masses which stain deeply with basic dyes. This may mean increased acidity and consequently an increased hydrophylic tendency on the part of the cell, particularly the nucleus. It is probable, however, that the cytoplasm is also involved inasmuch as certain of its constituents, namely the Golgi apparatus and the mitochondria are markedly modified within 5 hours after administration of the drug. Under such treatment the Golgi apparatus which ordinarily occurs in the carcinoma cell as a basket-like or looser thread-like mass at one side of the nucleus is dispersed into a cloud of fine droplets, and the usually thread-like mitochondria break up into small rods or granules. Whether the plasma membrane of the cell becomes more permeable to water is not yet known.

There is even noticeable change after some hours in the gross appearance of carcinomas in rats which have been given as high a dosage of colchicine as 0.2 mg per 100 g of body weight. The usual white, waxy appearance of the tumor is replaced by a decidedly reddish color, indicating considerable extravasation of blood. Experiments with various aqueous agents and with other types of tumor are in progress.

Summary. Following administration of colchicine to their hosts 15 hours earlier, Flexner-Jobling carcinomas were found in a majority of 103 cases to be rapidly destroyed by distilled water injected directly into their substance.

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A Note upon the Micro-Determination of Ammonia.*

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Several excellent procedures are available for the micro-estimation of ammonia of which the methods of Conway and Byrne[1] and Gibbs and Kirk[2] are notable examples. In connection with studies of tissue respiration it became desirable to determine quantities of ammonia of the order of 5 γ to 50 γ present in the Ringer fluid in which various

* Aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

1 Conway, E. J., and Byrne, A., *Biochem. J.*, 1933, **27**, 419.

2 Gibbs, G. E., and Kirk, P. L., *Mikrochemie*, 1935, **16**, 25.

tissues were suspended. Attempts were made to Nesslerize the deproteinized fluid with measurement of the resulting light transmission with the Evelyn photoelectric colorimeter (Filter 440) as in the micro-non-protein nitrogen determination adapted for this instrument.[3] These were frustrated by immediate clouding of the Nesslerized solutions as a result of the small amount of magnesium ion present in the Ringer fluid[4] employed. Inhibition of the precipitation was not obtained by addition of various stabilizers, *e. g.*, gum ghatti, gum acacia, etc., although the onset and rate of clouding was slightly delayed by adding a small amount of 50% Rochelle salt solution prior to Nesslerization. This slight delay sufficed to make the photoelectric determination feasible. The following technic was found to yield moderately accurate results in spite of interfering magnesium salts.

The fluid in the Barcroft vessel is transferred to a graduated 15 cc centrifuge tube by pipette transfer and the chamber washed 4 successive times with small amounts of distilled water (rendered NH_3-free by treatment with bromine), the washings, in turn, being transferred to the centrifuge tube, making the total volume about 10 cc. This fluid is ordinarily acidic, respiration having been stopped with appropriate quantities of strong acid. If this is not the case, 0.3 cc of NH_3-free 3N H_2SO_4 is added to the contents of the centrifuge tube. This is followed by the addition of 0.22 cc 10% sodium tungstate and 0.11 cc of a 50% solution of Rochelle salts, after which the volume is made to 11 cc with NH_3-free distilled water, and the contents well mixed by agitation with a clean glass rod. Precipitation of the protein is complete in 10 to 20 minutes, after which a clear supernatant fluid may be obtained by centrifugation. Ten cc of this is measured into an Evelyn photoelectric colorimeter tube. A "blank" tube is prepared, containing 1.36 cc of Ringer fluid (which has been made NH_3-free by treatment with bromine), 0.27 cc 3N H_2SO_4, or the corresponding quantity of acid used in stopping respiration, 0.2 cc 10% sodium tungstate, 0.1 cc 50% Rochelle salts, and NH_3-free water to make the final volume 10 cc. Filter 440 is now placed in the colorimeter, and the galvanometer adjusted to the expected center setting (*i. e.*, the reading which is to be obtained following withdrawal of the Nesslerized blank tube when the reading with the latter in place is 100). Two cc of Nessler's reagent are now added to the blank tube, mixed by

[3] *Evelyn Photoelectric Colorimeter; Notes on Operation*, p. 25, Rubicon Company, Philadelphia, Pa. 1939.

[4] Krebs, H. A., and Henseleit, K., *Z. Physiol. Chem.*, 1932, **210**, 36.

tapping, and the tube placed in the instrument *immediately*. The galvanometer is quickly adjusted to 100, the tube withdrawn and the immediate center setting value recorded. The whole operation should not occupy over 5 to 7 seconds. With the empty instrument adjusted to the proper center setting reading, the "unknown" tube is Nesslerized similarly, and the immediate galvanometer reading taken, ignoring the slow drift which subsequently occurs.

The "K" value is not sufficiently constant over a range of 5 γ to 50 γ to calculate the concentration as a function of the transmission from the Beer-Lambert law, hence a calibration curve is prepared by Nesslerizing tubes containing the reagents present in the blank tube plus graded amounts of a standard ammonium sulfate solution, plotting the *immediate* galvanometer reading against concentration. The concentration of ammonia in an "unknown" tube may then be read off the curve once the galvanometer reading is obtained. Multiplication of this by the factor 1.1 gives the total ammonia content of the fluid in the Barcroft vessel.

Table I shows the recovery of added quantities of ammonia from a series of Ringer fluids in which kidney cortex tissue slices have respired. In our experience, over the range of 10 γ to 50 γ the accuracy of the method is of the order $\pm 5\%$. For quantities below 10 γ the inherent errors in photolometric measurement considerably decrease the accuracy of the determination, which at concentrations of 5 γ is not better than $\pm 10\%$.

Although the reaction of Nessler's reagent with ammonia is a very rapid one, it is not entirely completed in the time interval before the galvanometer reading is made. Standardization of technic, in terms of the time intervals employed, eliminates this error by cancellation in the preparation of the calibration curve.

The advantages of this procedure include rapidity, the independence from dilute standard solutions, diminished risk of contamination, as well as the obviating of interference by magnesium salts.

TABLE I.
Recovery of Ammonia Added to Ringer Fluid in Barcroft Vessels; Averages of Duplicate Determinations.

γ NH_3 added	γ NH_3 recovered	% recovery
5.0	4.6	92
10.0	9.5	95
10.0	10.6	106
20.0	20.1	101
30.0	30.7	102
40.0	39.7	99
50.0	50.6	101