time alone will show whether we are correct in our assumptions. We can only be encouraged by our results of the past eight months. No little purpose will have been served if we have sown the seed for a new line of thought and research on the diagnosis and intensive treatment of early syphilis.

We wish to express our thanks to the Medical Director-General of the Navy, Sir Sheldon Dudley, for permission to carry out this work and for his support. Thanks are also due to Surg. Lieut.-Cmdr. Sloan Miller for all his laboratory co-operation, and to the sick-berth staff for their never-failing help.

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ASSESSMENT OF LEVEL OF NUTRITION: REVISED PROCEDURE FOR ESTIMATION OF ANEURIN IN URINE BY THE THIOCHROME TEST

BY

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The nutritional status of human subjects in vitamin B₁ can be assessed by examination of the urine before and after test dosing, the procedure being in principle analogous to that introduced previously for determining the level of vitamin C (Harris, Ray, and Ward, 1933; Harris and Ray, 1935) and later for nicotinic acid (Harris and Raymond, 1939). In its original form (Harris and Leong, 1936; Harris, Leong, and Ungley, 1938) the test for vitamin B₁ involved a biological assay of the urine, preferably by the bradycardia method (Birch and Harris, 1934). This has been used by various investigators—e.g., Wright and Baker (1939), Guha and Ahmad (1939). Later the perfecting of the chemical method of estimating vitamin B₁ by the thiochrome reaction (Jansen, 1936) has made possible a great simplification in the routine. In previous papers (Wang and Harris, 1939; Harris and Wang, 1941) we described a suitable technique for the estimation of the aneurin in the specimens of urine by a modified thiochrome test. This has been used satisfactorily by workers in other laboratories (e.g., Slater, 1941; Toverud, 1940). We checked its reliability, by comparison with biological assays, using the bradycardia technique, in several series of comparisons; and the agreement was found to be satisfactory (Wang and Harris, 1939; Harris and Wang, 1941).

Given a little experience, the method is found to work smoothly with nearly all specimens of urine; in rare instances, however, samples may be encountered that give an abnormally whitish-green blank and a dark-blue fluorescence on oxidation, which may make it tiresome to effect a satisfactory matching. To overcome this objection we tried treatment of the urine with H₂O₂, a reagent which had been previously employed by McFarlane and Chapman (1941) in estimating aneurin in green feeding-stuffs. This addition of H₂O₂ reduces the blank considerably and renders the final matching very easy even in the most troublesome specimens of urine (Wang and Harris, 1942). The treatment of the urine with the H₂O₂ does not cause any destruction of thiochrome and presents a distinct advantage, and we now use it in all our routine estimations of aneurin. The method is so simple and rapid to carry out

that it may be employed conveniently in regular clinical tests. For this reason it seems worth while to put together here the working instructions for the modified procedure, incorporating the foregoing and several other small improvements (Wang and Harris, 1942).

Method

1. *Preliminary Washing with Isobutanol.*—Measure 5 ml. of urine into a 15-ml. centrifuge tube, add a drop of concentrated HCl, and shake for one minute with an equal volume of H₂O-saturated isobutanol (redistilled). Centrifuge, discard the isobutanol (top) layer, and note the volume of the aqueous layer.

2. *Oxidation of Aneurin and Extraction of Thiochrome.*—Pipette two 1-ml. aliquots of the washed urine into two 25-ml. glass-stoppered cylinders marked A ("unknown") and B ("blank"). Into a third cylinder, marked C ("control"), introduce 1 ml. of a standard solution of aneurin containing 4 µg. per ml. (For urines collected after test dosing, it may be necessary to use a stronger standard—say, 10 µg. instead of 4 µg. On the other hand, with relatively deficient urines, containing little vitamin B₁, 2 ml. of urine may be taken for analysis instead of 1 ml. and added to cylinders A and B; in this case another 1 ml. of water should be added to the control (cylinder C) to equalize the conditions.) A stock solution containing 20 µg. of aneurin per ml. in 25% alcoholic N/100 HCl is kept for use in a cold place; the diluted standard should be freshly prepared from this. Add to each cylinder 2 ml. of methanol and mix well. To cylinder A add 1 ml. of 20% NaOH, mix quickly, and follow immediately by a 10% solution of K₃Fe(CN)₆, drop by drop, mixing thoroughly after each addition, until the colour of the K₃Fe(CN)₆ stays for more than 30 seconds. (Usually 2 to 3 drops are sufficient; large excess should be avoided.) Treat cylinder C in exactly the same way. To cylinder B, the "blank," add 1 ml. of 20% NaOH, but no K₃Fe(CN)₆. Now add 3 drops of 30% H₂O₂ to all three cylinders (if the urine gives an exceptionally high blank more H₂O₂ can be used). Mix well and allow to stand for half a minute. Then add 1 ml. of H₂O to each, followed by 10 ml. of redistilled isobutanol (if H₂O-saturated isobutanol is used the addition of H₂O may be omitted) and shake for 1 minute. Allow the layers to separate, then draw off and discard the bottom aqueous layer. Add 3 ml. of H₂O to each, shake gently for half a minute and again let the layers separate. Pipette off the top layer of isobutanol into three separate dry conical flasks and clarify with 2 ml. of 96% ethanol.

3. *Comparison of Fluorescence.*—Measure 10 ml. of the clear isobutanol extracts of the oxidized unknown A and the "blank" B into separate test-tubes. (These tubes must be of "non-fluorescent" glass, and uniform in diameter. A convenient size is one in which 10 ml. occupies a column of 6 to 7 cm. The bottoms of the tubes should be of the same shape.) Compare the fluorescence in the tubes, holding them in front of a source of ultra-violet light. The u.v. lamp should be enclosed in a case fitted with a Wood's-glass window, and the room in which the comparison takes place should be completely darkened. Incline the tubes at an angle of about 60 degrees to the horizontal and look down the tubes. The bottom of the tubes should rest on a black platform fitted to the Wood's-glass window. Never look for more than a few seconds, since prolonged exposure to the blue colour will temporarily desensitize the eye; but make a quick inspection and decide which tube has the stronger blue fluorescence. Reverse the position of the tubes and look again. By means of a micro-pipette add the standard of thiochrome (from cylinder C) to the "blank" little by little, mixing well after each addition by closing the tube with the thumb and inverting two or three times, until the fluorescence in both tubes is equal. (The thumb should be thoroughly cleaned with soap and water, as traces of grease may introduce considerable fluorescence.) Before the final matching add to the "unknown" an amount of isobutanol equal in volume to that of the standard added to the "blank." For further details concerning matching, including use of colour filters, see Harris and Wang (1941).

4. *Calculation.*—If U is the volume of urine taken for analysis (1 ml. or 2 ml.), v the volume of the standard used for the matching, x the total amount of aneurin (4 µg. or

10 µg.) in the standard solution, and r_1 and r_2 the volume in ml. of the extract before and after the preliminary washing with isobutanol, then the aneurin content of the urine is

$$\frac{xv}{10} \times \frac{r_2}{r_1} \times \frac{1}{U} \text{ µg. per ml.}$$

$$\left(= \frac{4v}{10} \times \frac{r_2}{r_1}, \text{ when, as in usual circumstances, } x=4 \text{ and } U=1 \right).$$

5. *Interpretation of Results.*—Past work has shown that a normal adult male* subject receiving not less than the reputed requirement of vitamin B₁ (300 I.U.=900 µg. of aneurin daily) excretes at least 90 µg. of aneurin daily (Wang and Harris, 1939). The excretion rises with each increase in the intake, rapidly adjusting itself to such changes. On a diet deficient in the vitamin the excretion quickly falls to a steady value of about 60 to 90 µg., where it is maintained for some time (Wang and Yudkin, 1940).

On the basis of observations such as the foregoing, nutritional status may be assessed by measurement of the "resting level of excretion" plus the response to daily test doses. A "resting" excretion of below 90 µg. combined with one or more days' delay before attainment of the plateau of excretion after the daily standard test doses (350 I.U.) can be assumed to indicate an insufficient intake. For further discussion of standards for excretion tests earlier papers from this laboratory may be consulted (e.g., Wang and Harris, 1939; Wang and Yudkin, 1940).

It is perhaps worthy of note that the differentiation between normal and deficient subjects by means of their urinary response is less steeply graded for vitamin B₁ than, for example, for vitamin C, and this means that saturation tests are somewhat easier for the latter than for the former. Thus, special emphasis has to be laid on the fact that the excretion of aneurin is relatively "labile," adjusting itself fairly rapidly to each change in the intake, as mentioned above. As a result, the "resting level" of excretion may sometimes do little more than reflect the intake during the few days immediately preceding the collection of the urine. With test doses the deficient subject satisfactorily answers to the test by giving less response than the normal subject on at least the first day, or first and second days, of test dosing; yet after a further day or so both deficient and normal will have reached an equal degree of excretion. (With extreme deficiency of long standing the response to test doses may be delayed a little further.) But for vitamin C, in contrast, the deficient subject may give little or no response at all to test doses for a period of some days, and full saturation will not be finally reached (in developed scurvy) until 7 to 10 days: thus the difference between normal and deficient is made more dramatically apparent than for vitamin B₁.

6. *Collection of Urine.*—If the urine is not to be tested within an hour or two of its being passed it can be preserved by the addition of a few ml. of glacial acetic acid (or HCl) and toluene.

7. *Avoidance of Certain Drugs.*—It is important that the patient should not take aspirin, quinine, or related drugs for some hours before or during the time of the test, since these may cause the excretion of highly fluorescent substances in the urine and so make an accurate determination of thiochrome impossible.

Summary

Working directions are given for the method of estimating vitamin B₁ in urine in the test for nutritional status, various improvements in technique being incorporated.

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* The minimal level of excretion by females may possibly be slightly lower (cf. Toverud, 1940; Robinson *et al.*, 1940). Precise figures for children, and the more detailed standards for varying intermediate degrees of deficiency, such as have now been worked out for vitamin C (Harris, 1943), still remain to be filled in.

LOCAL ORAL MEDICATION WITH SULPHANILAMIDE IN LOZENGE FORM*

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The local application of sulphonamides has been the subject of much interest lately, and this method of treating oral lesions is attracting attention, though a report on the use of sulph-anilamide following dental extractions was noted several years back in the *Journal of the Canadian Dental Association*. That the oral cavity may be a valuable site for absorption of drugs, and particularly in the case of oral lesions, should not cause much surprise. The rich vascular bed of the buccal mucosa is readily available, as witness the immediate relief of substernal pain in angina pectoris after the use of a tablet of nitro-glycerin sublingually. Intranasal application of drugs also has its advocates. Cattell (1943) points out "that no outstanding new discoveries in the field of sulphonamide therapy have occurred during the past year, but that, on the other hand, the period has been one of continual progress in the application of the sulphonamides."

It was a search for a suitable means of local oral application and absorption of the drug that prompted me, with the advent of sulphanilamide in 1937, to use it in a vehicle that would permit its slow release in the oral cavity and by direct contact promote local healing and defence against such organisms as are inhibited or destroyed by it. Then, as now, the mode of action of the drug cannot be regarded as fully settled, but justification for its application need not await complete elucidation of a *modus operandi*. The present report is based on the results of the direct application of sulphanilamide in a lozenge specifically for the purpose of testing its efficacy in cases of acute follicular tonsillitis, the aetiological agent being *Str. haemolyticus*, common at Fort Francis E. Warren, and the subject of much concern to all.

The manner of sulphanilamide administration as practised to-day calls for large doses of the drug—some 7 to 9 g. daily—for therapeutic effect upon a small area on the tonsils or adjacent pharyngeal wall. Other tissues resent playing host to the drug by producing an added lesion at times more deadly than the original condition. Stated in other words, an attempt was made to reverse the order of mass saturation preceding local therapeutics by a maximal local effect with a minimal blood concentration of the drug.

The vehicles used at the time were chicle (chewing gum) and lozenge. The lozenge proved the more satisfactory means. It could be held in the mouth for a period up to 45 minutes, was palatable, and liberation of the drug was slow and uniform. Several lozenges could be used daily, and no disturbing chewing manipulations were required. It was satisfactory both for children and for adults. A lozenge weighing 2 g. and containing 0.065 g. of sulphanilamide proved best suited for the treatment. Observations made at the time on mucosal staining, using prontosil and methylene blue in chicle and chocolate, agree with those of Arnett (1943).

The present study is a resumption of the above, though on a much larger scale and under better-controlled conditions. It is intended as a preliminary report, for it would have been more satisfactory had the work been carried through the entire winter season. The incidence of upper respiratory infection is as high during March as in any of the preceding months, and it is felt that enough clinical data have been obtained to warrant further investigation at a future date.

The increase in upper respiratory infections is an accompaniment of the expanding army. For the time being, fortunately, the diseases are mild, though at times of epidemic proportions. Nevertheless, they incapacitate fairly large numbers of new arrivals during their basic training period. This is inevitable owing to the close contact of the men, hence the rapid passage of infection from one individual to another. The problem

* This study was made possible through the assistance and co-operation of Col. D. W. McEnery, post surgeon, Fort Francis E. Warren, Wyoming.