by other investigators.[10, 11, 12, 14] During the control period the patients were placed on a daily intake of 1,500 to 2,000 calories with the diet containing 6 to 10 gm of salt and a fluid intake of 2,500 to 3,000 cc. Penicillin levels were determined after the third day of this regimen. During combined treatment the penicillin was given 20 to 30 minutes after the benzoic acid. At first sodium benzoate was tried, but it was found that the added base neutralized the effects of a controlled salt intake, and in addition the dose of sodium benzoate proved to be somewhat nauseating. On the normal diet 2.5 gm of benzoic acid equalled 6 gm of sodium benzoate in raising the serum penicillin level. During diet restrictions which were in effect 3 days before penicillin levels were determined, the patients received the same caloric intake as previously, but fluids were limited to 1,000 to 1,500 cc a day and salt to 3 gm or less a day. On the restricted fluid intake the urine volume commonly fell to 400 to 600 cc in 24 hours.

Six patients have been studied so far in this series. In addition to determining the degree of elevation and effective maintenance of blood levels, urine levels were done to check the per cent. excretion of penicillin in a 2-hour period following intramuscular injection. The experimental results of this phase of the study are presented in Table 1.

Results: The 1-hour peak serum levels using 20,000 units of penicillin alone were in the 0.07 units per cc range. Two-hour serum levels, however, were ineffective. Using the low salt and fluid diet plus benzoic acid 1-hour levels were 0.56 units per cc and 2-hour levels 0.28 units per cc. A fall from 56 to 22 per cent. in the 2-hour urine excretion of penicillin as a result of diet and benzoic acid treatment reflects a similar trend (Chart 1). This represents an eight-fold increase in, as well as a prolongation of, the penicillin blood level. In the work with adrenalin[6] using two and one-half times the dose of penicillin, the 2-hour levels were one fourth this amount, except for one case which showed the same level. With repeated injections every 2 to 3 hours, restriction of salt and water intake and administration of benzoic acid every 4 hours, the minimum levels obtained were 0.14–0.28 units per cc of serum. This is a five to ten-fold increase over the levels usually maintained when two to five times greater doses of penicillin are used. This method of treatment is being applied to penicillin therapy in acute, subacute and chronic conditions, and will be discussed elsewhere.

Summary: (1) Restriction of fluid intake to 1,500 cc and the salt intake to 3 gm a day doubles the penicillin blood level following interrupted intramuscular injections of penicillin. (2) The administration of benzoic acid to a patient on an unrestricted diet may double the penicillin blood level during similar treatment. (3) The combination of these two procedures results in a four- to eight-fold increase in penicillin blood level with a prolonged effective blood concentration.

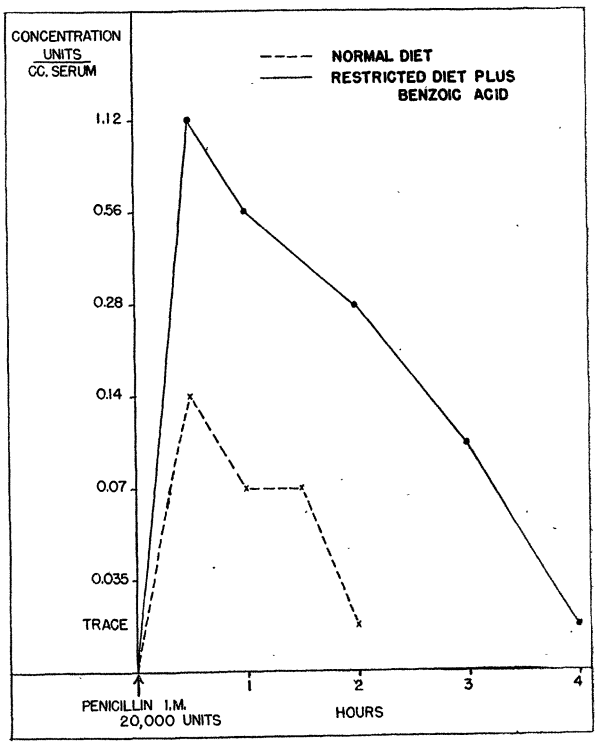

CHART 1

JACK BRONFENBRENNER
CUTTING B. FAVOUR

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE MICROBIOLOGICAL DETERMINATION OF CHOLINE[1]

HOROWITZ and Beadle[2] have reported a microbiological method for the determination of choline, based upon the growth response of *cholineless,* a mutant of *Neurospora crassa.* The procedure calls for pressing out on filter paper the mold mat obtained at the end of the incubation period, and drying the material to constant weight. The dry weights are reported to vary from 2 to 50 mg for choline concentrations of 0.5 to 20 mcg in 25 ml of media.

In our hands the above procedure gave erratic re-

[14] Sir A. Fleming, M. Y. Young, J. Suchet and A. J. E. Rowe, *Lancet,* 2: 621, 1944.

[1] Received for publication, April 11, 1945.

[2] N. H. Horowitz and G. W. Beadle, *Jour. Biol. Chem.,* 150: 325, 1943.

sults in tests of pure solutions of choline and of hydrolyzed extracts of biological materials before and after adsorption on and elution from permutit. Drying the mold mats on tared filter papers (Whatman, No. 50, diameter 11 cm) proved to be unsatisfactory since the papers showed losses in weight varying from 2 to 8 mg as a result of the filtration and washing operations. Furthermore, because of the time-consuming nature of these operations, the washings were inadequate. The medium contains approximately 28 mg of soluble solids per ml and the wet mold varies in moisture content from 90 to 95 per cent. so that failure to remove extraneous solids can account for variability in the weight of the small quantities of dried mold. Drying the mold in moisture dishes also gave erratic results, ± 20 per cent., since other than heavy mats could not be removed quantitatively from the filter paper and the dry material contained variable amounts of solids derived from the medium.

By the simple expedient of using fritted glass filters (30 ml capacity) of medium porosity, quantitative removal and washing of the mold growth is easily effected. The glass filters need be tared only occasionally, since the dry weights vary no more than ± 0.2 mg. Filtrations and washings are rapid since suction is employed. The mold suspensions may be stirred with a glass rod, thereby rendering the washings more effective. The relation between the dry weight of the mold and choline concentration is now consistently linear. The weight of dry mold for a given choline concentration is reproducible within ± 2 per cent. Tests on biological materials (yeast preparations, poultry feeds, pharmaceuticals, etc.) give good agreement among different assay levels.

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A QUICK TEST FOR FLOUR ENRICHMENT

ENRICHED flour under U. S. Government requirements must contain from 2.0 to 2.5 milligrams of thiamine, from 1.2 to 1.5 milligrams of riboflavin, from 13.0 to 16.5 milligrams of iron, and from 16.0 to 20.0 milligrams of niacin or its amide per pound of flour. Thiamine in flour is ordinarily determined by the thiochrome fluorometric procedure,[1,2] or microbiologically;[3] riboflavin, either fluorometrically[4] or microbiologically[5]; niacin, colorimetrically[6] or microbiologically;[7] and iron is ordinarily determined colorimetrically.[8] The time-consuming nature of all these tests strictly limits the practicability of applying such tests in the routine inspection of flour.

The colorimetric niacin determination is based on the König reaction[9] in which pyridine compounds react with cyanogen bromide and primary or secondary amines to produce complex colored compounds. The new rapid test described herein is based on the above reaction and, although not strictly quantitative, may be used to determine almost instantaneously whether a sample of flour is unenriched, partly enriched or fully enriched in respect to niacin (or its amide). The test requires the use of only two reagents: namely, a 4 per cent. aniline solution in ethyl alcohol and a 4 per cent. aqueous cyanogen bromide solution.

To make the rapid niacin test, place on white blotting paper or in the well of a porcelain indicator block about ½ to 1 gm of flour. Press the flour flat with a spatula so that the packed flour is about 3 mm thick. Drop 2 drops of the aniline solution onto the center of the flattened flour, thus causing a wetted portion about 6 mm in diameter. Drop onto this wetted portion 3 drops of the cyanogen bromide solution. Almost immediately a canary yellow color appears, the depth of color depending on the amount of niacin present in the flour. A simple comparison with flour having known amounts of niacin treated the same way will give a roughly quantitative test. This color comparison should be made 4 minutes after the addition of the reagents. Unenriched flour will develop a slightly yellow color only after 10 to 15 minutes, probably because the small amount of niacin naturally present in unenriched flour is chemically bound in some manner and is gradually liberated by the reagents used. Enriched flour, however, contains a relatively large amount of niacin (or its amide) which is in the free state because it has been added as such in the enriching process.

Since flour is ordinarily enriched by the addition of an enrichment "premix" containing the various enrichment ingredients in the proper proportions, a test for any one of these ingredients in flour usually indicates whether or not the flour is fully enriched. The actual degree of enrichment can be determined, of course, only by actual assay for all four enrichment ingredients. In the routine inspection of flour the rapid niacin test should prove to be very useful

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