Solids not protein	0.970
Ash	0.630
Lipoids (total)	0.115
Phosphatides (crude)	0.27
Fat, cholesterol, etc	0.88
Total extractive N	0.019
Lipoid N	0.004
Extractive N	0.015
Urea N	0.0106
Creatinine	0.0019
Basic N	0.0019

It is therefore shown that of the total solids in follicular fluid, the chief mass is protein. The concentration of the salts are apparently somewhat lower than in the blood and a considerable quantity of urea is present, but a trace only of phosphotungstic precipitable substances. The fat fraction amounted to 0.088%.

LABORATORIES OF THE UPJOHN COMPANY,
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A PALATABLE COD LIVER OIL CONCENTRATE POSSESSING THE THERAPEUTIC PROPERTIES OF COD LIVER OIL.*

BY HARRY E. DUBIN.

The therapeutic value of cod liver oil has been common knowledge for a great many years, but until recently its use was on a purely empiric basis.[1] With the indisputable proof at hand that cod liver oil is really a specific in the treatment of rickets, interest has been aroused in the possibility of isolating the active principle responsible for its therapeutic effect.

Regarding the mechanism of the action of cod liver oil, investigation has revealed the fact that the metabolism of calcium and phosphorus is in some way favorably influenced. It has also been found that cod liver oil has a stimulating effect on the intestinal mucous membrane and possibly on the formation of blood platelets.

Whatever the mode of action, the efficacy of cod liver oil is unquestioned. While it is a specific remedy for rickets, it has in addition a general restorative and vitalizing value. The vitamines inherent in cod liver oil are necessary for growth and health and for the normal development of bones and teeth. They promote growth and development in the adolescent period and are of great help in combating infections, by increasing body resistance.

The healthy adult does not need cod liver oil, but there are numerous cases where it could be used to advantage. While the adult does not suffer from active

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* Detailed experimental data obtained by Dr. Dubin and Dr. Funk appears in *J. Metabolic Research*, 4, 467 (1923).

[1] The results of the clinical investigation made by Dr. Louis Fischer, Director of the Infantorium and Nursery of the Heckscher Foundation, New York City, are published in *J. Metabolic Research*, 4, 480 (1923).

rickets, the same dietary deficiencies leading to its development in the child may also exist in the adult and impair his health.

The medicinal properities of cod liver oil were at first thought to be due to its nutritive value as a fat. Later its value was attributed to its mineral content. Recent research, however, makes it impossible to doubt that the therapeutic action of cod liver oil is due solely to the antirachitic and antiophthalmic vitamines present.

Recognizing the therapeutic efficacy of cod liver oil, attempts have generally been made to overcome its objectionable features, such as nauseous taste and odor. The customary procedure has been to prepare extracts and emulsions of various kinds. However, the usual alcoholic extract has little or no therapeutic value, while the emulsion is active only in proportion to its oil content; as for palatability—there is very little decided improvement.

Attention has therefore been directed towards the isolation of the active principle. Many investigators have been occupied with this problem and appreciable progress has been reported. We have been engaged in this work for almost three years, and although we have not actually identified the active principle of the oil, *we have nevertheless succeeded in preparing a highly concentrated product, free from oil, practically odorless and tasteless, non-toxic and equal in therapeutic value to fresh cod liver oil.*

Briefly, the method involves extraction of cod liver oil with an organic acid such as acetic or formic acid. The acid extract is freed from acid and the oil remaining, amounting to about 5 per cent. of the original cod liver oil, is saponified with alcoholic soda or potash. The soaps are extracted with ether and the ether evaporated off, leaving the active cod liver oil concentrate. The destructive effect of oxidation is avoided by carrying out the process in the absence of air. Exclusion of air is essential to assure the stability of the preparation.

By this method, from 1000 Gm. cod liver oil it is possible to obtain 0.1 Gm. concentrate which is practically as potent as the original quantity of fresh cod liver oil.

In the crude state, the cod liver oil concentrate is a brown semi-crystalline pasty mass. In combination with sugar it appears as a creamy white powder which may be compressed into tablets of any desired size and dosage. Because of the high degree of concentration, it is feasible for example, to prepare a 1-grain tablet to be the equivalent of 1 tablespoonful of fresh cod liver oil.

Almost 700 animal experiments have been carried out to prove the value of this cod liver oil concentrate. When rats are placed on a rickets-producing diet, they develop rickets in about 30 days. On administering an amount of cod liver oil concentrate corresponding to as little as 0.028 Gm. cod liver oil, the rachitic manifestations disappear in about 3 weeks. The same amount of concentrate will also prevent rickets in animals maintained on the above diet. In other words, the concentrate can act both as a curative and as a prophylactic agent.

The accompanying chart and radiographs illustrate briefly the antirachitic action of the cod liver oil concentrate, using the prophylactic type of experiment.

Each of the animals represents a series placed on the indicated rickets-producing diet. Rat No. 693, the control, developed rickets, while the others, receiving either cod liver oil or the cod liver oil concentrate, remained normal.

Rat.	Weight—Gm. June 6, 1924.	Weight—Gm. July 8, 1924.	Gain.	X-Ray.	Substance administered daily.	Diet Pappenheimer-Zucker diet D.	Gm.
680	42	75	33	Normal	28.0 mg. cod liver oil	Patent flour	80.9
684	40	64	24	Normal	0.074 mg. concentrate equal to 28.0 mg. cod liver oil	Egg albumin Butter fat	10.0 5.0
693	40	92	52	Rickets	Control	Salts	4.1

In the rachitic rat No. 693, note the enlarged epiphyses, A, and lack of calcification, as compared with the well-calcified bones, B, of the other rats.

Rat No. 680. Rat No. 684. Rat No. 693.

The antiophthalmic effect of the cod liver oil concentrate is illustrated by the following typical experiment:

Rat No. 726 was placed on a diet adequate in every respect except as to fat-soluble vitamines. At the end of 2 months the animal showed marked ophthalmia and had stopped growing. Daily administration of an amount of cod liver oil concentrate corresponding to 0.028 Gm. cod liver oil was started. The animal promptly resumed growth and at the end of 14 days its eyes appeared normal.[1]

Clinical experiments have also been made, showing clearly by means of X-ray and otherwise that the concentrate is as effective in children as in animals.[2]

By means of such a concentrate it is a simple matter to institute cod liver oil therapy in a more agreeable form, especially in the numerous instances where fresh cod liver oil is not tolerated.

There seems to be no good reason why infants, as early as one month old, should not be given cod liver oil or cod liver oil concentrate as a prophylactic against rickets.

It would be possible, therefore, to decrease the incidence of rickets to negligible proportions, and remove it as a menacing factor in the development of the child. At the same time, other nutritive disturbances due to lack of the vitamines found so abundantly in cod liver oil would be eliminated.

In this connection it is interesting to note that respiratory infections are quite prevalent among babies whose diets are deficient in these vitamines. The routine administration of cod liver oil to such babies has been strikingly effective in producing an immunity against these infections.

It has thus become increasingly evident that cod liver oil therapy, emerging

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from the haphazard to the scientific stage, is taking its place as an important element in the management of infant nutrition.

DISCUSSION.

Arno Viehoever: I had the privilege of looking over three papers submitted to me by Dr. Dubin for perusal. My first suggestion would be that the author reconsider the presentation of the paper to be submitted to the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION because in that paper there is only a brief abstract without any experimental data being given. I feel that all the members of the Association are interested in that work, and many of you no doubt will want to be informed as we have been this morning, as to the evidence which has been obtained in an experimental way on animals. I feel that the work which has progressed for three years has certainly produced results. There are, as the author mentioned, other extracts on the market.

He also points out in his paper that four methods exist. One is the saponification of the original oil, necessitating working in very large quantities. He also pointed out that another method consists in saponification of the alcoholic extract. The third method which he did not bring out this morning was developed by himself and Dr. Funk, and that is hydrogenation of the oil and then subsequent extraction. And finally, there is the acid extract of cod liver oil, the subject of the present paper.

It was stated that either acetic or formic acid had been found to be useful.

I may also say that after saponification of this acid extract, the residue is extracted with ether and in that way a concentrate containing the valuable vitamines are obtained. I may further say that this cod liver oil concentrate has been found to be free of nitrogen sulphur and phosphorus. Only the presence of carbon, hydrogen and oxygen has been demonstrated.

One of the questions I would like to ask at the end of my remarks is whether there is any close chemical relationship between the cod liver oil concentrate and the substance isolated from yeast by Dr. Eddy, who pointed out that the yeast vitamine contains nitrogen.

I was interested to find out that a distinct difference exists between the saponification of the original oil and saponification of the alcoholic extract.

I certainly want to compliment the author on the progress of the work which is evident from a study of his three papers and from the demonstration this morning.

A. C. Taylor: I would like to ask the author if I correctly understood that he distinguishes in his experiments between the antiophthalmic vitamine and the antirachitic vitamine which are recognized to-day as being distinct? Does the alcohol fail to remove both of them, or only the one; and does the acid extract that he speaks of remove both or only one; and does the concentrate contain both of these or one?

Dr. H. C. Wood: I certainly was very much interested in this paper. It represents a very marked advance and is a very important record.

I want, however, to protest against one phrase that the reader of the paper used, and that is when he speaks of this concentrate as representing all the therapeutic value of cod liver oil. It would perhaps be more accurate to speak of it as antirachitic value of cod liver oil. A very large proportion of the value of cod liver oil rests in the peculiar character of the fat which composes it. The fat of cod liver oil is more nutritious than most of our fats, because it is more readily absorbed from the intestinal tract than other fats. I maintain no preparation of cod liver oil which does not contain the fats can represent all the therapeutic value of the oil.

L. E. Warren: I would like to ask the author for a little more detail in regard to the method of the manufacture of this preparation. It seems to me that in the details that he has given and in the little more enlarged details that Dr. Viehoever has given, no chemist among you could follow his method and reproduce that preparation.

Robert J. Ruth: I would like to have a word concerning the stability of the preparation.

Harry E. Dubin: As regards Dr. Viehoever's remarks on the relationship between the cod liver oil vitamines and yeast vitamine, it is recognized that yeast contains one of the so-called water-soluble vitamines, while the cod liver oil vitamines are fat-soluble. Cod liver oil will cure rickets and ophthalmia, whereas yeast will cure beriberi. The available evidence is against any close chemical similarity between these two types of vitamines.

The only possible connection is that all vitamines are necessary for complete growth. The antiophthalmic vitamine has always been called the growth-promoting vitamine. We

must remember, however, that real growth on any particular diet will not take place unless all the vitamines are present. It is evident, therefore, that any one of the vitamines, or for that matter the mineral content of the diet, may be the limiting factor of growth.

Regarding the questions raised by Dr. Taylor, the alcoholic extract of cod liver oil, while it is antirachitic, is not antiophthalmic. The oil remaining after the alcoholic extraction still contains the major portion of the antirachitic and antiophthalmic vitamines originally present.

The acid extract is both antirachitic and antiophthalmic. The residual oil, after acid extraction, is no longer active. In other words, the acid has extracted the active principles of the oil.

The concentrate made by means of these acid extractions followed by saponification contains the antiophthalmic and antirachitic vitamines; therefore, this particular process is an advantage in that we have been able to extract both vitamines.

As regards the statement of Dr. Wood that the concentrate does not represent all of the therapeutic value of the cod liver oil, I feel that it is not valid, for this reason: The activity of the oil at one time was attributed to its nitrogen or phosphorus content. This has been disproven because the concentrate contains neither nitrogen nor phosphorus; and yet if it is mixed with sugar, for example, the therapeutic activity of the mixture is exactly the same as that of fresh cod liver oil. In other words, the oil has been replaced by sugar but the therapeutic effect is the same because of the presence of the concentrate.

The oil remaining after acid extraction is no longer effective in those nutritive disturbances for which cod liver oil is used. In view of the foregoing, we are justified in saying that the therapeutic value of cod liver oil, as far as experimental evidence is concerned, is due to its vitamine content.

As regards the stability, we have found that the product is stable even after seven or eight months, so far. As long as the concentrate is protected against oxidation, there is no loss of activity. This is assured by carrying out the process in a non-oxidizing atmosphere.

As to the details of manufacture, they are largely given in the detailed paper to be published shortly in the *Journal of Metabolic Research.*

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DIETHYLPHTHALATE III.*

BY J. A. HANDY AND L. F. HOYT.

1. INTRODUCTION.

A survey of the data on diethylphthalate appearing since our last paper on this subject (1) and a study of some recently proposed detection tests, have been made.

2. METHODS OF DETECTION.

A detection test for diethylphthalate involving the use of pyrogallol, quite different in its results from the fluorescein type test or the phenolphthalein test of Calvert, has been proposed by Eilles (2). This author deprecates the use of phenol-H_2SO_4 (*i. e.*, the Calvert Test) as being of little use (no reasons given) and states that the resorcinol- H_2SO_4 tests, (*i. e.*, like those of Lyons, Handy and Hoyt, and Andrew) are uncertain because of fluorescence in blanks. The Eilles test, which is official at the German Bureau of Internal Revenue (Hamburg) is carried out as follows:

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* Scientific Section, A. PH. A., Buffalo meeting, 1924.