inhibition. Similarly, it was found that cyanide in concentrations of 0.0166M inhibits the oxygen uptake to the extent of 84 to 86 per cent. in normal beets and of 79 to 80 per cent. in tumor beet tissue.

Previous work in this laboratory[1] has shown a differentiation between the healthy part and the tumorous part of the same beet, whereas the above work shows a differentiation between tumorous tissue and the tissue of an entirely healthy non-infected beet root.

Upon addition of cyanide to resorcinol, and *vice versa,* in the case of both healthy and tumorous beet root tissue, there is an increase in inhibition of 6 to 8 per cent. above that due to cyanide alone. These results are summarized in Table 1.

TABLE 1

EFFECT OF RESORCINOL AND CYANIDE ON THE RESPIRATION OF NORMAL AND TUMOR BEET ROOT TISSUE

Substance added	Per cent. inhibition Beet tissue slices	
	Normal	Tumor
Resorcinol	12–14	20–23
Cyanide	84–86	79–80
Resorcinol and cyanide ..	92	86

They suggest: (*a*) Tumors as well as healthy beets may have the following types of respiratory mechanisms: (i) cyanide insensitive system, (ii) resorcinol insensitive system, (iii) cyanide plus resorcinol insensitive system, as well as the corresponding sensitive systems, the relative proportion of the three systems in tumor and healthy tissue being different in each case. (*b*) The inhibitions brought about by cyanide and by resorcinol function to a certain degree independently of each other. (*c*) It appears possible that different active centers of the same enzyme (probably a heavy metal compound) are attacked by both inhibitors but to a different degree.

Experiments are now in progress to determine the nature of this differentiation between tumor and normal tissue.

The authors wish to thank the Carnegie Corporation of New York with whose financial assistance this work is being carried out.

MORITZ MICHAELIS
IRVING LEVI
HAROLD HIBBERT

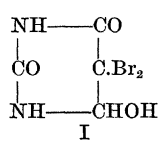
DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, McGILL UNIVERSITY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SENSITIVE COLOR REACTION FOR THE DETECTION OF URACIL AND CYTOSINE[1,2]

THE author will describe in this paper a modification of the well-known Wheeler and Johnson color test for the detection of the pyrimidines uracil III and cytosine[3] II, which has met with wide application since the date of its discovery in the Yale Laboratory.

The original test is based on the action of bromine on either of these two pyrimidines in aqueous solution, which reacts with them quantitatively with formation of 5,5-dibromoxyhydrouracil I. This hydropyrimidine is characterized by its reactivity towards barium hydroxide with which it reacts in aqueous solution to form (1)—isodialuric acid V and (2)—by rearrangement dialuric[4] acid VI. Both of these pyrimidines give insoluble, deep-purple colored barium salts by neutralization with an excess of barium hydroxide.[5]



I

Bromine water or liquid bromine are necessary reagents for the application of this useful test. There are, however, limitations in applying successfully the color test when uracil and cytosine are present in very small quantities; and furthermore, bromine is not always available in clinical laboratories for experimentation. The author also has experienced unexpected difficulties in detecting uracil and cytosine in the study of natural products containing mixtures of carbohydrates, purines and pyrimidines. He has, therefore, devised a new technique or modification of the original Wheeler and Johnson procedure[3] for testing for these two naturally occurring pyrimidines, which is an improvement on the original experimental procedure.

The new technique calls for only two common laboratory reagents, namely, concentrated hydrochloric acid and superoxol.[6] Advantage is taken, in this new application of the color test, of the known reactivity of hydrogen peroxide towards hydrochloric acid as is expressed in the equation below. The hypochlorous

$$HCl + H_2O_2 = HOCl + H_2O$$

acid formed in this reaction reacts immediately and quantitatively with uracil or cytosine if present in

[1] A. C. Neish and Harold Hibbert, *forthcoming publication.*

[1] Contribution from the Department of Chemistry, Yale University.

[2] "Researches on Pyrimidines," clxxx.

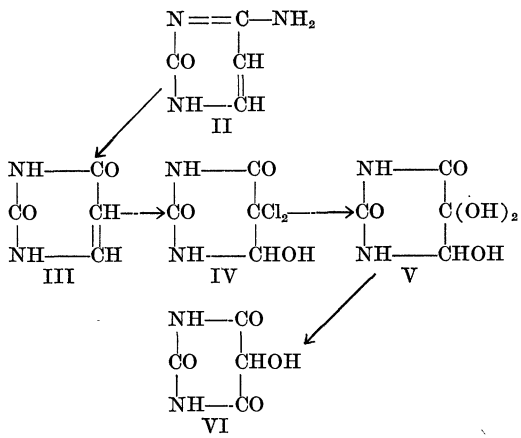
[3] Wheeler and Johnson, *Jour. Biol. Chem.,* 3: 183, 1907.

[4] Behrend and Koch, *Ann.,* 315: 246, 1901.

[5] Behrend and Roosen, *Ann.,* 251: 244, 1889.

[6] Hydrogen peroxide 30 per cent.—Merck and Company, Rahway, N. J.

such a solution with formation of 5,5-dichloroxyhydrouracil IV. This chloro-compound reacts with barium hydroxide in a similar manner as the bromo-pyrimidine I, yielding the characteristic, purple barium salt of dialuric acid VI. The pyrimidine IV has previously been prepared in this laboratory by the action of chlorine water on uracil, and by the oxidation of uracil with potassium chlorate in hydrochloric acid solution.[7] In other words, the original color test for the detection of uracil and cytosine was dependent upon the formation of 5,5-dibromoxyhydrouracil[8] I, while in this new modification of the test 5,5-dichloroxyhydrouracil IV functions as the key pyrimidine reagent. The sensitiveness of the test has been increased by introduction of this new experimental technique.



EXPERIMENTAL PART

The Formation of 5,5-Dichloroxyhydrouracil IV by the Action of Hydrogen Peroxide on Uracil in Hydrochloric Acid Solution:[8] One gram of uracil was dissolved in 20 cc of concentrated hydrochloric acid and 15 cc of superoxol (Merck) added at 0°. Heat was gradually evolved on standing accompanied by evolution of oxygen, but without development of color. After allowing to stand at laboratory temperature for several hours (12 to 15), the solution was then transferred to a glass evaporating dish and exposed to a draft of air in a hood. Beautiful, glistening prisms began to form and increased in quantity until 1.0 g had deposited. They were identified as pure 5,5-dichloroxyhydrouracil[7] IV. The compound contained chlorine and decomposed with effervescence at 218°. This pyrimidine reacted immediately in cold aqueous solution with barium hydroxide yielding a brilliant purple precipitate of the characteristic barium salt of dialuric acid.

Analysis: Calc'd. for $C_4H_4O_3N_2Cl_2 \cdot H_2O$ N, 12.90
Found: N, 12.80, 12.75.

[7] Johnson, *Am. Chem. Jour.*, 40: 26, 1908.

[8] See "Researches on Pyrimidines," clxxviii. Johnson, *Jour. Am. Chem. Soc.*, 65: 1943.

The acid solution remaining after filtering off the crystals of 5,5-dichloroxyhydrouracil IV responded immediately to the Wheeler and Johnson color test for uracil.

Method of Application of the Modified Wheeler and Johnson Color Test for Detection of Uracil and Cytosine: Use a 10–15 cc pyrex beaker for performing the test. Deposit on the bottom of this beaker 5–10 mg of uracil, or the mixed sample to be tested, and moisten with 0.5 or 1.0 cc of superoxol. There will be no apparent change or development of color. Then add from a pipette the same volume of pure concentrated hydrochloric acid (0.5 or 1.0 cc). Heat will be evolved and the uracil will finally dissolve, giving a perfectly clear solution. The mixture is then heated on a boiling water or steam bath when a violent reaction will set in with rapid evolution of oxygen. After subsidence of this reaction continue to heat at 100° until the mixture is reduced to a volume of about 0.5 cc and then cool. If uracil or cytosine were present in the test mixture, an immediate purple coloration or precipitate will be formed by addition of barium hydroxide solution until the reaction mixture shows an alkaline reaction. The whole operation can be conducted in an open laboratory hood, which is not the case when bromine is used in applying the test. Both uracil and cytosine are very sensitive towards hypochlorous acid.

In testing for the presence of uracil or cytosine in solutions of unknown concentration, a known volume should be evaporated almost to dryness before applying the hydrogen peroxide treatment for formation of 5,5-dichloroxyhydrouracil IV.

In Table 1 are recorded the results of a series of color tests applied to different reactants according to the new technique for detecting uracil and cytosine.

TABLE 1

A. *Reactants which gave positive tests confirming the presence of uracil or cytosine:*
   1. Uracil, uracil + thymine, uric acid + uracil, 4-methyluracil + uracil, cytosine hydrochloride, yeast nucleic acid + cytosine, 4-methyluracil + cytosine, glucose and uracil, thymine + cytosine.
   2. 2-Ethylmercapto-6-oxypyrimidine, 2-methylmercapto-6-aminopyrimidine.

B. *Reactants which gave no color test indicating the absence of uracil and cytosine:*
   1. Thymine, uric acid, 4-methyluracil, hydrouracil, 5-bromhydrouracil, 4-phenylcytosine, 4,5-dimethyluracil, yeast nucleic acid, 4-phenyluracil.

C. *Reagents used in applying the above color tests:*
   1. Superoxol, concentrated hydrochloric acid and barium hydroxide.

SUMMARY

(1) Uracil and cytosine are both transformed quantitatively into 5,5-dichloroxyhydrouracil by interaction with superoxol and concentrated hydrochloric acid.

(2) This 5,5-dichloroxyhydrouracil reacts with barium hydroxide immediately at ordinary temperature giving the purple colored barium salt of dialuric acid.

(3) Both uracil and cytosine can be detected successfully in minute quantities by application of the technique discussed in this paper.

TREAT B. JOHNSON

NEW HAVEN, CONN.

A NYLON BLOOD AND PLASMA FILTER[1]

THE storage of blood and plasma has become commonplace within the last few years. However, these products frequently develop precipitates despite the use of what appears to be an adequate amount of sodium citrate as the anticoagulant. Although it is desirable that fresh blood be filtered immediately before administration to the patient, this precaution is mandatory when stored blood or plasma is used.

Materials such as cotton gauze, fiber glass braid, glass beads and stainless steel screens have been employed for filtration with varying degrees of success. More recently, Novak[2] has advocated a viscose rayon cloth.

During the past eight months we have been using a nylon[3] filter which has the following desirable characteristics: (1) It removes all clots without clogging; (2) it does not shed lint into the filtrate; (3) it is simple to clean, assemble, and sterilize by autoclaving, and (4) it is inexpensive so that the filter bag may be discarded after being used once.[4] The filter fabric has approximately 30,000 orifices per square inch; a double row of stitches with very fine nylon thread eliminates any danger of leakage. The bag has a filtration surface of approximately 50 sq. cm.[3] This is more than ample for the complete filtration of at least 500 ml of blood or 3,000 ml of plasma. Our failure to observe frequent clogging of this fine mesh filter is probably related to the fact that the nylon filaments are very smooth with round cross sections and aqueous solutions spread over the surface of the fabric without absorbing much moisture. The filtration of blood through the nylon fabric yields results that are comparable to those obtained with a standard 200-mesh screen.[5]

Fig. 1 shows the assembled filter. In use, the cannula is inserted into the appropriate orifice in the rubber-stoppered blood bottle, and the filter with its glass-covered drip tube remains suspended from the inverted bottle. The reservoir, tubing and 18-gauge needle are filled with saline. When the flow into the vein is definitely established, the filter assembly is inserted into the saline reservoir tube and the rate of blood flow is adjusted with the clamp to about 10 ml per minute. The unit simplifies the administration of blood or plasma because it includes the filter, the Murphy drip principle and the saline reservoir for starting the transfusion. No instance of sensitivity to nylon has been observed in over 1,000 blood and plasma infusions. No reactions have followed the administration of plasma in over 200 cases and the incidence of reaction with the use of whole blood has been very low. A further analysis of these data will be presented in a separate communication.

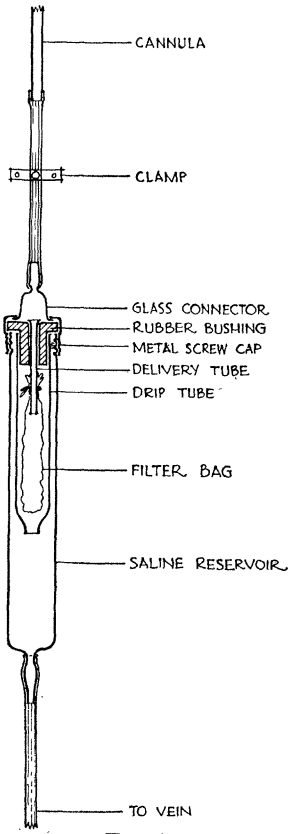


FIG. 1.

S. BRANDT ROSE

[1] Presented before the Academy of Surgery, Philadelphia, April 5, 1943, as a contribution from the Philadelphia General Hospital, Solution Room Committee, which also includes Dr. J. H. Clark, Dr. H. I. Hneleski, Dr. J. G. Reinhold and Dr. W. G. Turnbull.

[2] M. Novak, SCIENCE, 97: 248, March, 1943.

[3] The specially woven fine nylon fabric is known as "Hematex." The filter and fabric are available from Frederick H. Rhodes, Madison, N. J.

[4] Due to the current critical value of nylon the bags have been washed and used repeatedly. Immediately after use, the bag is rinsed blood-free in saline. At the end of the day, the accumulated bags are washed thoroughly in Dreft, a sulfonated fatty acid detergent marketed by the Procter and Gamble Company. The bags are then rinsed thoroughly in distilled water, boiled in distilled water for about 15 minutes, air-dried, assembled in sets with tubing and autoclaved. Each filter has been used at least twenty-five times.

[5] I am indebted to Dr. Max Strumia, Bryn Mawr Hospital, Bryn Mawr, Pa., for his kindness in performing this comparative test.