micrograms the bluish discoloration of the skin due to the first growth of normally pigmented hair shafts in the epidermis was noticed in from 5 to 7 days; with lower doses (50 micrograms), later. The appearance of the black fur then made rapid progress and the cure was practically complete with higher doses (75 to 100 micrograms) in from 5 to 7 weeks. In rats fed lower doses (50 micrograms), the cure was incomplete but the effect was still pronounced.

In summary, the statement appears to be warranted that pantothenic acid has a definitely curative effect on nutritional achromotrichia in rats fed a diet free from pantothenic acid.

PAUL GYÖRGY
C. EDWARD POLING[10]

THE BABIES AND CHILDRENS HOSPITAL,
AND THE DEPARTMENT OF PEDIATRICS,
SCHOOL OF MEDICINE, WESTERN RESERVE
UNIVERSITY, CLEVELAND

CRYSTALLINE VITAMIN A PALMITATE AND VITAMIN A ALCOHOL

THE preparation of crystalline vitamin A alcohol was first reported by Holmes in this journal.[1] From methyl alcohol yellow crystals were obtained, melting at 5–6° C, with an extinction coefficient at 328 mμ ($E^{1\%}_{1cm}$) of 2,000. Later Mead[2] repeated the preparation by Holmes's method and confirmed the melting point. Mead noted, however, that the crystals were not solvent free and suggested that solvent of crystallization might be present. This is to be noted in view of our findings. Extinction coefficients of 1800 and 1820 were found for the solvent-free A alcohol.

In this laboratory the need has long been recognized for a crystalline, natural ester of vitamin A suitable for use in correlating the spectrophotometric and biological assay of vitamin A, that is, in determining the conversion factor. It is now well established that vitamin A occurs in fish-liver oils, the chief source, esterified with long chain fatty acids. Tischer[3] has furthermore shown that vitamin A palmitate is one such ester. We, therefore, chose to make vitamin A palmitate, esterifying crystalline vitamin A alcohol to obtain the purest ester possible. This work has resulted in the preparation not only of the first crystalline fatty acid ester of vitamin A (so far as we know) but also of crystalline vitamin A alcohol, which melts much higher than hitherto reported.

In the preparation of vitamin A alcohol rich fish-liver oils were distilled in a cyclic molecular still. Distillates with extinction coefficients at 328 mμ of 400 or greater were combined and saponified, yielding vitamin A alcohol concentrates with extinction coefficients of 1,100–1,300. These usually crystallized readily, sometimes after redistillation, from their 10 per cent. solution in ethyl formate at −35° C. after sterols had been removed. After drying in a vacuum at low temperature, pale yellow prismatic crystals of vitamin A alcohol were obtained which were solvent free and melted at 63–64° C. Propylene oxide has also been used as a crystallizing solvent.

Vitamin A alcohol crystallized twice in this way has an average extinction coefficient at 328 mμ of 1,725. This is the average of eighteen preparations, some with extinctions measuring as high as 1,850. The average extinction coefficient at 328 mμ calculated from the blue value was slightly lower, namely, 1,700.

The ultraviolet absorption was measured with a Hilger quartz spectrograph, model E-498, with a Spekker ultraviolet photometer. Antimony tri-chloride blue values were determined with an Evelyn colorimeter standardized with a distilled vitamin A concentrate whose extinction coefficient had been repeatedly determined and checked by the non-saponifiable matter from U.S.P. reference oil.

The extinction coefficient of the vitamin A alcohol blue color with antimony tri-chloride was also determined using a Hardy recording spectrophotometer. The average value found was $E^{1\%}_{1cm}$ (622mμ) = 4700. Special precautions were taken to evaluate the transitory blue color at its maximum intensity.

Vitamin A palmitate was prepared by esterifying crystalline vitamin A alcohol with palmityl chloride and quinoline in chloroform solution at −15° C. The crude ester crystallized readily from a 2 per cent. solution in propylene oxide at −30° C, in pale yellow plates, m.p. 26–28° C.

After two crystallizations vitamin A palmitate has an average extinction coefficient of 940. The average extinction coefficient at 328 mμ calculated from the blue value was 933. This value of 940 corresponds to an extinction coefficient of 1,720 for vitamin A alcohol.

The extinction coefficient of the antimony trichloride blue color with crystalline vitamin A palmitate was found to be 2490 at 620 mμ. By calculation this gives a value of 4560 for vitamin A alcohol which is 3 per cent. lower than the value found by measurement for the crystalline alcohol.

Our value for the extinction coefficient of vitamin A alcohol is about 1,720, whether obtained by assaying the crystalline alcohol or by calculation from the extinction of the crystalline palmitate. This is about 4 per cent. lower than the value of 1,800 recorded by Mead from the assay of crystalline vitamin A alcohol and two esters, the anthraquinone-2-carboxylate and the 2-naphthoate. We do not exclude the possibility that our preparations are impure, but it seems

[10] S.M.A. Corporation fellow in biochemistry, assigned to the Department of Pediatrics.

[1] H. N. Holmes, SCIENCE, 85: 103, 1937.
[2] T. H. Mead, *Biochem. Jour.*, 33: 589, 1939.
[3] A. O. Tischer, *Jour. Biol. Chem.*, 125: 475, 1938.

more probable that the difference is due to a slightly different spectrophotometric technique and the use of different instruments.

Since the high-melting vitamin A alcohol and the crystalline palmitate present possibilities for biological research and the standardization of vitamin A preparations, it is important that their stability under various conditions be known. We have made preliminary storage tests on the palmitate, distilled esters from a fish-liver oil, vitamin A 2-naphthoate (kindly supplied by Dr. T. H. Mead) and beta carotene, the present international standard, and found that they are equally stable in refined cottonseed oil at comparable concentrations when exposed to air in the dark. The crystalline vitamin A palmitate decays more rapidly than the naphthoate on exposure to air. It is hoped to make a further report on the stability of these materials.

A preliminary biological assay of the crystalline vitamin A alcohol has shown that its potency is greater than 2,700,000 U.S.P. units per gram. It is planned to determine the biological potency of both crystalline vitamin A alcohol and crystalline vitamin A palmitate as precisely as possible. The results will be reported separately.

J. G. BAXTER
C. D. ROBESON

DISTILLATION PRODUCTS, INC.,
ROCHESTER, N. Y.

THE EFFECT OF SALICYLATE ON THE OXYGEN UPTAKE OF THE TUBERCLE BACILLUS

UNLIKE most other bacteria the tubercle bacillus does not readily oxidize carbohydrates, amino acids, hydroxy acids, etc., when these substances are added to suspensions in the Warburg apparatus. As shown in Fig. 1, the addition of 1.0 mg of sodium salicylate (o-hydroxybenzoate) to the bacteria suspended in 2.0 cc of M/20 phosphate buffer pH 6.7 more than doubles the oxygen uptake. A corresponding increase in CO_2 production also occurs. The bovine strain B_1 was used. It was grown on beef glycerine infusion broth and the floating masses were removed with a loop and suspended in sterile saline in Hopkins tubes. These were then centrifuged at 2,000 r.p.m. for 15 minutes and the saline replaced by sterile buffer, so that 0.1–0.2 cc of the packed bacteria were suspended in 1.0 cc of buffer. A glass rod the diameter of which was just smaller than the narrow part of the Hopkins tube broke up the cell masses and gave an even suspension. Fig. 1 shows that benzoate also has an effect on the oxygen uptake. On the other hand, p- and m-hydroxybenzoates and methyl salicylate are without action, as is acetylsalicylate until the acetyl group has been hydrolyzed off. p-aminobenzoate has no action and

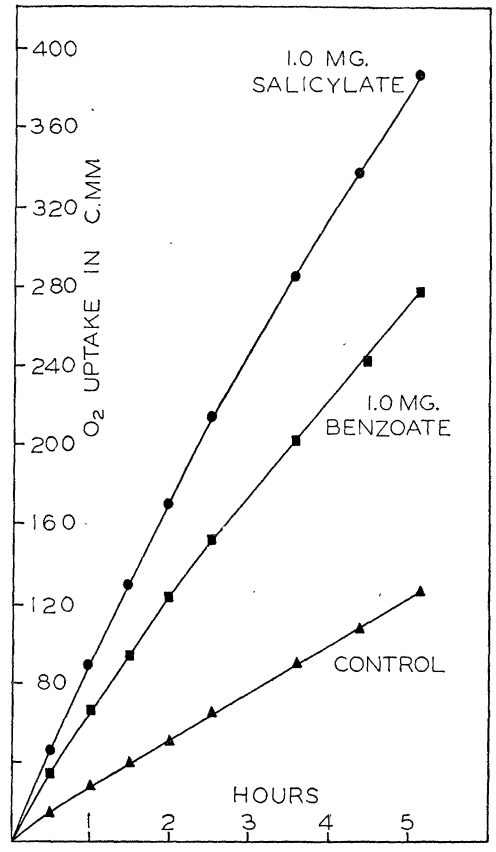

FIG. 1. The oxygen uptake of 0.5 cc of the suspension of tubercle bacilli alone and with salicylate and benzoate at pH 6.7 and 37° C.

o-aminobenzoate only a slight one, but when the latter is added with salicylate it inhibits the salicylate effect. o-aminosulfonic acids have not yet been tried.

When 0.1–0.2 mg of salicylate is used the oxygen uptake is proportional to the concentration, indicating that the salicylate is being oxidized as a substrate. No definite end-points were obtained. The results do not prove that salicylate is a normal metabolite of the tubercle bacillus but suggest that it or compounds of similar configuration may be important. The fact that p-aminobenzoate has been shown to be a metabolite of certain streptococci[1] indicates that substituted benzoates may play a part in bacterial metabolism.

FREDERICK BERNHEIM
DUKE UNIVERSITY MEDICAL SCHOOL

NORNICOTINE AS THE PREDOMINATING ALKALOID IN CERTAIN TOBACCOS

NICOTINE has long been considered the main alkaloid of *Nicotiana tabacum*, ordinary tobacco. This view is based upon the finding of Pictet and Rotschy,[1] in 1901, that only 2.5 per cent. of the total tobacco

[1] D. D. Woods, *Brit. Jour. Exp. Path.*, 21: 74, 1940.
[1] Amé Pictet and A. Rotschy, [Paris] *Acad. des Sci. Compt. Rend.*, 132, 971–2, 1901.