in from 5 to 50 seconds, the average being 19 seconds. The duration of paralysis is from 4 to 16 minutes, averaging 9 minutes and 10 seconds. The one death which occurred can probably be explained on the basis of the marked age of the animal. It will be noted that the duration of the paralysis is short enough to fall entirely within the post-metrazol confusion period. The dogs were next erythroidinized and then give previously determined convulsant doses of metrazol with results shown in Table IV:

The final recheck with metrazol alone to rule out the possible development of tolerance to the drug in the dosage previously used is given in Table V.

CONCLUSIONS

The results as indicated in Table IV show that a standard dosage of Beta-erythroidin hydrochloride about 4 mg. per Kg. was effective in markedly reducing the intensity of the metrazol seizure in dogs. The duration of the convulsion was variable but in the more prolonged instances included pauses between brief clonic seizures. In general the duration appeared to be decreased. Table V demonstrates the potency of the metrazol dosages used with the Beta-erythroidin hydrochloride thus proving that this drug has a paralytic action upon the animals that had received an otherwise convulsant dose of metrazol. No untoward side reactions such as fecal vomiting, defecation, etc., were observed.

Work is now in progress on the application of the method to schizophrenic patients.

SUMMARY

1. Paralytic doses of curare were given to two dogs on successive days without harmful effects. The curare, however, was found unsatisfactory because of difficulties in standardization and undesirable side-actions. The same procedure was followed with Beta-erythroidin hydrochloride, a substance having a curare-like action. The one death that occurred was in an aged dog who had reacted very poorly to a metrazol dose on the previous day and who died following Beta-erythroidin hydrochloride and metrazol.

2. A previously determined convulsant dose of metrazol was given to a series of seven dogs already paralyzed by Beta-erythroidin hydrochloride. The severity and, to some extent, the duration, of the metrazol convulsion were drastically reduced.

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Comparative Study of Vitamins and Constants of Free and Extracted Oils from Canned Sockeye Salmon*,†

*By Arthur W. Steers‡ and Louis Fischer***

Canned salmon is recognized as an important staple of food and commerce in all parts of the world. The world's output during the last ten years has reached an annual average of over ten and a half million cases. Every year a portion of the pack is stored and the greater part sold prior to the arrival of the new pack. It is of immense interest to know if the product undergoes any changes during this period. This led to the inception of a study, which is in progress, of the effects of storage upon the oil in canned salmon. The present report, however, concerns an investigation as to the proper procedure of removing the oil from the salmon and a comparative study of certain properties of the free oil (that which drains from the salmon upon opening the can) and the extracted oil (obtained by ether extraction). Of the five species used for canning, sockeye

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is considered the most important from the standpoint of aggregate value and hence was selected for this project in preference to the others. The two oils were separated by definite procedures and a comparative study of the vitamin A and D values, together with the constants, was made.

Numerous workers have reported varying amounts of vitamin D in salmon body oil, and some have found vitamin A to be negligible or much lower than the amount usually present in cod liver oil. The literature reveals that the vitamin content is considerably higher in the offal oil (that produced from waste products, such as livers, viscera, cannery trimmings, etc.) than in the body oil. A complete review of the literature, concerning the vitamin content and physical constants would be too voluminous for discussion in this paper. However, a few of the later reports, involving the body oil in which results have been expressed as U. S. P. or International units, the two being equal in activity to the rat, are given.

Schmidt-Nielsens (1), 1929, using biological methods, reported 20 to 30 U. S. P. units of vitamin A and 25 to 50 U. S. P. units of vitamin D per Gm., whereas the Rosenheim antimony trichloride reaction for vitamin A was quite negative. Tolle and Nelson (2), 1931, examined the oil from all five species of Pacific salmon and concluded that none of them were a particularly good source of vitamin A, but were equal to medicinal cod liver oil in vitamin D potency.

Devaney and Putney (3), 1935, investigated the vitamin content of the flesh of several commercial brands of four different species of salmon. They found vitamin A to vary from 0.25 to 8.0 and vitamin D from 1.9 to 8.0 International units per Gm.

Bailey (4), 1936, found the oil from sockeye and pink salmon to contain from 50 to 67 International units of vitamin D per Gm., and the oil of sockeye salmon to contain from 2.8 to 3.5 International units of vitamin A per Gm.

EXPERIMENTAL

SOURCE OF CANNED SALMON

These samples consisted of sockeye salmon packed at the Fishermen's Packing Corporation cannery, located at Anacortes, Washington. The fish were taken from a commercial catch obtained from the ocean waters near the Salmon Banks, located off the San Juan Islands in Puget Sound, on September 10, 1938.

The salmon were examined and found to be in perfect condition for canning. The identity of each dressed fish was maintained by separately packing it into four to six one-pound flat cans, depending upon its size, and stamping identification marks upon each of these containers. The cans were then processed in the same manner as that carried out in the cannery. This consisted of clinching the tops loosely, removing a greater part of the air by a vacuum machine and then rolling the seams tightly. The cans were next cooked in a retort at 240° F. for a period of ninety minutes and, upon removal, washed thoroughly in a lye solution followed by fresh water, and allowed to cool.

This manner of packing and marking made possible the use of the free or extracted oil from the entire edible portion of one or more fish in the subsequent chemical and biological tests. The amount of oil varies greatly in different parts of the same fish and it is possible the chemical characteristics and vitamin content may also vary. By using oil from entire fish possible errors due to sectional differences were eliminated.

EXTRACTION AND SEPARATION OF THE OILS

A. Free Oil for the Vitamin D Assay.—As soon as opened, the cans were inverted and the free oil allowed to drain for fifteen to twenty seconds. This liquid, from fifty cans and representing the entire edible portion of ten fish, was combined and the oil separated from the aqueous part by use of a separatory funnel. The oil was filtered into small bottles, anhydrous sodium sulfate added, and, after displacing the air with carbon dioxide, was kept tightly stoppered and in a refrigerator. All samples were held in a similar manner, and were filtered before proceeding with any of the described determinations.

B. Extracted Oil for Vitamin D Assay.—The oil was obtained using a slight modification of a method by Stansby and Lemon (5). The solids, remaining after the free oil was removed as previously described, were first passed through a meat grinder. Two 20-Gm. samples were taken from each can and the samples were transferred to eight-ounce wide-mouth bottles. Twenty-five grams of anhydrous sodium sulfate were mixed thoroughly with the flesh, 100 cc. of peroxide-free ether were added, and the air displaced with carbon dioxide. The bottles were agitated in a shaking machine for an hour and the solvent subsequently removed by expression through muslin. The ether solution was dried with anhydrous sodium sulfate and passed through a Jena glass filter. The solvent was evaporated under reduced pressure and the remaining traces of ether were removed by use of a slow stream of carbon dioxide.

C. Free Oil for Vitamin A Assay and Constants.—The method was identical as previously described under (*A*) above, but the composite sample represented ninety-six cans from the entire edible portion of twenty fish.

D. Extracted Oil for Vitamin A Assay and Constants.—A composite sample was prepared from forty-eight cans, comprising the entire edible portion of ten fish, by the following procedure. The free liquor was removed from three cans and the ground solids were triturated to a powder with 700 Gm. of anhydrous sodium sulfate in a large mortar. The material was then transferred to a five-liter wide-mouth bottle, one liter of peroxide-free ether added, the air displaced with carbon dioxide, and shaken at intervals for one hour. After decanting the ether solution and expressing the solid material through muslin the extraction was repeated a second time. The combined ether solution was dried with anhydrous sodium sulfate, filtered, and the solvent removed as previously described.

basal diet was supplemented with small amounts of lettuce or spinach twice weekly, except to lactating does. The young were weaned when they attained an average weight of 32 to 35 Gm. and were continued on the basal diet until they averaged 45 to 60 Gm. in weight. They were then transferred to the rachitogenic diet No. 2, U. S. P. XI, and maintained on this diet for twenty-one days, at which time a satisfactory degree of rickets was developed. Litters were then divided into groups; one-half of each litter received graded doses of the Standard (0.66, 1.33 and 2.66 unit levels daily); the other half received graded doses of the salmon oil (10, 20 and 40 mg. levels daily). Eight animals were used for each level in the assay of the extracted oil and seven for each level in the assay of the free oil. Preliminary work, in which widely varying doses were fed to small groups, indicated that the above levels would give approximately the desired healing. Both oils were diluted with corn oil so that 0.1 cc. carried the desired daily dose. This was con-

Table I.—Data for Vitamin D Assay of Free Salmon Oil

Sample	Daily Feeding Level	Total Amt. of Oil Fed, per Rat	No. of Rats	Bone	No Healing	Line Test Results 1	2	3	4	5	6	Av.
Reference cod liver oil	7 mg.	4 units	7	Ulna		3	3	1				1.64
				Radius		5	1		1			
Reference cod liver oil	14 mg.	8 units	6	Ulna		1	3	1	1			2.42
				Radius		1	2	2	1			
Reference cod liver oil	28 mg.	16 units	7	Ulna			4	1	2			2.86
				Radius			1	5	1			
Free salmon oil	10 mg.	60 mg.	7	Ulna		2	5					1.64
				Radius		3	4					
Free salmon oil	20 mg.	120 mg.	7	Ulna			3	4				2.64
				Radius			3	3	1			
Free salmon oil	40 mg.	240 mg.	7	Ulna			2	3	2			3.22
				Radius			1	3	2	1		
Control			9	Ulna	9							
				Radius	9							

Table II.—Data for Vitamin D Assay of Extracted Salmon Oil

Sample	Daily Feeding Level	Total Amt. of Oil Fed, per Rat	No. of Rats	Bone	No Healing	Line Test Results 1	2	3	4	5	6	Av.
Reference cod liver oil	7 mg.	4 units	8	Ulna		2	2	2	2			2.50
				Radius		2	2	2	2			
Reference cod liver oil	14 mg.	8 units	7	Ulna			1	3	2	1		3.50
				Radius			1	2	3	1		
Reference cod liver oil	28 mg.	16 units	8	Ulna			1	1	4	2		3.94
				Radius				2	4	2		
Extracted salmon oil	10 mg.	60 mg.	8	Ulna		1	3	3	1			2.69
				Radius		1	1	5		1		
Extracted salmon oil	20 mg.	120 mg.	7	Ulna				4	2	1		3.72
				Radius				2	4	1		
Extracted salmon oil	40 mg.	240 mg.	7	Ulna				1	3	1	2	4.72
				Radius				1	1	3	2	
Control			7	Ulna	7							
				Radius	7							

VITAMIN D BY BIOLOGICAL ASSAY

Procedure.—The determination of the vitamin D content of the free and extracted oils, obtained as previously described, was carried out by the bioassay method of the U. S. P. XI.

The stock colony of albino rats was reared according to the conditions suggested by Russell (6). The veniently fed from a one cc. tuberculin syringe using a blunt number eighteen needle. After six successive daily doses, the animals were killed on the eighth day and the line test performed on the distal end of the ulna and radius.

Results.—The healing of the ulnæ and radii was designated by numerical estimates using a chart

drawn up in the laboratories of the Pharmaceutical Society of Great Britain for comparison. The final results were calculated using a method described by Coward (7) and the free oil was found to contain 80 and the extracted oil 88 U. S. P. units per Gm.

VITAMIN A BY BIOLOGICAL ASSAY

Procedure.—The vitamin A content of the free and the extracted oil was determined by the method outlined in the U. S. P. XI. The rats used were reared as previously described and when individuals of the litters averaged 38 to 45 Gm. in weight they were put on the vitamin A deficient diet. The U. S. P. diet was prepared, using 18 per cent casein freed from vitamin A by Todhunter's method (8). Four and one-half per cent FRL Salt Mixture (9) was substituted for the designated mixture. The other ingredients consisted of corn starch 65 per cent, pure corn oil five per cent, Strain L yeast five per cent, purchased from the Anheuser-Busch Company, and vitamin D in the form of irradiated ergosterol.

The rats were continued on this diet until they were depleted of vitamin A as indicated by declining weight, at which time they were divided into groups of eight each, equally distributed as to sex and weight. Two groups were fed the U. S. P. diet supplemented with a daily dosage of 2.0 and 4.0 units of U. S. P. Reference cod liver oil, previously diluted with corn oil so that 0.1 cc. carried the daily dose. In the other groups (two for each oil) the diet was supplemented with 0.4 cc. and 0.8 cc. each of the free and extracted oil. They were given the supplement daily (six days a week) for 28 days and their final weight recorded on the twenty-ninth day. The average increase in weight of each group was calculated and the results interpreted as follows.

Table III.—Data for Vitamin A Assays

Sample	Daily Feeding Level	No. of Rats	Number Considered in Results[a]	Average Gain in Weight
Reference cod liver oil	2 units	8	0	....
Reference cod liver oil	4 "	8	7	36.7 Gm.
Free salmon oil	367 mg.	8	0	....
Free salmon oil	733 "	8	6	22.4 Gm.
Extracted salmon oil	367 "	8	7	25.4 "
Extracted salmon oil	734 "	8	7	46.4 "
Control	.....	8	8	0.0 "

[a] Those gaining more than 12 and less than 60 Gm.

Results.—A daily dose of 733 mg. of free salmon oil produced somewhat less growth than four units daily of Reference oil. This indicates that the free oil contains about 5.5 units per Gm.

The growth produced by 4 units daily of the Reference oil lies about midway between that produced by a daily dose of 367 mg. and 734 mg. of extracted oil. This would indicate that the extracted oil contains about eight units per Gm.

CONSTANTS OF FREE AND EXTRACTED SALMON OILS

A comparative study of certain constants of the two oils was made by determining these values using procedures outlined in the Methods of Analysis of the A. O. A. C. Before proceeding with the analysis, the oils were freed from carbon dioxide by exposing them in a shallow dish in a vacuum desiccator for twenty-four hours. Both samples were analyzed in duplicates and the average results listed in Table IV.

DISCUSSION

The results of the vitamin D assays show that the free oil contains 80 units and the extracted oil 88 units of vitamin D per Gm. This compares very favorably with cod liver oil which has a minimum of 85 units per Gm. Canned salmon is therefore a very valuable source of this vitamin. In view of the fact that most workers consider the vitamin D assay to be accurate within 20 per cent of the results and a few consider it accurate within 10 per cent, the difference between the vitamin D values of the two oils does not seem to be significant. The results have, moreover, been submitted to statistical analysis, and it is found that the method of Coward (7) shows no significant difference in the vitamin D content of the two oils.

The results of the vitamin A assays indicate that the vitamin content of the free and the extracted oils is very low as compared to cod liver oil. Therefore the results were not submitted to statistical analysis for ascertaining any significant difference in the vitamin A values of the two oils.

The results of the constants agree quite favorably with the reports by other investigators, and indicate only slight differences between the free and the extracted oil. In the values reported by other workers no

Table IV.—Chemical Constants of Free and Extracted Oils

	Sp. Gr. 25° C.	Ref. Index 20° C.	Sapon. No.	Unsap. Matter, Per Cent	Iodine No. (Hanus)	Acid Value
Free oil	0.9164	1.4783	183.8	0.53	134.5	0.52
Extd. oil	0.9173	1.4783	183.3	1.23	131.7	1.48

distinction was made between the free and the extracted oil with one exception of the acid value mentioned later. The specific gravity of the free oil, 0.9164, and of the extracted oil, 0.9173, indicate only a slight variation from the values reported by Holmes and Pigott, 0.9196 (10); and Truesdail and Boynton, 0.9145 (11). Likewise, the refractive index, 1.4783, for both oils compares closely to figures of Truesdail and Boynton, 1.4763; and Bailey, 1.4760 to 1.4775 (4).

The saponification value for the free oil, 183.8, and the extracted oil, 183.3, are practically the same and hence indicate little difference in composition. These values vary somewhat from those given by Holmes and Pigott, 187.7; and Truesdail and Boynton, 168.6.

A slightly higher amount of unsaturated substances is indicated in the free oil by its higher iodine number, 134.5. The value obtained for the extracted oil was found to be 131.7. Other investigators report quite wide variations; Holmes and Pigott, 142.2; Truesdail and Boynton, 160.0; and Bailey from 135.7 to 151.5.

The acid value shows the presence of more acidic substances in the extracted oil, 1.48, than in the free oil, 0.52. This difference is accounted for by the removal of substances with ether that are titratable by alkalies and were previously reported on by Brocklesby (12). Holmes and Pigott reported 0.7717 per cent free fatty acids; Truesdail and Boynton, 0.21 per cent; and Bailey gave for the acid value of the free oil 0.38 to 0.50 and for the extracted oil 1.74 to 2.22.

Slightly more unsaponifiable matter was obtained in the extracted oil, 1.23 per cent, than in the free oil, 0.53 per cent. This can be accounted for by the extraction with ether of unsaponifiable substances which are removed only with difficulty by the free oil. Bailey reported figures for this value between 1.10 and 1.21 per cent.

The differences in the constants found in this investigation from those given by other workers may be attributed to various factors such as time of year, locality where the salmon were caught and the method used in removing the oil.

SUMMARY

The free oil was separated from special packs of canned sockeye salmon and the remaining oil extracted with ether by definite procedures. The vitamin A and D content of the two oils was assayed biologically using albino rats.

The vitamin D content of the free oil was found to be 80 units and of the extracted oil 88 units per Gm. This difference is not significant.

The vitamin A content of both the free oil and the extracted oil was very low, approximately 5.5 and 8 units per Gm., respectively.

A comparative study of certain constants for each oil was made.

ACKNOWLEDGMENTS

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A Chemical and Pharmacological Comparison of the Menthols

By A. Richard Bliss, Jr., and H. Bryson Glass†*

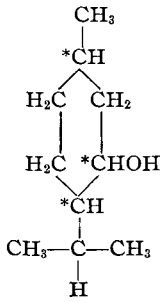
Many users of menthol are unaware of the fact that there are several menthols. The literature on menthol most commonly available is limited chiefly to "Menthol, U. S. P.," which is defined as "An alcohol obtained from oil of peppermint or other mint oils or prepared synthetically" (1). In manufacturing quarters the common impression is that there are two menthols, *viz.*, (*a*) "natural menthol" and (*b*) "synthetic menthol," and little attention has been paid to the fact that there are eight possible menthols, and that six of them have been isolated and characterized.

A major objective of the studies herewith reported was the determination of any measurable differences in the pharmacodynamic activities and in the toxicities of these menthols which might affect their employment as ingredients of creams, ointments, lotions, douches, nasal sprays, mouth washes, dusting powders, tooth pastes, shaving creams, cough drops, etc. Through the courtesy of a manufacturer,[1] generous supplies of the menthols used in these studies were obtained.

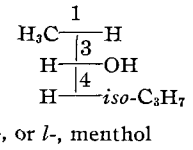
Until recently the world supply of menthol was obtained from Japanese peppermint oil produced from *Mentha arvensis*. According to Trease (2), Japanese peppermint oil is derived from *M. canadensis, var. piperascens*. *Mentha canadensis*, having a chromosome number of 27, is derived from *M. arvensis* (36 chromosomes) and *M. aquatica* (18 chromosomes). According to this writer, before the foregoing facts were elucidated, the Japanese plant was brought to England by Christy, and its affinity to *M. arvensis* being recognized by Holmes, he named it *M. arvensis, var. piperascens* (3), (4). Numerous attempts have been made to produce menthol from mint oil grown in other parts of the world, but the menthol content of these oils is too low to permit commercial production. A stereoisomeric menthol, *l*-menthol is the well-known U. S. P. material (1). As a general rule, organic compounds containing an asymmetric carbon atom which occur in plants are found in the optically active state. Usually, *only one* stereoisomer of a given compound is found.

Several synthetic menthols have been on the market for a number of years, but they have not been identical with the natural product, being optically inactive and of lower melting point than the natural product. These synthetic menthols also differ greatly from the natural in odor, taste and "cooling effect."

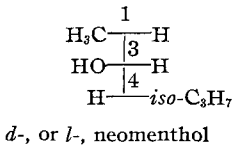
A very brief and uninvolved consideration of a portion of the chemistry of menthol will serve to explain these differences. From the constitution of menthol as developed by Beckmann, Semmler, Widman and others (5), it is evident that menthol contains three asymmetric carbon atoms (designated by *) as shown in the following structural formula:



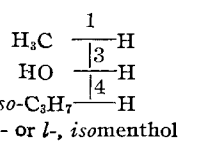
and is capable, therefore, of existence in *eight optically* active forms, *viz.*:



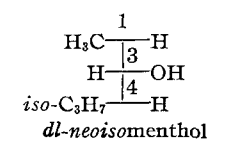
d-, or *l*-, menthol



d-, or *l*-, neomenthol



d- or *l*-, *iso*menthol



*dl-neoiso*menthol

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