

Detailed investigations have been undertaken to study the effect of reagent concentration on the intensity and stability of the colour involved. From the results obtained the following generalisations may be made:—

- (1) Increase in the concentration of dimethyl glyoxime results in increased intensity and diminished stability.
- (2) An increase in the concentration of ammonia has practically no effect on the intensity of the colour but has a marked stabilising influence on it.
- (3) Increased intensity and diminished stability are caused by lowering the concentration of Iodine.

In the light of the above observations various alterations in the procedure have been tried with a view to stabilise the colour and the following were found to be most satisfactory:—

- (1) Using 0.2 per cent. solution of dimethyl glyoxime in 80 per cent. "ammonia" instead of 0.1 per cent. solution in 50 per cent. "ammonia".
- (2) Substituting N/15 Iodine for N/10 Iodine.
- (3) Effecting the final dilution with 50 per cent. "ammonia" and not with distilled water.
- (4) Waiting for at least one minute after the addition of Iodine and for two minutes after treatment with the dimethyl glyoxime reagent.

The last item was found to be quite helpful in obtaining reproducible values for the drum differences. Full details giving further particulars will shortly be published elsewhere. Our thanks are due to the Works Manager, The Tata Iron & Steel Co., for permitting the publication of this note.

Research & Control Laboratory,  
The Tata Iron & Steel Co.,  
Jamshedpur,  
August 24, 1944.

G. V. L. N. MURTY.  
N. B. SEN.

1. Vaughan, E. J., The Institute of Chemistry Lecture on "Further Advances in the Use of the Spekter Photo-Electric Absorptiometer in Metallurgical Analysis," 1942, pp. 3 and 4.

### A BROTH CHOLERA VACCINE

It has become customary to prepare prophylactic vaccines against bacterial infections from growths of organisms on agar. As recently as 1940, the Cholera Advisory Committee of the Indian Research Fund Association<sup>1</sup> recommended that "the (Cholera) vaccine should consist of a suspension of the vibrios obtained by washing off the growth from a 24-hour agar culture with 0.85 per cent. saline solution". This recommendation was in accordance with the practice of the majority of laboratories preparing cholera vaccine in large quantities. The reason for the preference for growths on agar over growths in broth, must be due to the anxiety of workers to obtain their suspensions of organisms as free as possible from extraneous proteins.

The acute shortage of the supplies of agar in the country, brought about by the outbreak

of war against Japan, led us to investigate the possibility of preparing an effective cholera vaccine from growths in a liquid medium. The success of this effort needed the fulfilment of two conditions: (1) the availability of a liquid medium as free as possible from proteins and yet yielding good growth of the vibrio, and (2) the development of a reliable method of testing the protective power of the vaccine in experimental animals. In the acid hydrolysate of casein of Mueller and Johnson,<sup>2</sup> we have found an excellent liquid medium for the purpose. It gives a profuse growth of the vibrio, is easy to prepare, does not give biuret reaction, and what is more, costs less than half to prepare than the usual laboratory nutritive broths. We have been able to develop a protection test in white mice which gives repeatable results within narrow limits. Our mouse protection test determines the minimal dose of the vaccine required to protect 50 per cent. of the immunised mice against an infective dose of 10 m.l.d.'s administered intraperitoneally with mucin.<sup>3</sup>

In the several experiments we have performed so far the vaccine prepared from cultures in the liquid medium incubated at 28° C. for seven days, killed and preserved with phenyl mercuric nitrate, 1 mg. per 100 ml., gave a mouse protective dose of 0.00003 ml. Against this, the customary cholera vaccine made from 24-hour agar cultures of the same strain containing 8,000 million organisms per ml., gave a mouse protective dose of 0.0004 ml. Further our vaccine has a low toxicity, as much as 1.5 ml. per mouse (18-20 gm.) produced no deaths. However, we are working to still further detoxicate it by the addition of formalin. 0.8 ml. of agar vaccine killed four out of five mice.

The new cholera vaccine we have described is about ten times as potent as the customary agar culture vaccine, has low toxicity and has the great merit of being easier to prepare in large quantities than the agar culture vaccine. Haffkine Institute, S. S. SOKHEV.  
Bombay, M. K. HABBU.  
September 9, 1944.

1. Taylor, J., 'Cholera Research in India, 1934-40, under the Indian Research Fund Association, A Review,' The Job Press, Cawnpore, 1941, pp. 37-38. 2. Mueller, J. Howard, and Johnson, Everett R., *J. Immunol.*, 1941, 40, 33-38. 3. Griffiths, James J., *Pub. Health Rep.*, 1942, 57, 707-10.

### URINARY EXCRETION OF SULPHANILYL-BENZAMIDE

In continuation of our previous work (Bose and Ghosh, 1944)<sup>1</sup> where the toxicity and blood concentration of sulphanilyl-benzamide were studied, the present work was undertaken to find out the amount of urinary excretion of the drug in human volunteers. Along with this, the excretion of sulphanilamide was also studied for comparison.

Four laboratory workers volunteered for the study. Two of them were fed orally with 1 gm. of sulphanilyl-benzamide two hours after their morning meal. The other two volunteers were similarly fed with sulphanilamide. The

Urinary excretion of Sulphanilyl-Benzamide and Sulphanilamide in human beings  
Dose of each drug = 1 gm. orally

Name of Drug	Volunteer No.	Sample in hours	Total drug excretion in mg.		Percentage excretion of drug in 3 days		
			As free	As conjugated	Total	Free	Conjugated
Sulphanilyl benzamide	1	24	330.2	157.6	70.4	59.3	40.7
		48	78.7	93.1			
		72	8.7	35.1			
	2	24	446.4	233.2			
		48	52.9	107.9	87.6	57.0	43.0
		72	..	46.2			
Sulphanil-amide	3	24	213.2	240.6			
		48	94.0	107.0	71.5	46.7	53.3
		72	25.2	34.8			
	4	24	256.0	475.6			
		48	74.2	168.4	97.4	34.2	65.8
		72	..	..			

total 24 hours' urine was collected for three successive days, and the excretion of the drugs both as free and conjugated forms was estimated daily according to the modified technique of Marshall and Litchfield (1938).<sup>2</sup> The table gives the result of this investigation.

The observations on the urinary excretion as given in the table amply corroborate the rapid systemic absorption of sulphanilyl-benzamide in man (cf. Bose and Ghosh, 1944). The average percentage of the total excretion of the drug as apparent from the table was 79 per cent. in 72 hours, which indicate a fair amount of absorption and a moderately rapid excretion. Moreover, it is being found that the excretion of the drug is more as free (58 per cent.) than as conjugated from (42 per cent.). But in the case of sulphanilamide the reverse is being observed. Considering the excretion of sulphanil-benzamide more in the free state it is considered to be of interest to study the effect of this drug in different urinary infections.

The compound being a benzoyl derivative, it would also be worthwhile to study the excretion of hippuric acid, which might give a clue to the possible nature of its breakdown in the system. Work is already in progress.

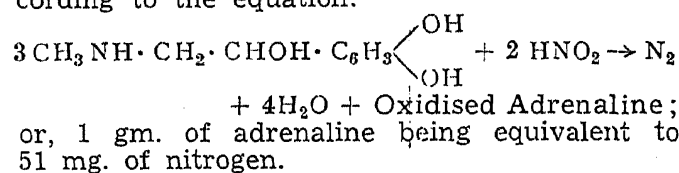
A. N. BOSE.  
J. K. GHOSH.

Bengal Immunity Research Laboratory,  
August 26, 1944.

1. Rose, A. N., and Ghosh, J. K., *Ind. Jour. Med. Res.*, 1944, **32**, 61. 2. Marshall, E. K., Jr., and Litchfield, S. T., Jr., *Science*, 1938, **88**, 85.

ESTIMATION OF ADRENALINE BY  
VAN SLYKE MANOMETRIC  
TECHNIQUE

PURE adrenaline is usually determined by Folin's method as modified by Culhane and Underhill.<sup>1</sup> The significance of the observations of Barker, Eastland and Evers<sup>2</sup> that ascorbic acid as present in suprarenal gland interferes in the oxidation of the catechol grouping as present in the adrenaline molecule, is now obsolete as most of commercial adrenaline is being produced synthetically and as such free from ascorbic acid. As Folin's method is virtually dependent on the oxidation of catechol part by the phenol reagent, it was thought that the same phenomenon may happen in presence of nitrous acid with the liberation of nitrogen gas (cf. Carter and Dickman<sup>3</sup>). The latter may then be an easy measure in the estimation of an adrenaline solution according to the equation:



On this basis 0.1 per cent. solution of pure adrenaline hydrochloride was treated with nitrous acid in Van Slyke micro-apparatus by the customary method as followed in usual Van Slyke amino nitrogen estimation. The acid reacted vigorously with the adrenaline solution with evolution of gas which was collected, washed with alkaline permanganate and the volume of residual gas left behind, was