

doses the patients may sleep from 3 to 10 hours and occasionally longer. Nausea, retching and vomiting have not been observed in any of the cases. Frequently patients when coming out of sleep become restless before they are fully conscious. Morphine administered in the usual amount exerts a quieting effect.

In those cases in which maximal anesthetic doses have been employed, in which it is desired that the patient should awake somewhat sooner and fully alert mentally, 25 to 50 mgm. of ephedrine sulphate injected intramuscularly, followed by 0.65 to 1 gm. sodium caffeine benzoate, one-half to 2 hours after the operation, has exerted the desired effect.

Detailed papers covering the clinical, pharmacological and biophysical-chemical aspects of the case will shortly be published.

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A Consideration of the Pyridine Test for Chloroform.

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A method for the quantitative measurement of minute amounts of chloroform has been described by Cole.¹ An attempt was made to use this test to determine the amount of chloroform in sea water, in which small marine organisms were being anesthetized. Certain difficulties were encountered, many of which arose from the fact that the accuracy of the test was not known. This led to the study of the accuracy of the test discussed below.

A series of 105 tests were made upon a given concentration of chloroform in sea water. The test consists of mixing 2 cc. of 20% NaOH in distilled water, 1 cc. of pyridine and 1 cc. of the chloroform solution in a test tube and placing the latter in a water bath at 100° C., a red or pink color appearing in the mixture at this time. Precautions are taken to eliminate errors due to evaporation. When the chloroform is in sea water a precipitate forms; this is thrown down with a hand centrifuge. When distilled water is used no precipitate is formed. The tube is then cooled to about 18° C. and placed in a comparator box so arranged that several tubes can be arranged one behind the other, the color within the tubes being summated to give an intensity of color greater than that of any one

¹ Cole, William H., *J. Biol. Chem.*, 1926, lxxi, 173.

of the tubes so arranged. The single tube containing the test solution is in this way compared with one or more standards and its chloroform content determined. The standards consist of tubes containing solutions of basic fuchsin in acidified 50% alcohol and are prepared according to Cole¹; they are of various color intensities and represent 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005 and 0.0001% of chloroform. From this series of tests it has been calculated that the mean deviation of a representative group of such measurements is not less than ± 0.001 .

If a concentration of 0.03% chloroform can be measured to the fifth decimal place by this method, then the eye should be able to detect the difference in intensity when a 0.00005% color standard is placed behind a 0.03% standard in the comparator box mentioned above. Ten persons have been tested, therefore, as follows. The 0.5% standard is placed in the comparator box and each of the lower standards placed behind it. The person tested chooses the lowest standard which will just perceptibly increase the intensity of the 0.5 standard when placed behind the latter. In the same way, that standard is chosen which will just increase the intensity of the 0.1% color standard when placed behind it, and so on for each of the other standards. Standards were chosen in this way by each of the 10 persons; the results were averaged and are given in the table below.

Standard (A)	0.5	0.1	0.05	0.01	0.005	0.001	0.0005	0.0001
Average—	0.17	0.03	0.007	0.0007	0.0002	0.0001	0.0001	0.0001
10 persons (B)								
B/A	0.34	0.30	0.14	0.07	0.04	0.10	0.20	1.00

The ratio B/A is the ratio of a color standard which will just perceptibly increase the intensity of another color standard divided by the color standard the intensity of which is thus increased. This ratio is taken as a measure of the sensitivity of the eye to the particular intensity of color, the sensitivity increasing as the ratio decreases. It is to be noted that the sensitivity is greatest at about the 0.005% standard and decreases as the color standards increase and decrease. From the table it is evident that a 0.03% chloroform solution can be measured with an accuracy no greater than that implied by a figure in the third decimal place.

Cole² uses the pyridine test to determine the percentage of chloroform in extracts of the brains of rats killed by the inhalation of chloroform vapor. Knowing the volume of the extract and the weight of the brain he calculates the quantity of anesthetic taken

² Cole, William H., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, *xxiv*, 340.

up per mgm. of brain and subsequently yielded to the extracting fluid. These results are carried to the fifth decimal place. From these figures it is concluded that the amount of chloroform present in the brains of rats at the time of death varies directly with the dosage. From the above table it is evident that the accuracy of the test does not justify the expression of such measurements to the fifth place. Furthermore, if one assumes that these figures are means of representative groups, each having a mean deviation of ± 0.001 and the individual measurements of each falling within ± 0.004 , they should differ by at least 0.008 to be significant. As the dosage of chloroform increases, however, from 0.2 to 5.0 cc. per liter of air, only twice does the amount of chloroform per mgm. of brain tissue increase by as much as 0.008. From this it must be concluded that the measurements referred to do not show sufficient difference to justify their use as a basis for prediction.