

While some degree of variation has been experienced in the different results, it is obvious that the samples of urine obtained from the physiological sources contain a marked excess of urobilin over that generally encountered in so-called normal urines.

SUMMARY.

(1) The administration of phenyldimethylpyrazolon over a certain period of time occasions the appearance of an abnormal quantity of urobilin in the system.

(2) It is believed that the disintegration of erythrocytes is considerably augmented by continued ingestion of the antipyretic, resulting in an increased proportion of the urinary pigment.

(3) Data are furnished, showing the relative amounts of the body existing in normal and physiological samples of urine.

(4) Future work on the effect of certain of other therapeutic agents upon urobilin production, is contemplated.

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COMMENTS ON SOME TESTS AND ASSAYS OF THE U. S. P. X.*

BY HERBERT C. KASSNER.

For the past two years, part of my work has been to conduct a course of laboratory instruction for large classes of third year students, candidates for the degree of Pharmaceutical Chemist; part of the course consists in the carrying out of many U. S. P. tests and processes. It has been repeatedly made evident to me that certain tests and assays caused considerable difficulty and seemed in need of improvement; for example, on introducing impurities into certain substances, even in reasonably large proportions, it appeared impossible to detect them by the prescribed U. S. P. tests; again, some tests appeared to be unworkable unless modifications were made. It seemed advisable to call attention to the difficulties encountered and for this reason this paper has been written.

The list of subjects criticized does not pretend to be by any means comprehensive; it contains only those tests and assays which were included as a part of the course because of their importance from the educational point of view and which were the cause of trouble in one way or another. If a systematic search were made of the Pharmacopœia, doubtless many more difficulties on similar lines would be encountered.

All the tests commented upon have been carefully examined, many series of experiments being carried out and suggestions for modifications made wherever possible. In some cases it is merely the wording of the Pharmacopœia which has been

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* Scientific Section, A. Ph. A., Rapid City meeting, 1930.

criticized, and alterations are recommended especially when ambiguities or contradictions are apparent.

The order in which the subject-matter has been arranged is that of the Pharmacopœia, page references being given in every case.

Acetylsalicylic Acid, p. 13.—In order that the melting point may be used as a standard, a maximum figure should be given in addition to the minimum of 132° stated in the U. S. P., which determines this in a bath previously heated to 120°. It has been suggested by Corfield and Self (*Pharm. Jour.*, 110 (1923) 361) that a melting point range of 133° to 138°, determined in the usual way, is advisable.

Alcohol, p. 39.—*Boiling point*. In the sentence "Alcohol is readily volatilized even at low temperatures and boils at about 78° C.," the first statement is redundant since the volatile character of alcohol has already been mentioned under the heading "Description." In the case of the monograph on the more readily volatile substance, Ether, p. 34, no statement to this effect is made, hence it is suggested that it should be deleted in this case and also in the monographs on Acetone, p. 10, Carbon Tetrachloride, p. 98 and Chloroform, p. 106.

The boiling point of Alcohol is required to be determined by Method I, p. 432, which defines the term "boiling point" as "that range of temperature within which at least 95 per cent, by volume, of the substance, distils." The monograph, p. 39, states that "alcohol boils at *about* 78° C.;" this statement, being extremely vague, leaves the analyst in doubt as to what range on each side of the figure 78° C. he can allow. It has been our experience that many samples of Alcohol, which meet all the requirements of the U. S. P., do not yield a distillate of 95 per cent by volume at 78° C.

It would be satisfactory if the U. S. P. gave a definite range for the boiling point, as in the case of Ethyl Chloride, p. 36 (between 12° and 13° C.).

Aldehyde or Oak Tannin Test.—In this test the phrase "at once" must be taken to mean what it literally implies, for the U. S. P. states on p. 2 that "In testing chemicals for innocuous impurities it is understood that five minutes shall be allowed for the reaction to be observed unless otherwise specified."

On conducting a series of tests with concentrations of acetaldehyde varying from 0.5% to 10%, we found that even a 10% concentration did not give a "yellow color" until after a period of 3 minutes, while, with a concentration of 3%, no tint was produced until 5 minutes had elapsed and 11 minutes was needed for the appearance of a distinct yellow color. From our series of tests, we found that, if a time of 30 minutes is allowed to elapse, a concentration of 1% can be detected, while 0.5% gives a positive reaction by the end of 50 minutes.

In the case of tannin, however, a concentration of 0.001% produces a faint yellow tint *at once*, the color deepening slightly on standing for 50 minutes; while a concentration of 0.0001% although giving no positive reaction *at once*, shows a faint yellow tint at the end of 50 minutes. This test then, as given in the U. S. P., will detect small quantities of tannin, but is unsatisfactory for aldehyde. It is suggested that aldehyde is best tested for by Murray's test, which is given below.

Test for Organic Impurities, Aldehyde, etc.—This test gives negative results with 1% Formaldehyde, 2% Acetaldehyde and 1% Acetone, but 1% Fusel Oil, 0.1% Furfural and 0.00001% Tannin are the minimum concentrations giving just more than the "faint brownish tint" that the U. S. P. allows.

Murray ("Standards and Tests for Reagent Chemicals") and Mallinckrodt ("Methods of Testing Reagent Quality Chemicals") use the following test for Aldehyde; "Mix 10 cc. of alcohol with 10 cc. of water, add 2 cc. of ammoniacal silver nitrate solution and allow to stand 12 to 18 hours in a dark place. No color or turbidity should develop." It is necessary, of course, for the ammoniacal silver nitrate solution to be freshly prepared. On carrying out a series of tests over a period of 18 hours, the smallest quantity of Acetaldehyde that we could detect by this means was 0.001%, which concentration produced a faint brownish tint.

With Murray's permanganate test for Organic Impurities, we were able to detect 0.5% Acetaldehyde, but not less than 3% Formaldehyde, 3% Fusel Oil and 0.07% Furfural.

Acetone Test.—This, inserted as a distinctive test for the presence of acetone, seems un-

suitable for this purpose since a violet or violet-red tint is produced by acetaldehyde as well as by acetone. Equal concentrations of these two substances produce almost identical colorations, and a concentration of 0.5% of either is the minimum quantity which can positively be detected. With a concentration of 1% or more, it is possible to distinguish between acetone and acetaldehyde by the addition of 5 cc. of ammonia T. S.; in the case of acetone, the color is almost completely discharged, while, with acetaldehyde, a yellow color appears. As the U. S. P. test is not specific for acetone, a better method for detecting it should be found.

Isopropyl Alcohol Test.—It has frequently been stated in the literature that this test is unsatisfactory, since pure isopropyl alcohol, under these conditions, produces a white, and not a yellow precipitate; it has been shown that a yellow precipitate is caused by the presence of impurities in the isopropyl alcohol. The appearance of a white or yellow precipitate, therefore, does not signify the presence of isopropyl alcohol, since various compounds produce such precipitates under the conditions of the test. The most recent work on this test is that of Matthes and Schutz (*Pharm. Ztg.*, 3 (1929), 44).

Benzaldehyde, p. 76.—The test for nitrobenzene in benzaldehyde, if carried out exactly as described in the U. S. P., is quite unsatisfactory; in the first place, if no nitrobenzene is present, no turbidity is produced on the addition of water to the alcoholic solution; secondly, using zinc and dilute sulphuric acid no evolution of hydrogen can be obtained, owing to the blanketing effect of the benzaldehyde on the zinc; hence it is impossible to finish the test.

If a color test for nitrobenzene is desirable, the following modification of the U. S. P. test can be used: "To 1 cc. of Benzaldehyde add 3 cc. of hydrochloric acid, and 0.5 Gm. of zinc dust and heat gently for 30 seconds; allow to stand for one minute, and dilute with 10 cc. of distilled water. Filter, and add 3 drops of potassium dichromate T. S.; no green color develops." The smallest amount detectable with certainty by this test is 2% of nitrobenzene; with concentrations of 2% to 5% the green color varies from an olive green to a deep bluish green, from 5% to 50% from a brownish-green to a dark brown; with a concentration of 10% a deep reddish purple color was obtained and this was the nearest approach to the "violet color" mentioned in the U. S. P.

The test for nitrobenzene, as described in the monograph on Oil of Bitter Almond, p. 249, was carried out on samples of benzaldehyde adulterated with nitrobenzene; this test slightly modified as follows, will detect a minimum of 2% of nitrobenzene: "Add 10 drops of Benzaldehyde to 5 cc. of alcohol in a 50 cc. flask; add 0.3 Gm. of zinc dust and 2 cc. of acetic acid and boil the mixture for 30 seconds; add sodium hydroxide T. S. until strongly alkaline, then 5 drops of chloroform, boil for 30 seconds and cool for 5 minutes; no odor of phenylisocyanide develops." This phenylisocyanide test is preferable to a color reaction since it is more definite and equally sensitive.

Chloral Hydrate, p. 104.—The formula for this substance is given as $\text{CCl}_3\text{CHO}\cdot\text{H}_2\text{O}$. Since it does not behave as a typical aldehyde and is not considered to contain the $-\text{CHO}$ group, the formula is generally written as $\text{CCl}_3\text{CH}(\text{OH})_2$, and should be the one used in the U. S. P.

Under the heading "Tests for purity" the following test appears: "Gently ignite 2 Gm. of Chloral Hydrate: no inflammable vapors are evolved (difference from chloral alcoholate)." If this test is intended as a test for purity it is unsatisfactory in that no less than 15% of chloral alcoholate can be detected with certainty by this means. From the wording, however, "difference from chloral alcoholate," it would appear that this test is intended as a "Test for identity;" if this is the case, it would seem to be superfluous since these two substances, when pure, differ greatly in crystalline structure and are readily recognizable. If a test is needed, it is suggested that it should be worded as follows: "Gently ignite 2 Gm. of Chloral Hydrate: no inflammable vapors are evolved and no residue of carbon remains (difference from chloral alcoholate)" and inserted under the heading "Tests for identity." If it is necessary to test for the presence of small quantities of chloral alcoholate as an impurity, a new test must be devised.

Colchicum Corm, p. 115.—In the assay of this drug, the first filtration is difficult owing to the presence of mucilage formed from the starch present in the corms, and it is impossible to obtain 200 cc. of filtrate without resorting to the use of suction; mention of this fact should be made in the monograph.

Since, in this assay, the alkaloid is neither weighed nor titrated, but its proportion only evaluated by difference, it is suggested that the literature should be searched and, if possible, a

direct method substituted for the present assay, which, from our experience, is by no means satisfactory.

Glycerin, p. 180.—In this monograph, tests for oxalate and chloride are directed to be made on an aqueous solution of glycerin; that for chloride should be reworded as follows: “—or of 2 cc. of diluted nitric acid and 0.5 cc. of silver nitrate T. S. (chloride).” From a neutral solution of silver nitrate, as given in the U. S. P., oxalates are precipitated as well as chlorides; since oxalates are separately tested for with calcium chloride, the silver nitrate test should be made distinctive for chlorides.

Corrosive Mercuric Chloride, p. 187.—In the assay of this substance, the use of carbon tetrachloride for washing the precipitate free from adhering sulphur is extremely unsatisfactory, sulphur being soluble to the extent of less than 1% at ordinary temperatures. Carbon disulphide, in which sulphur is readily soluble, should be substituted.

Modified Dakin's Solution, p. 225.—From information received from various sources it appears doubtful whether any retail pharmacist in the City of New York ever makes this preparation. Attempts were made to purchase “Modified Dakin's Solution, U. S. P.” from four of the largest wholesale druggists in the city; only one wholesaler could supply a sample; two others suggested the buying of a well-known proprietary article which is a stabilized hypochlorite solution.

The sample supplied by the wholesaler assayed only 0.303% of NaOCl (U. S. P. limits 0.45% to 0.50%), and showed an excess above the maximum alkalinity allowed by the U. S. P. tests for purity. From qualitative tests carried out, it would appear that this sample was not prepared by the U. S. P. method.

The proprietary article mentioned above assayed 0.997% NaOCl, being twice the U. S. P. strength; it also gave a red color in the “maximum alkalinity” test, showing that it was too strongly alkaline.

One of our largest manufacturers of surgical dressings markets the ingredients necessary for the extemporaneous preparation of a Dakin's solution which meets the Assay and Tests for Purity requirements of the U. S. P., though it is not made from the same initial chemicals; the ingredients supplied are a package containing a definite weight of sodium carbonate and a tube containing a definite quantity of liquid chlorine, which are to be added to a liter of water according to specified directions. This easily prepared solution assayed 0.482% NaOCl, and met the U. S. P. requirements for alkalinity.

Some method should be devised by which this important solution may be prepared more readily than is possible by the U. S. P. process.

Methenamine, p. 238.—In connection with the statement that “an aqueous solution of Methenamine is alkaline to litmus paper,” no guidance as to the strength of the solution to be used is given. On examination of four manufacturers' samples it was found that, in using litmus paper sensitive to N/250 alkali, 2% solutions gave no reaction, while 10% solutions showed such a very faint alkalinity that the result must be said to be doubtful.

If a test for the alkalinity of methenamine is important, it could be stated similarly to that given under Smallpox Vaccine, p. 418. The following wording is suggested: “An aqueous solution of Methenamine (1 in 20) gives a red color with phenol red T. S. and is colorless with thymolphthalein (1% alcoholic solution).”

The p_H value of the samples examined was about 8.5.

Oil of Bitter Almond, p. 249.—It is recommended that the nitrobenzene test should be modified as described in this paper under Benzaldehyde. A concentration of 2% of nitrobenzene is detectable by this means. The color test for nitrobenzene previously mentioned may also be used, a similar range of colors with different concentrations being obtained.

Oils of Caraway and Cinnamon, pp. 253, 256.—There are upon the market, from reputable distillers, samples of these oils, labelled U. S. P., which cannot be assayed by the U. S. P. X process. In changing the method from that of the U. S. P. IX, while the same quantity of oil was employed, the size of the flask was reduced from one of 200 cc. to one of 100 cc. capacity, and sodium bisulphite was substituted for acetic acid for the purpose of neutralizing. It is impossible, in the smaller sized flask or even in a 200-cc. flask, to add sufficient sodium bisulphite to neutralize the mixture, consequently only very low and by no means concordant results, which cannot possibly be correct, can be obtained. If the method of the U. S. P. IX is used, satisfactory

assays, yielding consistent figures, can be carried out. As an example, a sample of Oil of Caraway labelled U. S. P., was assayed, with the following results:

	<i>U. S. P. X Method.</i>	<i>U. S. P. IX Method.</i>
1st Determination	27% Carvone	54% Carvone
2nd Determination	38% Carvone	55% Carvone

Similar results were obtained by these methods on samples of Oil of Cinnamon. Hence a return should be made to the method of the U. S. P. IX.

Phenol, p. 283.—When assaying samples of liquefied Phenol by the method given in this monograph, the stated quantity of 1 cc. of chloroform may not be sufficient to dissolve all of the tribromphenol present; if the amount of chloroform were increased to "2 cc. or a sufficient quantity to dissolve the precipitate," the assay would be entirely satisfactory.

Spirit of Ethyl Nitrite, p. 349.—In this monograph, the directions for the assay show discrepancies when compared with the general method for "Gasometric Estimations" given on pp. 437-439. Since reference on p. 349 is made to p. 437, it is desirable that the same method of procedure should be given in both places.

In the case of Spirit of Ethyl Nitrite, as well as of Amyl Nitrite, p. 49, 5 cc. of diluted sulphuric acid (10%) is directed to be used, whereas in the general method, p. 437, normal sulphuric acid (4.9%) is specified; which is the analyst to use?

The two monographs on pp. 50 and 349 state that corrections for temperature and pressure must be made in the calculation of the percentage of active constituent, when these vary from the U. S. P. standard of 25° C. and 760 mm. If these corrections are applied to the "*quotient*," and if each correction is made separately and added or subtracted as necessary, as directed in the monographs, a different percentage is obtained from that which would result if the correction were applied to the *volume of gas* measured. On p. 438, *factors* are given for temperature and pressure corrections, hence the processes of addition and subtraction do not enter into the calculation, so it is immaterial whether the corrections are made on the volume of gas or on the percentage obtained, though for the sake of clarity and accuracy of wording the sentence "Correct this volume for temperature and pressure according to the following tables" should be inserted on line 7 of p. 438, between the words "-tube." and "Multiply."

On p. 438, the following passages occur:

"For pharmacopœial purposes, the determination will be sufficiently exact if the evolved gas is measured within 3° above or below 25° C."

"The barometric correction is important at any locality more than 250 meters above sea level."

Then follow tables giving Factors for Correction of Temperature and Barometric Pressure. It is inferred from these quotations that corrections for temperature are unnecessary between 22° C. and 28° C., and that small variations in pressure at altitudes below 250 meters are not "important," and therefore may be neglected. This interpretation is reasonable, since the percentage of active constituent in Spirit of Ethyl Nitrite may vary between 3.5% and 4.5% (a variation of about 25%), while temperature corrections between 22° (factor 1.010) and 28° (factor 0.990) only amount to 1% above or below the factor 1.000 for 25°; also, variations in pressure are unlikely to be more than 3% to 4% on either side of the factor 1.000 for 760 mm., unless the altitude is greater than 250 meters, since barometric pressure varies by about 25 mm. for a change of approximately 250 meters in altitude. It can readily be seen that small corrections such as these can only produce small changes in the percentage of active constituent, that is, changes in the second place of decimals. Such changes, however, when the results are close to the limiting figures of the U. S. P., may be great enough to cause the passing of a bad or the rejecting of a good sample.

Bearing these considerations in mind, the author suggests the following rewording for the monograph p. 349:

"—volume of gas collected; correct this volume for temperature and pressure, according to the Tables given on p. 438. Multiply this corrected volume in cc. by 0.307 and divide the product by one-tenth of the weight of the Spirit of Ethyl Nitrite taken; the quotient represents the percentage of ethyl nitrite in the liquid."

The subject of temperature and pressure corrections is again referred to in this paper under the heading "Gasometric Estimations."

Mercurial Ointments, pp. 413, 414.—The U. S. P. states that a colorless solution should result from the treatment of these ointments with nitric acid, when carrying out this assay; it is impossible to obtain this condition, a yellow color being always present. The required reaction with nitric acid is complete within ten minutes, and so the following rewording of this part of the assay is suggested: "warm the mixture gently for 10 minutes, when red fumes should no longer be evolved."

Gasometric Estimations, pp. 437-439.—Reference has already been made in this paper under the heading "Spirit of Ethyl Nitrite" to the corrections for temperature and pressure to be applied to the volume of gas measured in these estimations; the insertion of a sentence on line 7 of p. 438 has been suggested.

If, as stated previously, it is to be inferred from paragraphs 2 and 3 on p. 438 that the Pharmacopœia intends to neglect small variations in temperature and pressure, why should not paragraph 2 be worded as follows and paragraph 3 omitted? "No corrections are necessary for temperature and pressure if the evolved gas is measured within 3° above or below 25° C. and 10 mm. above or below 760 mm." Giving a definite range of barometric pressure for which correction need not be applied seems more satisfactory than stating that "the barometric correction is important at any locality more than 250 meters above sea level."

A 3° change in temperature and a 10-mm. change in pressure only affect the percentage result in the second place of decimals, but in order to obtain uniformity of results especially when the question of "borderline samples" arises, it seems more satisfactory to require corrections for temperature and pressure to be applied in every calculation, in which case paragraphs 2 and 3 on p. 438 should be omitted altogether.

As sodium nitrite is not estimated gasometrically, the name of this substance should be omitted from line 9, page 438, and its factors from p. 439.

Proximate Assays, pp. 452-454.—Under the monographs on Cinchona, Hydrastis, Ipecac and Nux Vomica, which four drugs are assayed by Type Process A, it would be more definite and easier to follow, if in each case, when reference is made to the paragraph "Extraction of the Drug" on p. 453, it were stated at which step the process is to be taken up. For example, in the case of Cinchona, Type Process A is to be proceeded with from the words "stopper tightly" on line 4; for Hydrastis and Ipecac, the process is used from the beginning "Place the —;" for Nux Vomica, a start should be made at the words "stopper securely" on line 2.

Lack of uniformity is found in references made in the monographs to the "specified solvent" mentioned in the paragraph "Shaking Out with Immiscible Solvent" on p. 454; this is exemplified in the following quotations:

Belladonna—using "chloroform as the immiscible solvent for extracting the alkaloid from the aqueous liquid."

Cinchona—"use chloroform for the final extraction."

Hydrastis—"use ether for the final extraction."

Hyoscyamus—using "chloroform for the final extraction of the alkaloids."

Ipecac—"use ether for the final extraction."

Nux Vomica—"using chloroform as the immiscible solvent in shaking out the alkaline liquid."

Stramonium—using "chloroform as the immiscible solvent for extracting the alkaloids from the aqueous liquid."

It would be less ambiguous if the paragraph on p. 454 were headed "Final Extraction with Immiscible Solvent," and the wording in all the monographs were brought into line with that used in the cases of Cinchona, Hydrastis and Ipecac.

When the alkaloid is to be estimated by titration, mention should be made of the strength of the standard acid to be used; a twentieth-normal solution seems suitable.

Some authorities have recommended that, in alkaloidal assays, the final ether or chloroformic solution, which has been in contact with ammonia during the shaking out, should be washed with a little water before evaporation; our experience has shown us that, when this washing is carried out, more uniform and satisfactory results have been obtained.

In Type Process B, the drug is to be mixed thoroughly with the solvent and ammonia in a small glass percolator, the outlet of which has been packed with cotton; maceration for one

hour followed by percolation is then directed; it is impossible to carry out the mixing thoroughly without displacing the cotton. This is obviated if the maceration for an hour with solvent and ammonia is carried out in a flask, in which thorough mixing is possible, and percolation then proceeded with as directed in the U. S. P., after transferring the mixture completely from the flask to the percolator. We have found that this modification improves the process of the U. S. P.

Caines and Evers ("Year Book of Pharmacy and Transactions of the British Pharmaceutical Conference," 1926, p. 384, and *Quarterly Journal of Pharmacy*, London, 1928, p. 326), in a comparison of methods of assay of Belladonna Leaves, suggest that the ether-chloroform mixture should be made in the proportion of 4 to 1 by volume instead of 3 to 1 as in the U. S. P.; they claim that less emulsification is caused when this change is made.

Volumetric Solutions, pp. 495-510.—Substances which are to be used as primary standards require definite specifications; these are given under the heading "Reagents," pp. 470-485.

In the preparation of Hydrochloric Acid, Normal, p. 498, "freshly standardized sodium carbonate" is directed for the standardization; in the case of Sulphuric Acid, Normal, p. 508, "reagent anhydrous sodium carbonate" is to be used. Although the wording under these two headings differs, it is assumed that reference in each case is to "Sodium Carbonate, Anhydrous" p. 483. The specification for this chemical would be better if some limit for the water content were given; for example, a sentence similar to that given under "Potassium Bitartrate" p. 480: "Heat 5 Gm. at 100° C. for thirty minutes: no loss of weight should result."

The U. S. P. IX gave specific details for the methods of standardization of all volumetric solutions; since in most cases these are omitted from the U. S. P. X, different analysts will follow methods from different books of reference, wherein small variations in procedure will occur. If a strict uniformity of results is desirable, would it not be advisable to include all details of methods as was done in the U. S. P. IX? This question is worthy of consideration.

The various series of tests and assays necessary for this paper were carried out with the assistance of Samuel S. Liberman and Marguerite C. Dimler, to whom I wish to express my sincere thanks.

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AMERICAN DENTAL ASSOCIATION ORGANIZES COUNCIL OF DENTAL THERAPEUTICS.

At its recent annual meeting the American Dental Association provided for the organization of a Council of Dental Therapeutics. The organization will cooperate with members of the dental profession in protecting them and the public against fraud and acquaint them with objectionable advertising of proprietary dental remedies. The Association's bureau of chemistry will serve the Council in reaching their decisions. The organization hopes to promote a larger use of the preparations of the U. S. P. and N. F. and it is also contemplated to examine drugs and dental proprietaries for inclusion in an "accepted list." The preparations which receive favorable recognition may be marked "Accepted by the American Dental Association." The latter action is worthy of careful consideration; reference is made to the Government experi-

ence under the Food and Drugs Act with its related legend—viewpoints are as subject to change as individuals.

The Council of Dental Therapeutics is composed of Paul J. Hanzlik, Stanford University; Percy R. Howe, Harvard University; Milan A. Logan, Harvard University; Arno B. Luckhardt, University of Chicago; John A. Marshall, University of California; Victor C. Myers, Western Reserve University; John T. Norton, University of Chicago; Ura G. Rickert, University of Michigan; Harold S. Smith, Chicago; and the following ex-officio members: Editor C. N. Johnson, *Journal of the American Dental Association*; Secretary Harry B. Pinney, of the American Dental Association; Samuel M. Gordon, chemist for the American Dental Association.