sorbent, and petroleum ether as solvent. Both the nonsaponifiable fraction and the whole oil were fractionated. In both instances a significant proportion of the material originally put on the column was not retained but appeared in the filtrate. On removal of the solvent this was a yellow oil which darkened and solidified on refrigeration. This fraction constituted 83.25% of the whole oil chromatogram. The same pale yellow filtrate appeared when the nonsaponifiable fraction was chromatographed. In this instance it constituted 76.4% of the total recovery.

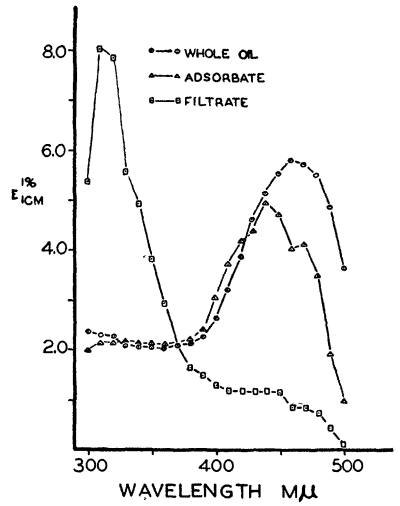

FIG. 1. Absorption spectra of plankton oil and of chromatographic fractions.

Spectrophotometric characteristics of this filtrate, of the combined eluate of the adsorbed pigments, and of the original whole oil are shown in Fig. 1. The adsorbed pigments show typical carotenoid absorption peaks. The primary ingredient of the filtrate material absorbs maximally in the neighborhood of 310 mμ in petroleum ether. There is no evidence of the 325–328 peak characteristic of vitamin A, nor are maxima found in the common carotenoid range.

TABLE 3
EFFECTS OF INCUBATION OF CECAE WITH PLANKTON OIL FRACTIONS

Time in hr	Filtrate	Adsorbate	Control
0	70 vitamin A/g	78 vitamin A/g	68 vitamin A/g
2	91	60	33
4	121	70	24
5	97	34	22

The biological activity of these two chromatographic fractions was determined by incubating them with homogenates of the pyloric cecae. When added to the cecal brei in amounts proportional to their concentration in whole oil the resulting increase in vitamin A potency is represented in Table 3. It will be seen that all the provitamin A activity resides in the noncarotenoid filtrate fraction of the plankton oil.

Thus it appears that some fishes can utilize zooplankton pigments other than the common carotenoids as raw materials for the elaboration of vitamin A.

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Pantothenic Acid in Copper Deficiency in Rats[1]

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According to the work of Free (*1*), the graying effect in rats could be due either to a lack of vitamins or to a deficiency of copper, and of several other elements. Henderson and his co-workers (*3*) reported that supplementation of the diet with 100 μg of calcium pantothenate per day had no effect on preventing the graying of piebald rats on a copper-deficient diet composed of whole milk supplemented with iron and manganese, whereas additions of 50 μg of copper sulfate corrected the condition. Other workers have studied the relationship between panthothenic acid and achromotrichia. Unna and Sampson (*4*) stated that doses of 5, 10, or 20 μg of calcium pantothenate were insufficient to prevent graying, whereas 40 μg gave inconsistent results. György and Poling (*2*) found that 75–100 μg of pantothenic acid daily caused definite restoration of pigmentation in 5–7 weeks when administered to rats deficient in pantothenic acid.

In our experiment, two groups of piebald and black rats, 22 days old, were placed on simplified diets designed primarily to study a comparison of the weight gains of the animals. In each group there were 15 animals with an average initial weight of 36 g. Group 1 was placed on a basal ration composed of whole dried milk (KLIM) 50.0%, sucrose 49.5%, NaCl 0.49%, manganous sulfate 0.0008%, ferrous sulfate 0.002%, and thiamine hydrochloride 0.00034%. Group 2 was fed the basal ration augmented with sufficient copper sulfate to give an analytical value of 20 ppm copper for the ration. The analytical value of copper obtained for the ration of Group 1 was less than 1 ppm. At the end of a 60-day trial, the two groups showed approximately the same rate of weight gain. The animals of Group 1 at the end of 7 weeks showed a consistent peculiar type of graying identical to

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that found in rats deficient in pantothenic acid. The change in the black rats of this group was more striking than in the piebald, although the pattern was the same. There remained a narrow stripe of black hair from the top of the head and extending along the middle of the back to the tail. Graying occurred in the remainder of the black hair. The animals of Group 2 maintained their normal pigmentation, indicating that the basal diet was adequate in pantothenic acid when copper was added. The basal diet had a calculated value of 12.5 μg of pantothenic acid/g of feed.

After maintaining the animals of Group 1 on the basal diet for a total period of 4 months without a fatality, several of the animals were placed on different levels of calcium pantothenate supplement. At the end of 5 weeks of oral administration, the results indicated that a 10-μg daily dose is without effect on repigmentation and a 20-μg daily dose exerts some effect, whereas 30-μg and 40-μg doses cause a pronounced effect, with restoration of most of the normal color. A microdetermination of copper showed that there was less than 1.1 μg of copper in the daily administered dose.

Graying of the hair occurring in black and piebald rats on a copper-deficient ration has thus responded to the administration of either copper or calcium pantothenate. This suggests some metabolic relationship between them, and implies a possible accentuation of pantothenic acid requirements by a deficiency of copper. The absence of any cases of spontaneous recovery in those animals maintained on the copper-deficient ration further emphasizes the importance of copper as a micronutrient concerned with normal hair pigmentation in rats.

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The Isolation of a Mucopolysaccharide from *Aerobacter aerogenes*

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The production of bacterial mucopolysaccharides which are depolymerized by testicular hyaluronidase has been limited to groups A and C streptococci (*4, 6*). More recently (*5*) an attempt to utilize polysaccharides from *Bacillus megatherium, Leuconostoc dextranicum, Rhizobium radicicolum,* and *Azotobacter chroococcum* as substrates for hyaluronidase was unsuccessful.

In the course of our investigations on the ability of bovine testicular hyaluronidase to depolymerize bacterial polysaccharides, a mucopolysaccharide was isolated from a strain of *Aerobacter aerogenes* which was attacked by hyaluronidase. The present paper is a brief account of these observations.

The strain of *A. aerogenes* gave a mucoid growth on nutrient agar and produced acid from dextrose, sucrose, mannitol, galactose, maltose, and inositol. Large capsules were demonstrable by the moist India ink method (*1*).

For the isolation of the polysaccharide a medium was used composed of 0.3% beef extract Difco, 1% Wilson SM peptone and 0.5% NaCl. The medium was adjusted to pH 7.4 and sterilized after distribution in 500-ml quantities into Fernbach flasks of 2800-ml capacity. The flasks were heavily seeded with an 18-hr to 24-hr nutrient agar slant culture and incubation was allowed to proceed for 5 days at 37° C. The cultures were centrifuged, and the viscous supernatant liquid was treated with three volumes of acetone. A white, stringy precipitate immediately formed which remained overnight in the icebox.

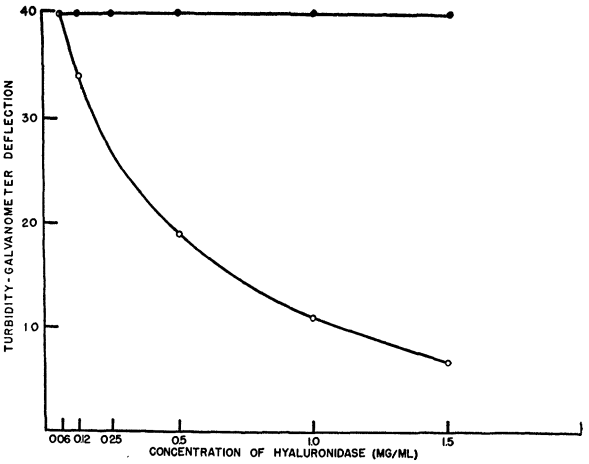

FIG. 1. The depolymerization of *Aerobacter aerogenes* polysaccharide by hyaluronidase. ○——○ = active enzyme; ●——● = enzyme inactivated by heat.

The precipitate was separated by centrifugation and the supernatant discarded. The precipitate was further purified by an acetic acid-acetate-alcohol procedure described by Pike (*7*) for streptococcal hyaluronic acid. A total of three reprecipitations were performed in this manner. The precipitate was next dissolved in distilled water, dialyzed against running tap water for 48 hr, and freeze-dried. The material, a cream-white, amorphous substance, yielded 75 mg to 100 mg per liter of broth.

Solutions of the substance gave negative protein tests with the Biuret, Hopkins Cole, and trichloracetic acid reagents. Reducing substances were not present as shown by negative Fehling and Benedict tests. A Molisch test for polysaccharide was strongly positive in a dilution of 1: 20,000.

The nitrogen content of the polysaccharide as determined by the micro Kjeldahl method was 7.9%; organic phosphate 1.27%; ash 10.5%; sulfur was not detectable.[1]

Bovine testicular hyaluronidase prepared by the method of Hahn (*2*) was used in the depolymerization studies. The enzyme preparation contained 700 turbidity reducing

[1] I am indebted to Mr. Wilhelm Reiss for this data.