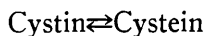


we had previously prepared. We next fed phenyluramino cystine which we had synthesized, to rabbits, to see whether this compound would be excreted or some of it changed into the cystine derivative. We found that much of it had been oxidized to the cystine compound.

This shows that the reaction



may be a common metabolic reaction and easily as well as precisely controlled by the living cell. It is interesting in as much as it is closely allied with the finding of Hopkins⁵ on his glutathione work and is strongly corroborative.

175 (2135)

A micro colorimetric method of estimating the hydrogen ion concentration of the blood.

By V. C. MYERS, H. W. SCHMITZ* and LELA E. BOOHER.

[From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York City.]

A discussion of the bicolorimetric principle was first presented to this Society in November, 1921,¹ at which time we had the present work in mind. The method described below is essentially an adaptation of the colorimetric method of Cullen² for the determination of the P_H of the blood plasma (or serum) to the bicolorimeter described by one of us.³ As modified the final determination is carried out on 0.1 c.c. of plasma, and does not require more than 10 minutes after the blood has been obtained. The color comparison can be made with an accuracy of $\pm P_H 0.02$.

⁵ Hopkins, F. G., *Biochem. Jour.*, 1921, xv, 286.

* Medical Fellow of the National Research Council.

¹ Myers, V. C., *Proc. Soc. Exp. Biol. and Med.*, 1921, xix, 78.

² Cullen, G. E., *J. Biol. Chem.*, 1922, lii, 501.

³ Myers, V. C., *J. Biol. Chem.*, 1922, liv, 675.

Blood is drawn without stasis in a narrow 5 c.c. Luer glass syringe containing sufficient mineral oil to fill any air spaces, and is at once delivered into a centrifuge tube of special design under oil. This tube is made of Pyrex glass and has at the bottom a bulb of 2 c.c. capacity (30 mm. in length with an internal diameter of 11 mm. and a neck of 4 mm.). Tubes with bulbs of 1 and 5 c.c. capacity have also been used, the latter being employed when a simultaneous estimation of the CO_2 content of the plasma is to be made. One drop of neutral 20 per cent. potassium oxalate is dried in the tube, after which three drops of mineral oil are added. In transferring the blood from the syringe to the centrifuge tube, the point of the needle is placed under the oil and sufficient blood delivered to bring the oil into the neck of the bulb. With the slight pressure exerted the blood readily takes up the oxalate and does not clot. The tube is centrifuged at moderate speed for about two minutes to separate the plasma.

As a check on the possible influence of the oil on the P_H under these conditions, specimens of blood plasma, saturated with CO_2 at alveolar tension, have been similarly centrifuged under mineral oil. P_H estimations were made both before and after the centrifuging, but disclosed no appreciable change in the P_H .

A 0.9 per cent. solution of sodium chloride in CO_2 free water, to which has been added 10 c.c. of 0.02 per cent. phenol red solution for each 100 c.c., is adjusted to a P_H of 7.4 with sodium hydroxide and then kept under oil in a paraffin lined bottle. Two c.c. of this solution are allowed to flow into the cup of the bicolorimeter under oil. A small portion of the separated plasma is now drawn into a 0.5 c.c. tuberculin syringe graduated in 0.01 c.c. (the point of the needle can best be cut off), the air spaces of which are filled with mineral oil. One tenth c.c. of the plasma is immediately discharged into the saline solution in the cup. This solution is stirred with a small glass rod and is then ready for color comparison.

For this purpose the two wedges of the colorimeter are filled with Sørensen's buffer phosphate solutions, containing 2 c.c. of 0.02 per cent. phenol red for 20 c.c. of phosphate solution, the front wedge having a P_H value of 8.0 and the second wedge of 6.8. The wedges are calibrated, as already described,² with a series of buffer phosphates ranging from P_H 7.0 to 7.8 and differing by 0.1 P_H . The readings made with the wedge con-

taining the dominant color (alkaline, P_H 8.0) are employed in plotting the curve from which the calculations are made. Although the solutions apparently keep for some time in the wedges, they should be checked at frequent intervals.

In several instances where the plasma has been slightly cloudy a third wedge containing finely suspended barium sulfate has been employed to equalize the fields. In this way perfect color matches have been obtained.

The factors worked out by Cullen to correct the P_H values to body temperature (38°C) have been employed.

FIGURES FOR THE P_H AND CO_2 CONTENT OR COMBINING POWER OF THE BLOOD PLASMA IN SEVERAL NORMAL AND PATHOLOGICAL CASES

Case	Age	Sex	Date 1923	P_H	CO_2 combining power	Diagnosis, remarks
1. A. S.	60	M	4/13	7.37	c.e. per 100	Normal findings.
2. J. S.	9	M	4/13	7.40	65	
3. M. M.	30	M	4/13	7.41	67	
4. S. G.	22	M	3/28	7.23	22	Diabetic coma. 4 hrs. after insulin. Next morning.
			3/28	7.33	28	
			3/29	7.275	30	
			4/18	7.35	47*	
5. J. B.	6	M	3/30	7.21	21	Diabetic coma.
6. R. M.	45	M	4/5	7.15	20	
7. W. T.	35	M	4/16		31	Chronic nephritis.
			4/17	7.30	40*	
8. E. L.	47	F.	4/20	7.30	63*	Cardiac decomposi- tion.
			4/23	7.40	63*	
9. N. M.	52	F	4/9	7.53	98	Cholecystectomy, had received 40 gm. HNaCO_3 , second specimen on 4/9 taken 6 hrs. after first.
					87	
			4/10	7.46	83*	
					79	
					79*	

* CO_2 content.

In a series of 25 miscellaneous hospital cases, in which abnormal value for the P_H were not anticipated, the plasma figures obtained with the method described varied between P_H 7.35 and 7.43, with an average close to 7.39. Illustrative figures on three cases with normal findings and on several interesting pathological conditions are given in the table. In two cases of diabetes in coma the P_H values were 7.23 and 7.21, the figures

harmonizing with the CO_2 combining power in both instances. In the first of these cases there was a rise to P_{H} 7.33 four hours after administration of insulin. In case 6 suffering from uremia following a bladder operation, and dying several hours after the test, the P_{H} was 7.15 and the CO_2 20. In case 7 suffering from advanced nephritis with marked nitrogen retention (blood creatinine of 9 mg.), the P_{H} was 7.30 and the CO_2 content 47. In the case with cardiac decompensation there appears to be a slight reduction in the P_{H} . Case 9, following a cholecystectomy operation and alkali therapy, showed a CO_2 combining power of 98. Six hours later when a P_{H} of 7.53 was observed the CO_2 had fallen to 87. On the next morning the P_{H} was 7.46 and the CO_2 was 79.

176 (2136)

Some studies on the vital staining of blood cells.

By R. SPIRIDONOVITCH (by invitation).

[From the Department of Anatomy, Cornell University Medical College, New York City.]

There are many papers on the vital staining of the blood cells but, considering the many papers of the conventional method used in the examination of the blood, one may say that the field of the vital staining is rather unexplored. It is surprising to find how few have actually studied living cells. Most of the work on vital staining is in reality *supra vital*, that is to say the living cells on taking up the stain died. I will mention of many investigators the names of Rosin and Biebergeil, Sabin and E. Cowdry. These writers observed cytoplasmic granules which took up certain dyes, while the cells containing them continued to live.

I have made a few comparative studies of the effect of certain vital dyes on the cytoplasmic granules in the white blood cell of man. To introduce the dye into a drop of blood diluted with Ringer's solution, I used the following method, which was