from these reservoirs gave evidence of disturbed metabolism, resulting possibly from malaria-control measures or other operations of the project.

Attention was also given to the effect of larvicidal oil (4 parts kerosene and 1 part black oil) as used routinely for mosquito control during 1939. Comparisons were made with kerosene (plus a dye as a marker) and with pyrethrum larvicide. The three materials showed similar results in that their deleterious effect was chiefly confined to the amphipods, of which a large proportion was destroyed. The larvicidal oil was toxic to vegetation when applied in excessive quantities, but resultant damage was more or less of a temporary nature, except that there was some evidence to indicate a tendency toward prevention of seed formation. Kerosene appeared to be the toxic constituent.

At a meeting of the participating agencies in Chattanooga, Tennessee, on February 28, 1940, it was agreed that the joint field study had resulted in the coordination of interests and that it should be continued during the summer of 1940. Investigations to be included are: (1) the relation of various species of aquatic vegetation to the production of *A. quadrimaculatus;* control of species of vegetation inimical to mosquito control and wildlife interests; and experimental plantings of species of vegetation desirable for wildlife propagation and relatively innocuous in mosquito production, (2) the biological influence of fluctuation of water-levels, (3) the source, rate of accumulation and the form in which arsenical residues occur in the bottom of the reservoir and the toxicity of these residues to aquatic fauna and flora, (4) the direct influence of Paris green and larvicidal oil on fish life, (5) natural predators of anopheline larvae, (6) the utilization of such supplementary malaria-control procedures as dykes, low-head dams, screening and mosquito-proofing; the restriction of land use to daytime occupation, and (7) new mosquito larvicides.

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Chairman, Policy Committee

SPECIAL ARTICLES

PANTOTHENIC ACID AND NUTRITIONAL ACHROMOTRICHIA IN RATS

DEPIGMENTATION of the fur, so-called "graying" in black and piebald animals and "rusting" in albino animals, can be produced with great regularity in rats fed a vitamin B-free basal diet (casein 18 per cent., sucrose 68, melted butterfat 8, cod liver oil 2, salt mixture 4) supplemented with thiamine, riboflavin and vitamin B_6 in an amount of 20 micrograms of each daily.[1-4] If the diet is devoid of vitamin B_6, this syndrome is less frequently observed, owing probably to the phenomenon of competition of vitamin-deficiency diseases—a B_6 deficiency *versus* lack of the anti-gray-hair factor.[5]

It has been shown[4] that purified but still crude concentrates of pantothenic acid with a degree of purification varying up to 50 per cent. had a definitely curative effect on this nutritional achromotrichia in rats. Thus it has been concluded that these concentrates contained a specific factor concerned in the cure of depigmentation of the pelt. This factor has been found to be heat labile; autoclaving of an alkaline solution destroyed the activity of the concentrates.

Although the parallelism between biological activity and grade of purification as well as heat lability of pantothenic acid favored the assumption that the specific anti-gray-hair factor is identical with pantothenic acid in the concentrates tested, the corresponding final conclusion had to be deferred until the experiments could be repeated with pure pantothenic acid.

Recently Nielsen, Oleson and Elvehjem[6] have reported the isolation of a crystalline substance which, when fed at 15 micrograms per rat per day, prevented achromotrichia. This substance was different from pantothenic acid. These authors point out that "the factor preventing nutritional achromotrichia should be clearly differentiated from pantothenic acid."

With the production of synthetic pantothenic acid,[7] sufficient amounts of the pure substance became available[8] for the study of its activity in nutritional achromotrichia in rats.

A large number (30) of rats suffering from deficiency of pantothenic acid with its different manifestations[4, 9] was treated with graduated doses of pantothenic acid. In this group 12 piebald and black animals were included, 6 of which had localized and 6 more extensive, generalized achromotrichia. The therapeutic effect of pantothenic acid on the depigmentation of the fur was prompt and definite. With the administration of daily doses of from 75 to 100

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[5] P. György and H. Goldblatt, *Jour. Exper. Med.*, 70: 185, 1939.
[6] E. Nielsen, J. J. Oleson and C. A. Elvehjem, *Jour. Biol. Chem.*, 133: 637, 1940.
[7] R. J. Williams and R. T. Major, SCIENCE, 91: 246, 1940.
[8] Synthetic pantothenic acid was generously put at our disposal by Merck & Co., Inc., Rahway, N. J.
[9] P. György and R. E. Eckardt, *Nature*, 144: 512, 1939.

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.

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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.

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