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THE CHEMISTRY OF VITAMINS[1]

THE existence of substances to which the name vitamin has been given was first recognized some fourteen years ago, and since that time much information has been obtained concerning their source, distribution, physiological and other properties, but comparatively little in regard to their chemical structure. This has been due to the fact that until recently it has not been possible to isolate any one of them in a relatively pure condition.

The difficulties in connection with the purification of vitamins have been due, among other things, to the complex character of the raw materials in which they occur, the small percentage present, the destructive effect of many of the reagents and laboratory manipulations used for their fractionation and finally, the want of a reliable chemical test by which the success of the purification steps could be controlled. In the absence of a chemical test it has been necessary to rely upon feeding experiments, which require much time and do not give uniformly dependable results.

In spite of the difficulties which have and will be encountered in the chemical study of vitamins, it is one of the most attractive problems which can occupy the attention of an investigator. The several vitamins perform essential functions in the nutrition of animals and on them depend growth, health, reproduction and completion of the life cycle. There is evidence that they are not of excessively complex or fugitive character, and the prospects of success in determining their chemical constitution is encouraging. Such information when obtained will undoubtedly contribute more to human welfare than can now be appreciated.

The proper selection, preparation and conservation of the food supplies of man and of domestic animals is intimately connected with a knowledge of the chemical properties of vitamins. Future modifications of food products will undoubtedly exceed those of the past. A comparison of the foodstuffs of primitive populations with the package products of to-day will give a faint hint of the extent of the improvements which may be expected in the future.

The modification of natural food products for the purpose of conservation and economical preparation for consumption is a field of endeavor which is deserving of unlimited effort. When there is also con-

[1] Presented before the Division of Medicinal Products at the 67th meeting of the American Chemical Society, April 22, 1924.

sidered the possibility of eliminating specific diseases and reducing the prevalence of others by increasing resistance to infections, through a knowledge of vitamins, the importance of research on their chemistry can not be overestimated.

On account of the very small amounts of vitamin required for striking physiological effects, it has been suggested that they are related to enzymes. Acceptance of this view has undoubtedly turned many from the problem, since the possibility of isolating an enzyme appears now to be very remote. Fortunately, the evidence is increasing that vitamins are less complicated structures than enzymes. The work which it is my privilege to report to-day furnishes additional proof that the characteristic physiological activity ascribed to vitamin B resides in a molecule of reasonably simple composition.

Before directing your attention to the work on vitamin B I wish to summarize briefly the status of current chemical studies on the other recognized vitamins.

VITAMIN A

Considering first the so-called fat soluble vitamin A, which has been found to be especially abundant in cod liver oil, it is now well established that by saponification of the oil, the activity remains unimpaired in the non-saponifiable fraction. This latter is largely composed of cholesterol. In addition there are present lipochromes, fatty acids and substances which give a characteristic blue violet color when the sample is dissolved in petroleum spirit or similar solvent and a drop of sulfuric acid is added. Drummond and Watson[2] have obtained evidence that in liver oils there is an association between the presence of vitamin A and the power to give the typical color reaction.

In an attempt to isolate vitamin A from the non-saponifiable fraction of cod liver oil, Takahashi[3] obtained a semi-crystalline product by the following procedure. The oil was first saponified with alcoholic potassium hydroxide and insoluble calcium soaps precipitated by addition of calcium chloride. The mixture was then saturated with carbon dioxide, after which the alcohol was removed, and the residue extracted with ether. The ethereal extract was treated with dilute hydrochloric acid and the liberated fatty acids separated from it with the aid of alkaline aqueous alcohol. The ether was then distilled off and the residue dissolved in methyl alcohol. This solution was cooled and the cholesterol which separated was removed by filtration. Additional impurities were eliminated by treatment with digitonin and the final filtrate concentrated to a viscous syrup. This was dissolved in a small quantity of methyl alcohol and cooled to — 20° C. From this solution there separated a semi-crystalline product which, when tested on mice, was active in daily doses of 0.08 milligram. It contained C, H and O but no N. Its properties indicated that it was of aldehydic nature.

These experiments are very encouraging and show that a more intimate knowledge of the chemistry of vitamin A only awaits additional experimental work.

VITAMIN C

In regard to the antiscorbutic vitamin C no exhaustive work on its isolation and identification has, so far, been reported. The ease with which it is destroyed by alkalinity and oxidation makes experiments with this vitamin very difficult. Attention should, however, be called to the experiments of Bezssonoff[4] on cabbage juice. His process consists in subjecting cabbages to hydraulic pressure and immediately treating the expressed liquid with neutral lead acetate. The excess of lead is removed from the solution with hydrogen sulfide and the filtered liquid evaporated to dryness at 35° under diminished pressure. The final product is dried over sulfuric acid in a vacuum. There is obtained 2.5 grams of slightly yellowish very hygroscopic powder from each 100 cubic centimeters of the cabbage juice. An apparatus is used which permits all the operations to be conducted in the nearly complete absence of oxygen. The product contains 33 to 46 per cent. of reducing sugars and 52 to 65 per cent. of total sugar. There is present about 7.5 per cent. of ash. It is free of fats and proteins and is active for guinea pigs in daily doses of 0.1 gram.

Recent work by Zilva[5] on the chemical character of the antiscorbutic fraction of desiccated lemon juice has shown that the invert sugar, which it contains, can be eliminated almost completely, without destroying the activity of the solution, by fermenting with yeast in an atmosphere of carbon dioxide. Thus he has obtained a product containing fewer impurities than any potent antiscorbutic so far described.

VITAMIN D

The latest announcements of interest in connection with the chemistry of vitamins are those concerned with the recently identified vitamin D. This product is characterized by its power to accelerate the growth of yeast and is probably identical with the "bios"

[2] J. C. Drummond and A. F. Watson, *Analyst*, 47, 341–9 (1922).

[3] K. Takahashi, *J. Chem. Soc.* (Japan), 43, 828–30 (1922); 44, 580–605 (1923).

[4] N. Bezssonoff, *Compt. rend.*, 175, 846 (1922).

[5] S. S. Zilva, *Biochem. J.*, 17, 410, 415 (1923); 18, 182 (1924).

of Wildiers.[6] Three groups of investigators have recently reported the isolation of crystalline or otherwise well-characterized compounds, which greatly promote the growth of yeast. The most surprising of these is the work done at the University of Toronto and reported by W. Lash Miller.[7] According to these experiments an infusion of malt house "combings," when purified by addition of alcohol and the filtrate precipitated with barium hydroxide, gave two products, neither of which was separately active, but a mixture of the two promoted the growth of yeast almost as well as did the original infusion. The fraction which was carried down by the baryta was designated "Bios I" and the one left dissolved was called "Bios II." The outstanding behavior of each was described, as well as their relative distribution in various products. It was later found that Bios II could be divided into two fractions, thus showing the presence of at least three separable constituents, all of which must be present in the medium to accelerate the growth of yeast.

A more recent announcement is that of Eddy, Kerr and Williams,[8] who report the isolation of a crystalline substance having the properties of bios. The essential feature of their method consists in a preliminary purification of autolyzed yeast by Fuller's earth, which removes other substances than bios, followed by the use of colloidal iron hydroxide. This latter carries down the bios, and can later be dissolved, leaving the bios free of contaminating ions. These authors expect to report more fully on their work at the present meeting.

Finally, attention should be called to a paper by Suzuki and Suzuki,[9] in which it is stated that vitamin D was separated from vitamin B by the use of aluminium cream and other precipitating agents.

VITAMIN B

Returning now to studies on the isolation of the antineuritic vitamin B, it should be mentioned that a very complete summary of the experiments made up to 1922 is given by Professor Sherman in his book "The Vitamins."

The original method of Funk, which has been followed more or less closely by the majority of those who have devoted themselves to this problem, consists in extracting the raw material with acidified water or aqueous alcohol. The resulting extract is concentrated and, after removal of extraneous material, the solution is subjected to successive precipitations with such reagents as phosphotungstic acid, silver nitrate, tannic and picric acids. In all cases in which crystalline products were finally obtained they proved to be well-known compounds, which did not possess the physiological properties of the antineuritic vitamin, or else the activity which they exhibited could be accounted for by impurities still present. Recent work following this same procedure is reported by Tsukiye.[10] A gray white powder, curative for hens in doses of about 10 milligrams, was finally obtained. It exhibited great similarity to histidine in most respects except the diazo reaction. As compared with pyrimidine bases and allantoin, it possessed some like properties and others which were different.

A consideration of the general procedure mentioned above shows that the desired separation of the vitamin from the numerous substances with which it is associated depends in the first place upon a fractional solubility in water or alcohol, and secondly upon the formation of insoluble complexes of the vitamin with certain precipitating reagents.

It is apparent that the first step, which depends upon solubility differences, is only slightly selective. Consequently any improvement at this point would be a distinct advantage. Realizing this and knowing of the discovery by Professor John Uri Lloyd that Fuller's earth exerts a selective adsorption of alkaloids, I tested, several years ago, the adsorptive power of this reagent for the antineuritic vitamin, and luckily found that it could be successfully used for separating this vitamin from the larger part of the substances with which it may be associated in solution. The vitamin-Fuller's earth adsorption complex or "activated solid," as I have called it for convenience, undoubtedly contains the vitamin in a purer form than any other product available as a starting point for isolation experiments.

Fortunately the active material can be recovered from its combination with the Fuller's earth by a relative simple procedure,[11] and the concentrated extract thus obtained has, in my opinion, advantages for precipitation experiments far superior to those of extracts prepared in any other way. All my subsequent work has, therefore, been based upon the utilization of this remarkable power of Fuller's earth, and any measure of success that may have been attained is due to this exceptionally favorable initial step of the process. It is interesting to note in this connection that selective adsorption has recently been utilized with great advantage in the purification of insulin, in which case charcoal was used as the adsorbent.

[6] Wildiers, "La Cellule," 18, 313 (1901); Amand, *ibid.*, 21, 327 (1904); Devloo, *ibid.*, 23, 361 (1906).

[7] W. Lash Miller, SCIENCE, 59, 197, 1924.

[8] March 26th meeting of the Society for Experimental Biology and Medicine, New York.

[9] U. Suzuki and B. Suzuki, *J. Chem. Soc.* (Japan), 44, 225 (1923).

[10] S. Tsukiye, *Biochem. Z.*, 131, 124–39 (1922).

[11] Seidell, *J. Am. Chem. Soc.*, 44, 2043 (1922).

PHYSIOLOGICAL TESTS

As mentioned previously, the circumstance which has perhaps retarded most the isolation of vitamins has been the necessity of depending upon physiological tests for controlling the fractionation procedures. In the early days the test for antineuritic activity was made by restricting a bird to a diet of polished rice until symptoms of polyneuritis developed, and then administering the unknown sample and noting the effect. It was later recognized that these curative tests were subject to unaccountable irregularities and many erroneous conclusions had been drawn. Gradually it has become evident that protective tests are much more reliable. In these cases the bird is placed on a vitamin B free diet and simultaneously given, at regular intervals, measured doses of the sample to be tested. A decline in weight indicates that the unknown contains less vitamin than required for maintenance. It has furthermore been found[12] that the indications of this test are even more trustworthy, if the birds are kept on a vitamin-free diet and given such doses of a standard vitamin preparation ("activated solid"), as are just sufficient to prevent loss in weight. When now the standard vitamin is replaced by the sample to be tested, any diminution in vitamin content below that of the standard is promptly and uniformly indicated by a loss in weight. Using pigeons, accurate conclusions as to the activity of a given sample can be drawn within a period of ten days, and in cases where the dose is markedly inadequate, an unmistakable decline in weight occurs within six and sometimes within four days. By this procedure the birds can be used repeatedly for the tests, provided care is taken that no more than moderate losses in weight are permitted. Many of the pigeons used for the tests depicted in the charts which follow have been kept for more than a year on an exclusive diet of polished rice, supplemented only by highly purified vitamin B preparations. It is interesting to note in this connection that as the pigeon reaches old age its vitamin requirement becomes less. Pigeons of three or more years may sustain no loss in weight when receiving only one half the vitamin required to maintain a one-year-old pigeon.

SILVER PRECIPITATION

The first precipitant which was used for removal of the active constituent of the vitamin extracts, prepared as above outlined, was silver nitrate.[13] Although the experiments showed that the active material was undoubtedly precipitated, especially by ammoniacal silver nitrate, it was found that a large proportion of the vitamin remained in the liquid. Furthermore, it became evident that inactive material was precipitated simultaneously with the active. Thus, on decomposing the silver precipitate and testing the resulting silver free product, there was no significant increase of activity over that of the original extract used for the precipitation. These unfavorable results finally made it necessary to abandon the silver method.

PICRIC ACID PRECIPITATION

Of the other reagents which were available, picric acid was selected as the most promising. The conditions for the nearly complete precipitation of the active material have been gradually developed and the resulting crude picrate subsequently separated into two crystalline compounds, one of which is highly active. There has thus been obtained for the first time ample quantities of a well-characterized antineuritic compound, suitable for detailed studies of the chemical structure of this one of the at present recognized vitamins.

A preliminary account of the preparation of the active picrate has been recently published,[14] and it will only be necessary at this time to outline briefly the steps involved and call attention to the evidence on which is based the conclusion that a crystalline compound, possessing the antineuritic properties of vitamin B, has been obtained.

REMOVAL OF POTASSIUM SALTS FROM THE VITAMIN EXTRACT

The crude vitamin extract prepared from brewers' yeast by the Fuller's earth method and used for silver precipitations during more than a year had been tested for activity on numerous occasions and its high potency well established. At the time the experiments with picric acid were begun, it was unexpectedly found that a large proportion of a potassium salt was present in the extract. The source of this was undoubtedly the Fuller's earth, from which it would be displaced by a substitution of bases during the extraction with barium hydroxide. The removal of the potassium from the extract was accomplished as follows.

The ash obtained from an aliquot of the extract was titrated with standard sulfuric acid, using methyl orange as indicator. On the basis of this titration an amount of sulfuric acid, equivalent to the total fixed bases, was added to the main portion of the extract and the solution evaporated nearly to dryness. The residue was digested in 66 per cent. ethyl alcohol and the insoluble potassium sulfate removed by centrifugation.

[12] Seidell, Public Health Reports, 37, 1919–23, June 23, 1922.

[13] Seidell, Public Health Reports, 36, 665–70, April 1, 1921; *J. Am. Chem. Soc.* 44, 2045 (1922).

[14] Seidell, Public Health Reports, 39, 294–9, February 15, 1924.

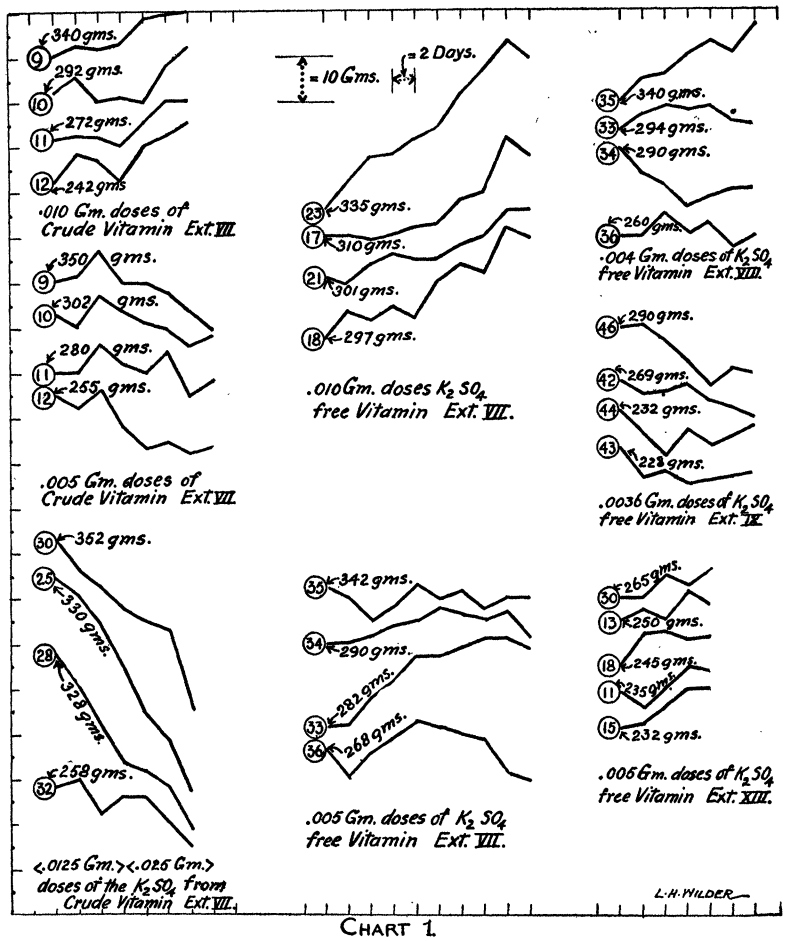

CHART 1.

This chart shows the changes in weight of pigeons fed on polished rice and given each second day the specified quantities of vitamin extract, before and after removal of the potassium salts, and also of one sample of the removed K_2SO_4. The figures in circles give the number, and the others the weight of the pigeons at the beginning of the test.

That this procedure exerted no injurious effect on the vitamin is demonstrated by the results in Chart I. An examination of this chart shows that 0.010 gm doses of the crude vitamin extract VII, given on each alternate day, caused pigeons on a diet of polished rice to slowly gain weight. One half this dose was insufficient to prevent the gradual loss in weight of another group. Corresponding doses of the extract from which the potassium salt had been removed gave results indicating increased activity, thus showing that the process for removal of the potassium salt had not caused an appreciable destruction of the vitamin originally present. The potency of three other samples of vitamin extract, freed of potassium salts by the described procedure, is shown by results in the chart. Finally, the inactivity of the removed potassium sulfate is demonstrated by one group of pigeons.

It is to be noted that the pigeons used for the tests recorded in Chart I were of unknown age and had been kept on an exclusive diet of polished rice for some time. It was afterwards found that young pigeons, of one year or less in age, required distinctly larger doses of vitamin to protect them from loss in weight.

PREPARATION OF THE CRUDE PICRATE

Having obtained a highly active vitamin extract, free of potassium salts, the next step was to prepare a picrate of it and determine what proportion of the total active material is present in the resulting compound. After many experiments the following procedure was adopted.

To the concentrated 66 per cent. alcoholic solution of the extract is slowly added, with stirring, an amount of picric acid, dissolved in warm methyl alcohol, approximately equal in weight to the solids pres-

ent in the extract. There is then added a volume of water equal to about one third that of the mixed alcoholic solution. This causes the separation of an additional amount of yellow precipitate. The mixture is then subjected to more or less rapid evaporation of the alcohol at a moderate temperature. Additional quantities of water are added to replace the alcohol from time to time, until no further clouding occurs on addition of the water. The yellow precipitate will then have assumed a more or less granular condition and can be conveniently removed by centrifugation. It should be washed by stirring once with about an equal volume of water and again centrifuging. The washed picrate, after pressing between filter paper and drying in a vacuum desiccator, will usually weigh somewhat more than the solids of the vitamin extract used for its preparation.

ACTIVITY OF THE CRUDE PICRATE

In testing the picrates prepared in this manner, as well as the subsequent crops obtained by evaporating the liquid decanted from the first crop, the samples have been administered as such, without regard to what effect, if any, the picric acid contained in them might have on the pigeons. It was, therefore, necessary to test this point, and groups of pigeons fed on polished rice were given, respectively, (1) "activated solid," (2) "activated solid" plus large doses of picric acid and (3) Fuller's earth plus picric acid. The resulting changes in weight are exhibited

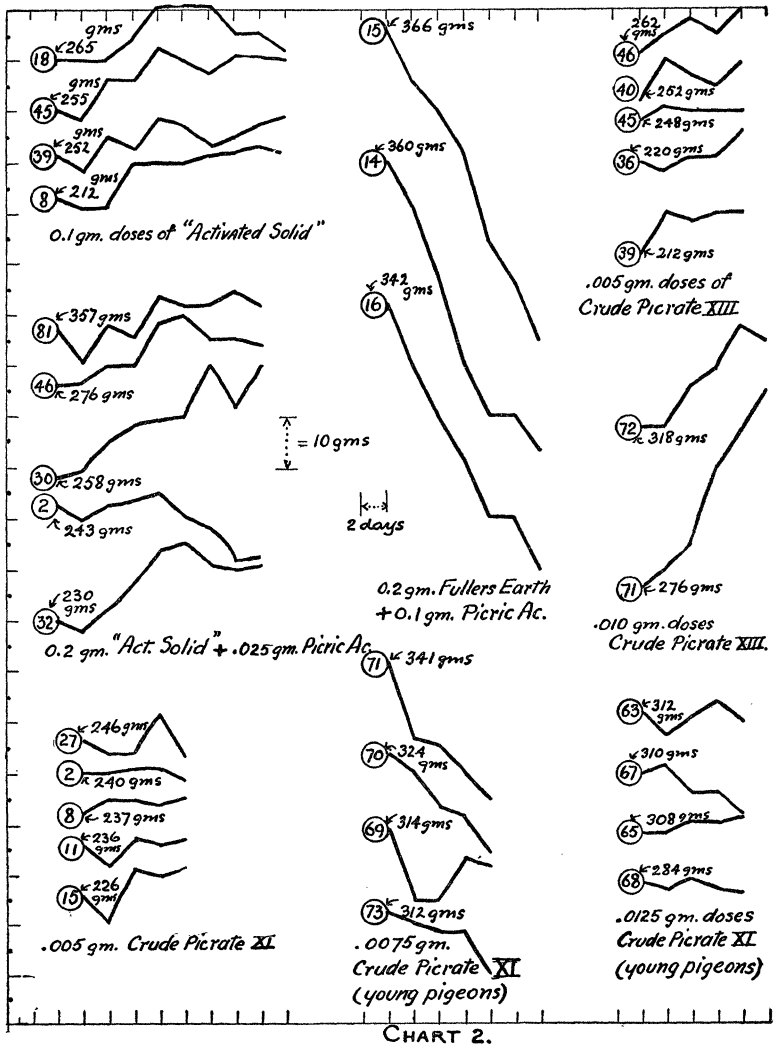

CHART 2.

This chart shows the changes in weight of pigeons fed on polished rice and given each second day the indicated quantities of various vitamin preparations. The figures in circles are the number, and the others the weight of the pigeons at the beginning of the test. Two of the above tests were made with pigeons one year or less in age.

in Chart II. There is also shown in this chart the weight curves of several groups of pigeons which were given doses of crude picrates prepared from vitamin extracts as described above.

It will be noted that the administration of picric acid in doses of 25 milligrams, which is four or more times the maximum quantity present in the largest doses of the active picrates, exerts no appreciable deleterious effect. A group of pigeons receiving picric acid without protective vitamin declined rapidly, thus serving as controls and showing that picric acid itself exerts absolutely no protection. Hence it is evident that in the case of picrates which prevent loss in weight on a rice diet, the activity must be due solely to the base united with the picric acid.

Attention should also be called to the variation in results obtained with young pigeons. Thus in the case of crude picrate XI, doses of 0.0125 gm were required to protect the young pigeons, whereas doses of 0.005 gm adequately protected the older ones.

The chart also shows that under the conditions of the test as here employed a period of eight days is sufficient for obtaining dependable information as to the activity of a given sample. It should be remembered, however, that in all feeding tests there are likely to be minor ill-defined factors which modify the results, and it is not always possible to obtain uniformly concordant figures for the activity of different preparations. Correct conclusions can be drawn only from an extended series of tests. In the present case a large number of experiments, made during many months, indicate that the crude picrate prepared as described protects pigeons of about one year in age from loss in weight on polished rice in doses of approximately 0.010 gm given on alternate days.

The solution separated from the above described picrate, when evaporated, yields further quantities of solid picrates, but these usually fail to protect pigeons in doses of two or three times the protective dose of the picrate which first separates. The solids of the final mother liquor also show a low protection. Approximately quantitative results indicate that about 80 per cent. of the vitamin originally present is retained by the first crop of crude picrate obtained as described.

Fractionation of the Crude Picrates

A superficial examination of the dried yellow granular picrate indicates that it is not homogeneous. Under the microscope there may be seen prismatic needles; bright reddish colored particles and numerous amorphous masses which may or may not show projecting spines. There is apparently more than one picrate present, and the relative amount of each probably depends upon the ratio of picric acid and concentration of alcohol present at the time the picrates separate from solution.

The product is only slightly soluble in water but dissolves readily in mixtures of acetone and water. It has been found that by digestion in acetone containing a small proportion of water the more active portion dissolves first. A separation of the active from the inactive constituents, by means of fractional solubility in 95 per cent. acetone, may be made as follows.

Ten grams of the crude picrate are stirred with 20 cc of 95 per cent. acetone until disintegration is complete and the mixture centrifuged. The supernatant acetone solution is decanted and the picrate stirred a second time, using 10 cc of acetone. The mixture is centrifuged and the second acetone extract decanted. This is repeated with 5 cc and 3 cc portions of 95 per cent. acetone. To the combined acetone extracts 20 cc of water are added and the liquid evaporated under diminished pressure. A yellow precipitate soon appears and increases in amount. Small quantities of water are added from time to time to replace the evaporated acetone. When the further addition of water fails to produce a clouding the yellow solid is filtered, pressed between filter paper and dried in a vacuum. The yield is usually about 5 grams. This procedure is then repeated on the once recrystallized acetone soluble picrate, using amounts of acetone, reduced in accordance with the weight of the once purified picrate available. There will now be obtained about 3.0 grams of twice crystallized acetone soluble picrate. This, as mentioned later, melts with decomposition at a temperature usually below 150°.

The acetone insoluble residue, when recrystallized from warm water or dilute acetone, yields well-formed prismatic needles which melt sharply at 202–3° and after complete dehydration at 206°. An investigation of the identity of this product is being made by Dr. W. O. Emery, of the Bureau of Chemistry.

Activity of the Acetone Soluble Picrate

Physiological tests of the fractions obtained by the above procedure were made on young pigeons of about one year in age. The results are given in Chart III. An examination of this chart shows clearly that the acetone insoluble part of the crude picrate possesses very little if any activity. Doses up to 20 milligrams did not prevent a rapid decline in weight, whereas doses of the acetone soluble fractions as low as 1 milligram exerted an appreciable effect. It is apparent that a fully protective dose of the acetone soluble fraction is about 4 milligrams, which is two and one half times less than the average quantity of the crude picrate required for protection.

Assuming a content of about 50 per cent. of picric

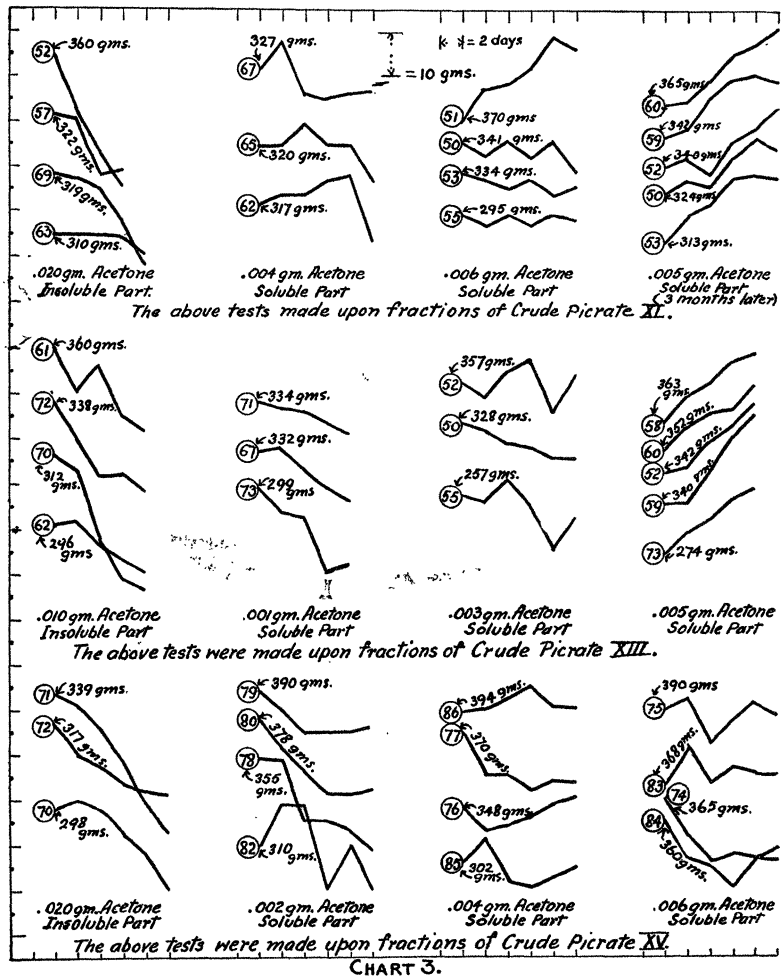

CHART 3.

This chart shows the changes in weight of pigeons of about one year in age fed on polished rice and given each second day the specified quantities of the 95% acetone soluble and insoluble fractions obtained from three samples of crude picrate. The figures in circles give the number, and the others the weight of the pigeon at the beginning of the test.

acid in the active fraction, the daily protective dose, calculated to uncombined vitamin base, would be approximately 1 milligram. For older pigeons probably less than one half this quantity would be sufficient. This is close to the previous figure calculated from nitrogen determination upon purified fractions obtained by the silver method[15] and shows that in both cases a distinctly significant amount of vitamin base is required for protection. It appears improbable, therefore, that the physiological action of vitamin is due to far smaller amounts of superactive substances of the nature of enzymes.

One other point worthy of note in connection with the present tests is that, in one case (see Chart III), a sample of the active fraction lost none of its protective power on being kept for a period of three months. It may, therefore, be concluded that the acetone purified picrate is a relatively stable form of antineuritic vitamin.

[15] Seidell, Public Health Reports, 37, 1523 (1922).

THE CHARACTER OF THE ACTIVE PICRATE

The first question which arises in connection with the acetone soluble picrate, obtained as described above, is whether its activity is to be ascribed to the one or probably more bases in combination with picric acid, or to some substance present as an impurity. An analysis of the process by which this final product is obtained indicates that the latter possibility is remote. If, for example, it is assumed that an unknown substance X is present, it will be necessary to ascribe to it exactly the same solubility relations exhibited by the acetone soluble picrate. Although it is possible that a compound, not a picrate, dissolves and sepa-

rates from solution in exactly the same manner as the picrate here described, it is not at all probable. As has been mentioned in connection with the fractionation process, another picrate (the sharply melting prismatic needles), no doubt closely related chemically to the active one, is almost, if not completely, separated from it by solubility differences in acetone. It would indeed be strange if some compound of an entirely different type should remain associated with only one of these two picrates, throughout the entire series of operations.

The possibility of such an attainment being due to an adsorption appears not to be well founded, since the solid picrate, on which it would be necessary to assume the adsorption occurs, separates from the same clear solution from which the hypothetical substance X is supposed to be subsequently adsorbed. Furthermore, the several crops of picrate which are successively obtained differ appreciably in activity and consequently, if the activity of the earlier crops is due to an adsorbed impurity, the latter crops should also obtain their share of it. The available evidence, therefore, points to the absence of adsorbed physiologically active impurities in the picrate obtained as described.

The question as to whether the recrystallized acetone soluble picrate is free from small amounts of a closely related picrate or degradation product of the active compound is still unsettled. The proper conditions for obtaining well-formed crystals have not been learned. The crystals that are obtained appear as aggregates of thin pale yellow plates with irregular outline. These show indices of refraction[16] of about 1.5 in the alpha direction and 1.8 + in the gamma. There is present, however, in all samples so far obtained a certain proportion of apparently potentially crystallizable material, which has not assumed a definitely crystalline character. This is probably due to the manner in which the crystallization occurs. As mentioned previously, water is added to the acetone solution of the picrate in repeated small quantities during the crystallization until its further addition causes no clouding. It has been found that this addition of water is essential, probably not to supply water of crystallization, since the final product has none, but to yield the proper dilution of acetone. As the solvent evaporates there first appears a yellow cloud which soon collects as a voluminous amorphous mass, throughout which, after a time, glistening facets can be distinguished. The volume gradually contracts and the crystalline appearance of the separated solid becomes more and more pronounced. There evidently takes place a gradual transition from the amorphous to crystalline state, and this process does not go to completion under the conditions which have so far been provided. Although this is to be regretted from the standpoint of accurate characterization of the product by its physical properties, it need not be a serious handicap to the identification of the compound. It is probably that the composition of the amorphous material is essentially the same as that of the crystalline portion, and analytical tests applied to samples containing the two forms should give trustworthy information concerning the identity of the active product.

Determinations of the melting point of the samples give unsatisfactory results since the product decomposes without melting sharply. The decomposition range varies somewhat with different samples but usually begins between 125° and 160° C. After an initial contraction, effervescence without discoloration occurs, and this increases slowly, accompanied by darkening, and ends in a greatly expanded mass of black foam.

The combustion analyses which have been made on the active picrate have not as yet given results from which it has been possible to calculate a satisfactory empirical formula. An approximate determination of amino nitrogen by the Van Slyke method indicated that a part of the nitrogen is present in this form. Tests of the rotatory power of the vitamin extract, before preparation of the picrate, showed an undoubted, although a very small, deviation to the left. Other qualitative tests for various properties and constituent groups have been applied, but an indication of the real identity of the compound has not as yet been obtained.

SUMMARY

There has been a notable revival of interest in the chemistry of vitamins within the past few years. The recent work has led to the isolation of more or less completely crystalline compounds having the properties ascribed to vitamins A, B and D. The evidence now available appears to definitely establish the conclusion that vitamins are not to be classed with enzymes. The physiologically active crystalline products which now for the first time have been isolated make possible intensive studies of the constitution and chemical properties of the several vitamins. Although many years have been required to obtain these elusive compounds in the state of purity now reported, it is probable that their identification will be accomplished in a much shorter time. With the completion of this latter phase of the problem we may confidently look forward to the eventual synthesis of the vitamins and their extensive application to the nutritive needs of man.

ATHERTON SEIDELL

HYGIENIC LABORATORY,

U. S. PUBLIC HEALTH SERVICE

[16] These estimates were made by Dr. E. T. Wherry, of the Bureau of Chemistry, to whom I wish to express my appreciation for his assistance.