In this experiment, the animals of the control group (III C) suffered more severely from pneumonia than did the saccharin-fed animals, as is shown by the progressive drop (continuous line, Chart 5) in weight commencing with the 28th week and by the fact that these animals lived an average of only $6^3/_4$ months as compared with an average of life of 11 months of the saccharin-fed animals.

DISCUSSION.

If Experiment I stood alone, the impression would be gained that the animals fed saccharin were less resistant to pneumonia than the controls; and that the diminution in resistance was in proportion to the amount of saccharin: though it must be admitted that the quantities used were so large as to render this experiment of no practical value as far as consumption of saccharin by human beings is concerned. Curiously enough, in Experiment III, the relations as to weight and duration of life are exactly the reverse. This probably shows that the incident and severity of the pneumonic involvement had nothing to do with the saccharin given; but was due to some other factor, such as possibly the location of the cages in relation to the window. It so happened that in Experiment I Cage A, the animals in which suffered most from pneumonia, was nearest to the window; while in Experiment III Cage C containing the controls, which suffered most from pneumonia, was nearest the window. This window was kept slightly open at all times, summer and winter.

While the gross figures of the average life span in Experiment II show quite a difference, decidedly unfavorable to the control animals, it would be unfair to conclude that saccharin lengthened the life-time of the animals that received it. Correcting the figures in the light of the necropsy findings gives a remarkably close correspondence of the average life period.

CONCLUSIONS.

Feeding saccharin to rats in even relatively enormous doses, and for a life-time of these animals, does not produce lesions appreciable either macroscopically or microscopically, nor does it interfere with the development of these animals or of their progeny, or shorten their span of life.

THE RESORCIN TEST FOR METHYL ALCOHOL.*

BY A. B. LYONS.

The tests most commonly used for the detection of methyl alcohol depend on the conversion into formaldehyde by oxidation of a portion at least of that compound, formaldehyde itself being easily recognized by characteristic reactions.

In case ethyl alcohol also is present the acetaldehyde which results from its oxidation may interfere with some of the distinctive tests for formaldehyde, as in the case of the very striking morphine test, where the purple color produced by formaldehyde is masked by the brown due to acetaldehyde.

The test which is the subject of this paper is not wholly free from this objection, yet fallacious conclusions from its indications are not liable to be reached. Although it has been somewhat neglected of late years, it is one of unique intrinsic interest.

* Scientific Section, A. Ph. A., Cleveland meeting, 1922.

It will be worth while for one who is not familiar with it to make the following experiments. Put into a 500 cc measuring flask 1 cc of the official solution of formaldehyde and fill the flask to the mark with water. From this solution (1:500) prepare successive dilutions 1:1000; 1:2000; 1:4000; 1:8000.

Put into each of 5 test-tubes 1 drop of 1% solution of resorcin, add 1 cc of solution No. 1, followed by 1 cc of strong sulphuric acid. If this is added all at once, and the fluids are quickly mixed by shaking the tube, there will appear momentarily a milky clouding of the mixture, giving place almost at once to a deep red color, with separation promptly of a dark red precipitate, which being specifically lighter than the fluid rises toward the surface, leaving the solution presently nearly without color. If the solution is now diluted with two or three times it volume of water, and thrown upon a filter, it will be seen that the filtrate is quite colorless.

The red precipitate is not perceptibly soluble in either water, dilute acids, alcohol, ether, chloroform, or any similar solvent, so far as I have carried my experiments. The manner of making the test may be modified. A part only of the acid, 10 to 20 drops, from a 1 cc pipette may be added at first, producing a milkiness, with shortly a separation of a nearly white precipitate, which on addition of the remainder of the acid becomes red. Again the acid may be added in such a manner that it flows down the side of the inclined test-tube to form an underlying stratum. In this case a red precipitate will form at the plane of contact of the fluids, while if the strata are made to commingle a little by a gentle motion of rotation, a distinct milky zone will develop above the red ring, the separating floccules changing from whitish to deep red.

Test in succession in the same manner the weaker solutions, and observe the gradual increase in duration of the stage of milkiness, and the corresponding decrease in the quantity of red precipitate which finally separates. At the last there is no immediate separation at all of such precipitate, although on dilution and filtration presence of red floccules is made evident. In the course of a few hours the red particles will aggregate, forming a distinct cloud if the dilution is not greater than 1:20,000 of HCHO.

Thus far we have been studying the reactions of formaldehyde uncomplicated by the presence of the products of oxidation of ethyl alcohol. Prepare now highly dilute solutions respectively of methyl alcohol, ethyl alcohol, and a mixture of these two, and oxidize them with potassium permanganate by the method substantially of Robert M. Chapin.*

For solution (A) add 1 cc of a ten per cent. (vol.) solution of methyl alcohol, 5 cc of water, 1 cc of "dilute" phosphoric acid (one volume of the official acid diluted with water q.s. to make 5 volumes), and 2 cc of 3% aqueous solution of potassium permanganate. Let the mixture stand at room temperature 30 minutes, add 1 cc of a 10% aqueous solution of oxalic acid, and after 2 minutes, drop by drop slowly, strong sulphuric acid q.s. to decolorize. Finally add water q.s. to make 50 cc.

Solution (F) is prepared in the same manner except that in addition to the 1 cc of 10% methyl alcohol, 2 cc of a 10% solution in water of official alcohol is used, and the quantity of the permanganate solution is increased to 3 cc.

In solution (K) use in place of the methyl alcohol of (A) an equal quantity of ethyl alcohol of official strength (95%).

* *Jour. Ind. & Eng. Chem.*, June 1921, p. 544.

From (A) prepare dilutions B, C, D and E by adding, respectively, 8, 6, 4 and 2 cc of it to water q. s. to make 10 cc. Place in each of 5 test-tubes 1 drop of 1% solution of resorcin, add to the first 1 cc of (A), to the second 1 cc of (B), and so on. Finally add to each tube 1 cc of strong sulphuric acid, shake the tubes in each case at once and note the color changes that occur in one minute and in 30 minutes. The first tube shows momentarily a milky turbidity, giving place to a dark red color, with prompt separation of a dark red precipitate. The last tube develops at first a slight milkiness, changing gradually to a pure red color, with or without the separation within the time limit of a red cloud. If the solution is diluted after cooling with twice its volume of water, and filtered, it yields a perfectly colorless filtrate.

From (F) prepare a series of dilutions corresponding with those from (A), designated respectively as G, H, I and J, and apply 1 cc of each the resorcin test. Compare the results with those of the former series.

Note that while the separation of a red precipitate occurs in much the same manner as in that series, there is a great difference in the color of the resultant solutions particularly when the quantity of methyl alcohol is small; in place of a pure red we have now shades of amber, orange or yellow. If the solutions when cold are diluted with twice their volume of water and filtered, there will remain on the filter traces at least of a red precipitate, while the filtrate will be yellowish or pale yellow.

From (K), which is practically free from formaldehyde, prepare also a series of dilutions, and apply to these the resorcin test. Neither milkiness nor a red color is produced in any of them, a clear solution of a more or less pronounced yellow color resulting in each case. In the strongest the color is an intense golden yellow, showing a strong green fluorescence. The same color, it will be remembered, is produced by Hehner's test in its several modifications, where in place of resorcin, peptone, egg albumen or milk is used, and appears also when either test is applied to oxidized solutions of acetone and other derivatives of ethyl alcohol.

Familiarity with these facts is presupposed on the part of anyone applying the resorcin test for presence of formaldehyde.

For the practical testing of a sample of spirituous liquor suspected to contain methyl alcohol the following routine procedure is proposed:

Prepare the sample if necessary by diluting with an equal volume of water and distilling slowly to obtain a distillate of the same volume. To 1 cc of this add water q. s. to make 10 cc. Measure of this diluted distillate exactly 1 cc into a small flask. Add 5 cc of water, 1 cc of "dilute" phosphoric acid (1 volume of the U. S. P. strong acid diluted with water to make 5 volumes) and 3 cc of a 3% aqueous solution of potassium permanganate. Let this mixture stand at room temperature 30 minutes, then add 1 cc of a 10% aqueous solution of oxalic acid. After 2 minutes add from a 1 cc pipette 5 drops of strong sulphuric acid, and after 1 minute 5 drops more, continuing this until the color of the solution is discharged. Add then water q. s. to make 50 cc. Label the solution (X).

Prepare a standard oxidized solution (A), using 1 cc of a 10% volume solution of pure methyl alcohol, 2 cc of 10% ethyl alcohol, 1 cc of "dilute" phosphoric acid, and 3 cc of 3% potassium permanganate, following the same routine as above and making up the solution to 50 cc.

From this standard solution prepare dilutions (B), (C), (D) and (E) by adding to 8, 6, 4 and 2 cc of it water q. s. to make 10 cc. Place in each of 5 test-tubes 1

drop of a 1% solution of resorcin, in the first 1 cc of (A), in the second 1 cc of (B), and so on; finally add to each 1 cc of strong sulphuric acid. Label respectively: Color Standard, 100% (or 10%); Color Standard, 80% (or 8%); Color Standard, 60% (or 6%); Color Standard, 40% (or 4%); and Color Standard, 20% (or 2%).

Prepare now for comparison a color solution of the sample by adding to 1 cc of (X), 1 drop of the resorcin solution, and 1 cc of strong sulphuric acid. Compare the results with those of the color standard series. If there is exact correspondence with one of these the per cent. of methyl alcohol in the sample will be that stated on the label (the first figure).

When coincidence is not exact in any case, make an interpolation by methods familiar in colorimetry and so arrive at a close approximation to the correct percentage. (Note that the "color" in this connection is used in a broad sense, including all the observed phenomena of the reactions.)

When the indicated percentage is less than 20, modify the procedure by taking 5 instead of 1 cc of the diluted distillate to prepare our oxidized solution, omitting the 5 cc of water and making up the volume to 25 instead of 50 cc. It will be seen that the indicated percentages will be just one-tenth of those given in the first figures on the color standard labels—the figures therefore in parenthesis on those labels.

If the percentage is above 20 resort to interpolation to obtain reasonably correct figures. It will not often happen in the examination of a distilled spirit (*e. g.*, whisky) that the per cent. will be much above 40.

In case no ethyl alcohol is present, indicated by absence of yellow color in results of tests of highly dilute solutions, modify the procedure by omitting ethyl alcohol in preparing the standard oxidized solutions, and use for the oxidation only 2 instead of 3 cc of the permanganate solution.

It is to be understood that the quantitative results arrived at in these tests are necessarily only approximate, for the reasons that oxidation of wood spirit by the method employed is not strictly quantitative, and that the tests made under conditions as nearly as possible identical show capricious variations, particularly as to rapidity of precipitation.

Further, we are to remember that methyl esters and some other compounds, including some of the higher alcohols, may yield formaldehyde by oxidation. It is safe to say, however, that a wholly negative result is conclusive as to the practical absence of methyl alcohol.

Whether useful results can be reached by isolating and weighing the precipitate is an interesting question, the investigation of which I have not yet attempted.

Comparisons of results may be made roughly in case the precipitates are copious by noting the effect of dilution, using as a criterion the separation or non-separation of floccules within 30 minutes, but slight differences in the conditions present influence the results to such a degree that the margin of uncertainty is very wide. Collection of the floccules on a filter, when the solutions are very dilute, may enable a practiced eye to judge of the relative quantity of such precipitates.

Results by the latter mode of comparison may be checked by one in which the quantity of acid used in the test is reduced until only a milkiness is produced instead of a distinct reddish or red precipitate.

A baffling problem is that of determining with reasonable exactness the proportions of methyl and ethyl alcohol when in simple mixture. Complete oxidation

requires a much greater quantity of oxygen in the former case than the latter, and a simple combustion method should not be difficult to devise.

The resorcin test, however, is capable of giving results of practical value and it need not consume much time. Standard solutions may be prepared containing respectively 20, 40, 60 and 80 per cent. by volume of methyl alcohol, the remainder consisting of 95% ethyl alcohol. Five cc of each of these solutions is to be diluted with water to make, after cooling, 25 cc.

To 1 cc of each of these dilutions is added 5 cc of water, 1 cc of "dilute" phosphoric acid, and 2 cc of potassium permanganate solution (3%). The further routine given already in detail for producing an "oxidized" solution is to be carried out, and thus standard color solutions obtained with which to compare similar solutions prepared from the sample under examination.

PRACTICAL CONCLUSIONS.

The resorcin test for methyl alcohol in spirituous liquors is easily made. The reactions involved are strikingly characteristic and not more subject to misinterpretation than other similar tests depending upon the oxidation of methyl alcohol to formaldehyde.

Ethyl alcohol influences the result of the test, but rarely so as to invalidate the conclusions drawn therefrom.

In case of mixtures pure and simple of methyl and ethyl alcohols it is practicable to determine approximately the proportions of each. This is true even when water also is present.

LABORATORY NELSON, BAKER & CO.,
July 3, 1922.

THE ANTIDOTAL EFFICACY OF FERRI HYDROXIDUM CUM MAGNESII OXIDO, U. S. P. IN ARSENICAL POISONING.

BY HUGH McGUIGAN, H. V. ATKINSON AND G. A. BROUGH.

INTRODUCTION.

This investigation was undertaken at the request of the U. S. Pharmacopœial Revision Committee, through Dr. Torald Sollmann. The Committee is interested in the antidotal efficacy of Ferri Hydroxidum cum Magnesii Oxido, U. S. P. in arsenical poisoning.

Bunsen and Berthold,[1] in 1834, recommended the use of freshly precipitated ferric hydroxide in the treatment of acute arsenical poisoning. This work has been the basis for treatment ever since. De Busscher,[2] in 1902, after an investigation of the problem, claimed the antidote was without beneficial effect.

In some cases his animals, even without antidote, survived for several months and therefore can hardly be called acute cases. In addition, laboratory animals may die from other causes when they are kept in cages this length of time. Before giving the arsenic, he did not attempt to have their stomachs practically free from food. All of these factors might be raised to render the interpretation of his results uncertain. Therefore, we have thought it advisable to reinvestigate the essential parts of his work before rejecting this standard treatment. It is not necessary to determine the smallest lethal dose; this has already been adequately done by de Busscher,[2] Morishima,[3] and also by Schwartze.[4] However, to test a treatment, we should know that a dose of arsenic sufficient to cause death has been given. Then, if the antidote is useful, we can evaluate it. We have, therefore,