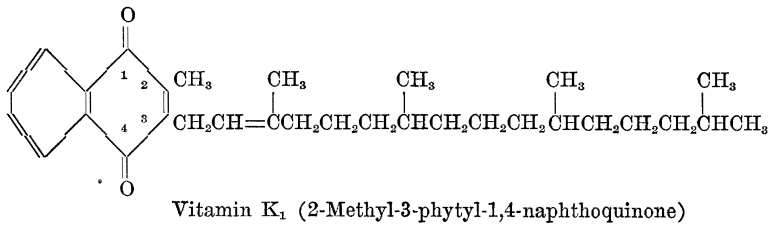
the grouping $C \cdot C \cdot C\ (C)_2$ characteristic of isoprene and of isoprenoid substances such as the terpenes, rubber and vitamin A. Following an extended study of lawsone, interest in lapachol had led to an immensely gratifying association with the late Samuel C. Hooker, whose remarkable investigations in the lapachol field are a classic of masterly experimentation. An outcome of this inspiring contact was the synthesis of lapachol and later (with J. T. Dunn) of plumbagin.

When the vitamin K problem emerged from chemical obscurity into a field actively explored in our laboratory for some eighteen years, we considered at once ways in which the accumulated experience, information and supplies could be employed in advancing the problem. Doisy had found that vitamins $K_1$ and $K_2$ correspond closely in spectrum and they therefore must be of the same structural type. Since they both possess antihemorrhagic activity, even though they differ considerably in composition, it occurred to us that simpler quinones of the proper general molecular pattern might show vitamin K activity. It was also apparent that the distinctive absorption spectra of the vitamins should provide an excellent guide for discovering the type of quinone required. The behavior on hydrogenation noted by Doisy was reminiscent of my past experiences with lapachol and suggested the presence of an unsaturated side chain. Since the allyl group ($—CH_2CH=CH_2$) is the simplest such chain which can be introduced by synthetic operations, we undertook to synthesize certain allyl quinones of the benzene and naphthalene series.

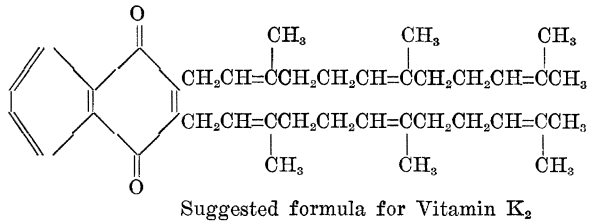
Our experiments were started on May 18, arrangements having been made for assays in the Merck laboratories. On the nineteenth Dr. R. N. Jones, who had surveyed the literature for pertinent spectrographic data, presented an array of comparisons which indicated that the vitamins are α-naphthoquinones. The vitamin factors are characterized by intense bands centering at 246 and 265.5 mμ and a broad band of low intensity at about 322 mμ, and among quinones previously investigated such a spectrum had been encountered only in the α-naphthoquinone series. Dr. Jones noted that the parent quinone and its 2-methyl derivative correspond only roughly, for they lack one of the two intense bands, but that 2,3-diacetoxy-1,4-naphthoquinone has a spectrum strikingly similar to that of the vitamins, the measurements of Macbeth, Winzor and Price (1935) indicating intense maxima, a 246 and 265 mμ and a broad band at 330 mμ. It seemed probable from this comparison that the vitamins are 1,4-naphthoquinones with alkyl substituents at the 2 and 3 positions, and the presence of such blocking substituents would account for an inertness to certain reagents noted by Almquist. Confirmation was provided a few days later in a letter from Dr. Byron Riegel, Northwestern University, who had found that the oxidation-reduction potential of vitamin $K_1$ is about that expected for a 2,3-dialkyl compound and who independently had conceived the idea of testing simpler substances of this type.

With this concrete basis for speculation, it occurred to us on the twenty-third that all the known facts could be explained on the hypothesis that vitamin $K_1$ is

2-methyl-3-phytyl-1,4-naphthoquinone, or a methyl homologue, and that vitamin $K_2$ is 2,3-difarnesyl-1,4-naphthoquinone (see formulas). Of prime importance



Vitamin $K_1$ (2-Methyl-3-phytyl-1,4-naphthoquinone)



Suggested formula for Vitamin $K_2$

was the belief that the vitamins are derived by a simple process of biogenesis from building units of recognized natural types. A naphthoquinone nucleus seemed clearly indicated, as with the known yellow pigments mentioned above, and analogy to lapachol suggested the presence of unsaturated isoprenoid side chains. From a somewhat daring interpretation of Doisy's hydrogenation results it was inferred that in these side chains vitamins $K_1$ and $K_2$ have one and six double bonds, respectively. The problem then resolved itself into picking isoprenoid alkyl groups having the proper carbon content, degree of unsaturation and composition, and inspection of the following list of known isoprenoid alcohols will show that there was little choice: citronellol ($C_{10}H_{19}OH$, 1 C:C), geraniol ($C_{10}H_{17}OH$, 2 C:C), farnesol ($C_{15}H_{25}OH$, 3 C:C), phytol ($C_{20}H_{39}OH$, 1 C:C), vitamin A ($C_{20}H_{29}OH$, 5 C:C). The alfalfa principle was known to contain approximately 21 carbon atoms in addition to those of the naphthoquinone unit. If two $C_{10}$-groups or one vitamin A residue were assumed as supplying the remaining carbon complement the unsaturation would be greater than that required. A phytyl and a methyl group, however, would give not only the right carbon content and composition, but exactly the degree of unsaturation noted. The fish meal principle must have a total of approximately 30 carbon atoms and 6 double bonds in the side chains, and both requirements, together with the composition, are accurately met by the assumption of the presence of two farnesyl groups, and again there is no alternative. From certain observations of Hooker it could be inferred that the yellow substance has no double bond adjacent to the quinone ring, and the absence of characteristics in the spectrum associated with the system C=C—C=C showed that the double bonds are not adjacent to one another. The symmetrical farnesyl pattern provides a rational distribution admissible on both scores, and indeed it can hardly be a coincidence that only one known alcohol meets all these exacting requirements.

The idea seemed all the more attractive from the evident analogy to vitamin E, a condensation product of phytol with a hydroquinone of the benzene series. It seemed reasonable that the vitamin occurring in green leaves should be the one derived from phytol, for phytol is a constituent of the green leaf pigment chlorophyll. Furthermore, the farnesyl group postulated as present in the fish meal principle is the building unit of the hydrocarbon squalene, which occurs in certain fish oils.

Analogy in support of the structures was soon forthcoming in the observation that the simple model compound 2,3-dimethyl-1,4-naphthoquinone has the vitamin K type of spectrum and possesses definite antihemorrhagic activity. With this favorable indication, a joint communication with Dr. Riegel and his associates was presented on June 12 setting forth the above theory. This was the first suggestion of specific structures and the only one concerning vitamin $K_2$ which has yet appeared. On the appearance of the July *Journal* we learn that a week after our report Doisy and co-workers had presented degradative evidence establishing the naphthoquinonoid character of vitamin $K_1$ and making probable the presence of the phytyl group. Arguing in part from a preliminary characterization of a certain degradation product, they suggested the structure of 2-ethyl-3-phytyl-1,4-naphthoquinone. We felt that a choice between the methyl and ethyl structures was still open and regarded the former as the more likely because of the analogy to plumbagin and phthiocol.

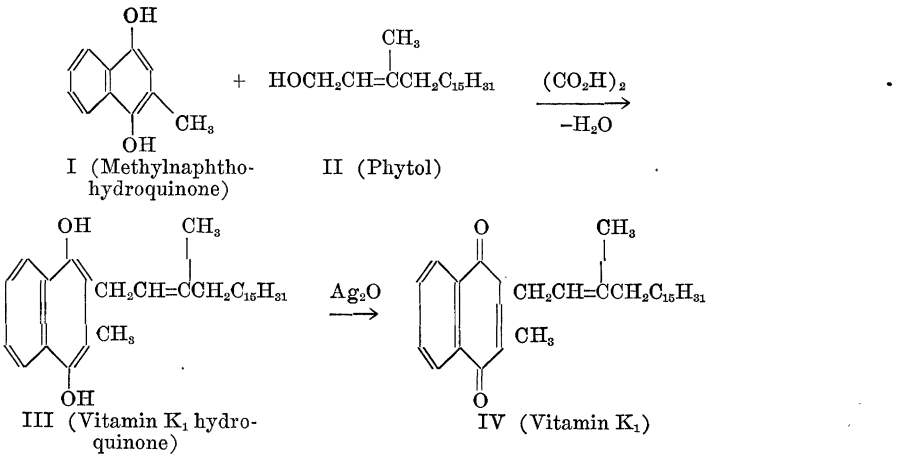
By the end of June we had observed that the allyl-naphthoquinones synthesized as possible models exhibited the color phenomenon stated in Karrer's laboratory to be characteristic of vitamins $K_1$ and $K_2$, and the reaction was recognized as an indication of the presence of a β-unsaturated side chain as postu-

lated. Certain of the models also showed detailed features of the complex vitamin K spectrum. All efforts were then directed toward the synthesis of 2-methyl-3-phytyl-1,4-naphthoquinone. We considered and briefly tried various rather elaborate schemes but always came back to the idea that there must be some simple way of causing phytol to condense directly with methylnaphthoquinone in either the oxidized or reduced form, for this seemed the obvious course of the biosynthesis. Good use was made of model compounds in exploring possible reactions, for the simpler products are more easily identified. It was found that methylnaphthohydroquinone is better suited to the reaction than the quinone, that alkaline condensation can be employed successfully but gives dark products in poor yields, and that mineral acids are too destructive and carry the condensation too far, giving products of the vitamin E rather than K type. A milder acidic reagent seemed called for, and we eventually found that the desired reaction can be brought about using oxalic acid in dioxane solution.

The condensation of phytol with methylnaphthohydroquinone apparently proceeded in the right direction to give some of the desired product III, but this was evidently mixed with by-products of similar properties and with unchanged starting materials. It appeared at first that the separation of the product either as such or as the quinone would be about as difficult as the isolation of vitamin $K_1$ from alfalfa concentrates by existing methods, and this is indeed the case if oxidation by the air is not prevented by suitable use of sodium hydrosulfite. By keeping the materials in the reduced condition the isolation is accomplished in an extremely simple and efficient way. By extraction with dilute alkali the starting hydroquinone I is separated completely from the weakly acidic phytyl-substituted hydroxy compounds, and on treating the residual dark oil with petroleum ether the desired hydroquinone III separates as a white solid and all the impurities and by-products pass into solution. The white solid is collected and washed by centrifugation, and oxidation with silver oxide gives the quinone IV as a light yellow oil in a directly pure condition. The whole operation is easy and the yield good.

This synthesis of 2-methyl-3-phytyl-1,4-naphthoquinone was completed on August 1, and it was at once apparent from a positive color test that it was of the vitamin K type. Within a few days the synthetic quinone was found to give the expected analysis, the absorption spectrum corresponded precisely in every detail with that of the natural vitamin, and in the chick assay the antihemorrhagic activity was comparable with that of the alfalfa principle. The hydroquinone diacetate, prepared for comparison with the crystalline derivative described by Doisy, was obtained as an oil which eventually crystallized in colorless needles having the correct analysis and melting point. This evidence was all complete on August 10, and, after ascertaining that a mixed melting point comparison could not be obtained without some delay, the results were communicated on the twelfth.

The exact correspondence in color reaction, analysis, spectrum, assay and melting point of the diacetate provided a strong indication of identity but not a complete proof. If the vitamin were the ethyl compound as postulated by Doisy one would anticipate correspondence on the first three points and possibly on the fourth. From observations with model compounds we could say that the agreement in melting point of the diacetates probably ruled out the ethyl structure, but probability is not proof. A mixed melting point determination was desirable, even though with fatty substances the absence of a depression may not be an absolute guarantee of identity. Pure natural vitamin $K_1$ was required for the direct comparison, and it occurred to us that the isolation might be accomplished more easily than heretofore by processing the material in the reduced form by the method found



so convenient in working up the synthetic reaction mixtures. It soon developed that a good way to discover how best to isolate a vitamin is first to synthesize it! A small sample (5.3 g) of 3 to 5 per cent. alfalfa concentrate kindly supplied by Dr. Riegel was reduced and put through extraction and precipitation procedures worked out with the synthetic substance, and in a few hours' working time 60 mg of pure vitamin $K_1$ was isolated. This sufficed for analysis, color test, assay, determination of the spectrum and for the preparation and analysis of the crystalline diacetate. In every instance direct comparison of the synthetic and natural substances indicated exact correspondence, and there was no depression in the mixed melting point determination. Finally, 2-ethyl-3-phytyl-1,4-naphthoquinone was synthesized and found devoid of vitamin K activity, an observation which effectively disposed of the only alternate possibility. We were thus in a position on August 25 to state positively that vitamin $K_1$ is identical with our synthetic product and has the structure of 2-methyl-3-phytyl-1,4-naphthoquinone. The structure had been fully established in a program of synthetic research independent of any work on the vitamin itself.

The provisional evidence of Doisy and co-workers pointing to the presence of an ethyl group was found by these investigators to have been in error, and in a communication of August 21 they reported completion of degradative experiments which provided full proof of the structure defined above. They thus announced the structure ahead of us, for we were not able to provide the completing evidence of the mixed melting point determination until a few days later. The St. Louis workers reported a second synthesis, by an alkaline method of condensation, of a product isolated as the hydroquinone diacetate, and on the twenty-fifth they recorded ample evidence of the identity of this derivative with that of vitamin $K_1$.

The elucidation of the structure of the vitamin was one important goal of the chemical investigations; a second was the development of a practical synthesis suitable for the manufacture of the pure vitamin in such quantity as may be required in medical practice. The objective seems to be fully met by the synthesis devised at Harvard, and it may be noted that this first synthesis is the only one which as yet has been demonstrated to afford the pure vitamin as such, rather than as a derivative. Trial batch preparations have been carried out in a commercial laboratory giving in a short working period quantities of vitamin equivalent to as much as 30,000 pounds of dried alfalfa. The synthetic material has been found completely innocuous in toxicity tests and has given excellent results in clinical trials.

The testing of simple quinones for antihemorrhagic activity has been conducted in several laboratories in this country and abroad, and Almquist was the first to discover activity in such a substance (phthiocol). Particularly striking is the discovery of S. Ansbacher and E. Fernholz that methylnaphthoquinone is three or four times as potent in the chick assay as vitamin $K_1$, and it is possible that this available and inexpensive substance may find use in therapy as a substitute for the natural principle. There are reasons for reserving judgment, however, for although the compound apparently is quite safe for administration in small doses and has shown high potency in numerous chick assays it does have a certain toxicity and irritating action on the skin not found in the vitamin, and the results in the early assays were so irregular as to lead three research groups to regard the substance as at most feebly active. The high sensitivity and chemical reactivity of the quinone may present an interfering factor, and if an inexpensive substitute is required it might be preferable to employ a substance more closely related to the vitamin, for example, 2-methyl-3-geranyl-1,4-naphthoquinone. Water-soluble derivatives may ultimately prove of particular value, and a series of hydroquinone esters having this property has been prepared in our laboratory.

Much remains to be learned concerning the origin, functioning and fate of antihemorrhagic compounds. The paradoxical high potency of methylnaphthoquinone may possibly be accounted for on the hypothesis that the administered material becomes reduced and provides one of two components required for a synthesis of vitamin K in the organism paralleling that achieved in the laboratory. Another interesting possibility is that the phthiocol isolated from tubercular bacilli after alkaline hydrolysis was not originally present as such but arose as a degradation product of the antihemorrhagic principle shown by Almquist to be produced by the bacteria. At least it has been proved in our laboratory that vitamin $K_1$ can be converted into phthiocol by gentle treatment with alcoholic alkali.

# OBITUARY

## JONAS BERNARD NATHANSON

ON November 25 death came without warning to Jonas Bernard Nathanson, of the Carnegie Institute of Technology. He collapsed while preparing to attend the theater with his family, and death came shortly after the arrival of the physician. Thus "An able teacher, an accomplished scholar, an earnest seeker after truth, a good and loyal friend is gone from our midst."

Jonas Bernard Nathanson was born on November 5, 1889, in Vilna, Lithuania. He came to this country when quite young and received his early education in