be 0.273 gm. per hour, while 10 days later, after recovery, it had gradually fallen to 0.095 gm. hourly. After 6 days of fasting, at which time the animal had attained the same body weight as that which prevailed during polyneuritis, the basal nitrogen output amounted to 0.095 gm., thus indicating that the high nitrogen excretion during polyneuritis is not attributable to the starvation at this time. Subsequent investigations now in progress on the same animal seem to indicate a relationship between the basal nitrogen content of the urine and the extent of vitamin B starvation.

It may be concluded that vitamin B starvation *per se* does not alter the basal metabolism, although when complicated with polyneuritis an increased metabolism is evident; an increased nitrogen catabolism is also found in the latter condition, as shown by urinary nitrogen excretion.

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Studies on the chemistry of cod liver oil. II. A cod liver oil concentrate manifesting both antirachitic and antiophthalmic properties.*

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In recent years, the demonstration of the specificity of cod liver oil for rickets has provided a stimulus for further attempts to isolate and identify the active principle of the oil. Though the goal has not been reached, nevertheless appreciable progress has been reported by various investigators.

The problem has been approached by devious routes, but the most promising finding has been the demonstration that the vita-

* This investigation was started in January, 1922, and is still being continued.

mines known to be present in cod liver oil—the antirachitic and antiophthalmic—are to be found in the ether-soluble, non-saponifiable portion of the oil.

On saponifying cod liver oil, Steenbock, Nelson and Hart[1] obtained a product that was curative for both rickets and ophthalmia. Using an analogous process, Zucker, Pappenheimer and Barnett[2] and also Takahashi[3] reported results somewhat like those of Steenbock, Nelson and Hart. Some time later, Zucker[4] extracted cod liver oil with alcohol and then saponified the alcoholic extract. He thus obtained a product which contained only the antirachitic vitamine, the antiophthalmic vitamine having been destroyed in the procedure.

By means of a special extraction and saponification process we have prepared from cod liver oil a concentrate manifesting both antirachitic and antiophthalmic activity. The residual cod liver oil is entirely free from vitamines.

The crude concentrate—0.5 gm. from 1000 gm. cod liver oil—is obtained as a brown syrupy mass which, on standing, crystallizes in light yellowish brown needle-like crystals radiating from a central point.

This highly concentrated substance, which is insoluble in water but freely soluble in the ordinary organic solvents, contains practically all of the antirachitic and antiophthalmic vitamines originally present in the fresh cod liver oil.

In other words, if this 0.5 gm. vitamine concentrate is incorporated in 1000 gm. cottonseed oil, the resultant product is practically equivalent in vitamine activity to the original cod liver oil. The same is true if this 0.5 gm. concentrate is incorporated in 1000 gm. of an inactive base such as sugar.

On eliminating cholesterol and other inert substances, the above 0.5 gm. concentrate is reduced to 0.1 gm.—a concentration of 1 in 10,000. By working with extreme care, it is possible to obtain an active product showing a concentration of 1 in 15,000.

Thus far we have been unable to establish the exact chemical composition of this highly concentrated vitamine substance

[1] Steenbock, Nelson and Hart, *Am. J. Physiol.*, 1921-1922, lviii, 14.

[2] Zucker, Pappenheimer and Barnett, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 167.

[3] Takahashi, *Proc. Jap. Chem. Soc., J. Chem. Soc.* (Japan), 1922, xliii, 828.

[4] Zucker, PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 136.

since it is undoubtedly still far removed from the actual chemical entity.

However, the presence of C, H, O and S have been demonstrated. N and P are absent, as are also the halogens. The H_2SO_4 reaction is obtained just as with fresh cod liver oil, showing that the vitamine activity of the concentrate has been retained. Further, it is not toxic, even in large doses.

The advantages of such a concentrate are twofold. First, it provides a starting material for chemical work directed to the actual isolation and identification of the vitamines present in cod liver oil. Second, it provides an effective method of carrying out cod liver oil therapy, whether experimental or practical.

Experiments with over 300 rats kept upon a variety of vitamine-free and rickets-producing diets have shown that the cod liver oil concentrate described above is capable of both curing and preventing rickets and ophthalmia. The concentrate was administered apart from the diet, mixed either with an inactive oil or with sugar. The results were controlled by x-ray of the tibia and by microscopic examination of rib sections.

Clinical experiments[5] have revealed ample evidence that the concentrate is equally effective in children. In this work, the concentrate was mixed with sugar and administered in the form of 1 grain tablets, arbitrarily prepared to be the equivalent of half a teaspoon of cod liver oil. Due to the high degree of concentration, it is of course possible to prepare a small tablet containing as much or as little concentrate as may be desired for the particular experiment at hand.

It is our belief that infants, as early as one month after birth, should be given cod liver oil, or its equivalent in the form of a concentrate, as a prophylactic against rickets and other nutritive disturbances traceable to the lack of fat-soluble vitamines in the diet.

Detailed data of the above work will be published shortly.

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[5] Conducted by Dr. Louis Fischer during the past year at The Infantorium, Heckscher Foundation Building, New York City. These results are reported in the next paper.