and probably the hemoglobin in such birds. While these results strengthen the assumption that the pigeon response is a measure of the substance or substances effective in pernicious anemia, limited clinical comparisons indicate that the pigeon

TABLE II.—RED CELL AND HEMOGLOBIN RESPONSES.

Experiment.	Dose.	Days after 1st Inj	Retic. %.	Red Cells, Million Per Cu. Mm.	Hemoglobin, Sahli %
3*c*	Single R, in 3 injs.	0	12.1	3.12	122
		7	23.3[1]	4.54	123
		14	19.7	5.00	135
4*c*	Single R, 1 inj.	0	15.4	3.40	
		7	22.4	3.81	Unchanged (?)
		12	24.1[2]	5.02	Increased[3]
		20	17.0	4.08	
5*a*	Histidine, tryptophane, 100 mg. in 2 injs.	−3	15.8	3.84	83
		−1	15.6	3.74	
		1	16.0		
		3	16.8	3.14	
		7	19.3[4]	3.39	90
		13	15.0	3.36 (10/31/32)	
5*b*	Crystals (L. E.) 44 mg. 3 injs.	0	14.8		
		9	19.2[5]	4.91 (1/4/33)	
10*d*	Half R, 1 inj.	−2		4.37	156
		0	14.0[6]		
		9	15.1	4.63	158
11*a*	Single S_8, 1 inj.	0	14.4	4.77	
		4	23.1[7]	5.23	
		11	15.6	5.77	

Remarks: [1] Max., 24.0%, 6th day. [2] Max. [3] Figures (Newcomer) omitted because of error in reading. [4] Max.; R. P. rise not typical (?). See discussion. [5] Max., 22.5%, 5th day. [6] Max., 18.5%, 5th day. [7] Max.

effectiveness may not parallel the clinical response. Further clinical comparison is necessary to settle the question of specificity or practical utility.

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A METHOD FOR THE DETERMINATION OF MINUTE AMOUNTS OF ALDEHYDES IN ETHER.*

BY M. W. CAREY, L. W. GREEN AND R. E. SCHOETZOW.

Several investigators (1, 2) have shown that the U. S. P. X test, for aldehydes in ether, is not sensitive to small amounts. The use of solid potassium hydroxide instead of the solution which is directed by the U. S. P. X, will increase the sensitivity so that between 50 and 100 parts per million of acetaldehyde may be detected

* Scientific Section, A. PH. A., Toronto meeting, 1932.

We desired a method capable of detecting as well as estimating much smaller quantities of aldehydes than those detectable by the U. S. P. X and similar tests.

A search of literature seemed to show that the most sensitive tests for aldehydes in ether are those employing fuchsine sulphurous acid, alkaline silver nitrate and Nessler's reagent.

The silver reduction test is performed with reagents composed of silver nitrate, sodium hydroxide and ammonia. The disadvantages are that reducing substances in general will react with ammoniacal silver nitrate and also if care is not exercised to immediately destroy the test materials, accidents may result, because the materials may explode upon standing. The test appears to be sensitive to one part per million, but, since it is not specific for aldehydes, we discarded it from consideration.

Nessler's reagent, likewise, although equally sensitive is not specific for aldehydes. The other impurities, which may be present in ether, such as peroxide will affect the test. Even alcohol, a substance which is normally present in anesthetic ether, reacts with Nessler's reagent within a few minutes. For these reasons, we discarded it, notwithstanding its official recognition in some foreign pharmacopœias.

The fuchsine sulphurous acid reagent has been used by Phelps and Rowe (3) in a quantitative manner with a sensitivity of about 30 parts per million.

Articles by Leffman and Pines (4) and Leffman and Trumper (5) showed that a fuchsine sulphurous acid reagent formulated by Fincke was extremely sensitive to aldehydes in ether; so sensitive in fact that they stated that it might be necessary to fix a toleration limit for practical control. We studied the use of this reagent and found it very sensitive. Although we found by experiment conditions under which it could be used in a quantitative manner capable of estimating aldehydes within two or three parts per million, yet it was not entirely satisfactory, because we had to run the test at a low temperature. Even at that temperature, some slight oxidation of the reagent seemed to occur, which gave rise to questionable readings at times.

Middleton and Hyman (6) published an excellent review of the various tests for aldehyde in ether. In this article, they stated that in testing ether, the use of Schiff's reagent (7), which is a fuchsine sulphurous acid reagent, had been limited by uncertainty as to its significance owing to the color showing a progressive increase in time, but that this objection could be removed by the addition of 0.1% pyrogallol, which does not decrease sensitivity, but does restrain oxidation.

Recently we substituted the decolorized fuchsine solution of the British Pharmacopœia, 1914 edition, with the addition of 0.1% of pyrogallol for the Fincke reagent which we had been using for some time and found that a definitely negative test could always be obtained with ether specially treated so as to be aldehyde-free. At the same time this reagent is sensitive enough to disclose aldehydes when added to the same ether in the proportion of one part per million. The test has the advantage that it may be run at room temperature while previously we had been obliged to use a lower and accurately controlled temperature.

The desirable features of this method are as follows:

1. It is sensitive to less than one part per million of aldehyde in the hands of the average operator and can be used to estimate somewhat larger quantities of

aldehydes with an accuracy of plus or minus two parts per million and perhaps plus or minus five parts per million for quantities up to about 50 parts per million.

2. The reaction is specific for aldehydes while compounds other than aldehydes likely to be present in ether give negative results. The compounds tried included peroxides, acetone and alcohol.

3. Results obtained were readily checked.

The details for carrying out the test are as follows:

PREPARATION OF FUCHSINE SULPHUROUS ACID TEST SOLUTION.

Prepare decolorized fuchsine solution according to the formula given in the 1914 edition of the British Pharmacopœia, which is as follows:

"Dissolve one gram of Fuchsin in five hundred millilitres of hot Distilled Water; add slowly twenty millilitres of a saturated aqueous solution of Acid Sodium Sulphite, and then, also slowly ten millilitres of Hydrochloric Acid, the mixture being kept well shaken. Cool and add sufficient Distilled Water to produce one thousand millilitres." To this add 0.1% of pyrogallol.

NOTES: 1. Basic fuchsine dyes of different brands vary in sensitivity.

2. Certain samples of fuchsine cannot be entirely bleached with sulphurous acid. We have found the resultant amber-colored solutions less sensitive than the colorless ones, which may be prepared from better brands of fuchsine.

3. Fuchsine sulphurous acid solution should be kept in a refrigerator to prevent decomposition.

PREPARATION OF ALDEHYDE-FREE ETHER.

Shake continuously equal parts of ether and sodium bisulphite solution (30%) for one hour. Separate and discard the sodium bisulphite solution. Then add to the ether, one-fifth of its volume of sodium hydroxide solution (10%) and shake for ten minutes. Separate and discard the sodium hydroxide solution. Test the ether according to the method given below. A colored line or band must not be produced.

PREPARATION OF ALDEHYDE STANDARDS.

(*a*) Prepare aldehyde-free alcohol and the primary standard acetaldehyde solution therefrom containing 1 Gm. acetaldehyde per 100 cc., according to the Methods of Analysis of the A. O. A. C. (8).

(*b*) Accurately measure 1 cc. of the standard aldehyde solution prepared under (*a*) and dilute to 100 cc. with aldehyde-free ether. Mix thoroughly. This solution contains 100 p. p. m. of acetaldehyde. Dilute this solution further with aldehyde-free ether to prepare dilutions containing 50, 30, 10 and 5 p. p. m. of acetaldehyde. After a preliminary test using these standards, other standards can be prepared to match the sample under examination.

NOTE: The primary standard acetaldehyde solution as prepared under (*a*) and the secondary or dilute standards as prepared under (*b*) should be kept in a refrigerator. The former will retain its strength for at least two weeks. The others must be prepared freshly.

THE QUANTITATIVE ALDEHYDE DETERMINATION.

Place 5 cc. of the ether under examination, 5 cc. of each of the appropriate standards, including one containing aldehyde-free ether as a blank, into separate

8″ x 1″ test-tubes preferably arranged in a rack under good light and provided with a white background. Then add slowly to each tube a 5-cc. portion of decolorized fuchsine test solution, holding the tip of the pipette just over the surface of the ether so as to float the ether quietly over the test solution. Do not shake or disturb the tubes. At the end of twenty minutes, observe the color at the junction of the two layers. The aldehyde content of the ether under examination is the same as that of the standard which it matches.

If necessary, repeat the comparison with stronger or weaker standards.

NOTES: 1. A match or nearly a match must result before making a decision as to the aldehyde content of the ether. This is necessary, since the color is not directly proportional to the aldehyde content. For instance, the ether containing 50 p. p. m. of aldehyde will not give twice as heavy a red band as one containing 25 p. p. m.

2. Do not shake the tubes nor disturb them until the time of examination; it causes the faint-colored bands produced by minute amounts of aldehyde to be dissipated throughout the aqueous layer and, practically, to disappear.

The substitution of the British Reagent plus pyrogallol for the Fincke's Reagent has occurred so recently that we have not had so much experience as desired, but we believe it the most satisfactory of all we have worked with and will continue our work with it.

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NOTE: This paper was read at the Toronto meeting of the A. PH. A. in 1932. Since that time, we have found it advisable to standardize the temperature at 20° C. at which to perform the test. We have also found it advisable to use redistilled acetaldehyde and aldehyde-free ether in the preparation of the aldehyde standards instead of the methods mentioned above.

ANALYTICAL LABORATORIES,
CHEMICAL & PHARMACEUTICAL DIVISION,
E. R. SQUIBB & SONS.

A. Ph. A. Resolution No. 10. Thanks to Council on Medical Education and Hospitals of American Medical Association.

Resolved, that the thanks of the AMERICAN PHARMACEUTICAL ASSOCIATION be expressed to the Council on Medical Education and Hospitals of the American Medical Association for the favorable attitude expressed by resolution of this body on the subject of hospital pharmacies and their supervision and be it further

Resolved, that the AMERICAN PHARMACEUTICAL ASSOCIATION continue its endeavors to provide for the supervision of all pharmaceutical work in hospitals by registered pharmacists.
