STUDIES IN FISH LIVER OILS

Part I. Seasonal Variation in (*a*) Vitamin A Content, (*b*) Some of the Physical and Chemical Constants of Some Fish Liver Oils

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IN Europe as well as in America, work on the seasonal variation in the yield and vitamin A content of the oil has been attempted by very many workers. Bills, *et al.* (1934), Haines and Drummond (1936), Evers, *et al.* (1936), Shortland (1938) and Molteno, C. J. and Rapson, W. S. (1939). In India work on the same line has been undertaken by Seshan (1940), Niyogi, *et al.* (1943 *a*) and Sarangdhar (1942).

It was observed in our laboratories that during the extraction of the oil from the livers of different species of fish that are available in Bombay coastal waters the colour of the oil varied from yellow to brownish red. On subjecting the various coloured liver oils for the assay of their vitamin A content using Lovibond tintometer great variations in the vitamin values for the same species were observed. It was also noticed that there was great variation in the colour as well as vitamin content from oils obtained from the livers of same fish collected and extracted at different periods of the year. It was observed that in the majority of cases the colorimetric assay gave more blue values for brown coloured oils than for pale yellow ones.

A careful study of the literature available revealed the fact that other than some stray references no systematic work has been done to correlate vitamin A value with different physical and chemical constants of the liver oils.

Embree (1939) has stated that cyclized vitamin A—the material formed by the treatment of vitamin A with hydrochloric acid in alcohol has been found to occur naturally in tuna and other fish liver oils. He further states that this cyclized vitamin A appears to have no growth-promoting power, but has considerable ultra-violet absorption at 328 μ and gives practically the same antimony trichloride reaction as vitamin A; he calls this 'Spurious vitamin A'.

Niyogi. Patwardhan and Acharya (1943 *b*), subjected the liver, oils from the two fish, *viz.*, mushi (*Scoliodon Sorrokowah*) and ghol (*Sciæna miles*) to the tintometric and biological assay for vitamin A. They used the factor 4·2 (Bomskov, 1935) to convert the blue values (as read on the Lovibond tintometer) to International Units. The two methods gave values which agreed in the case of mushi liver oil, but in the case of ghol liver oil the biological value was however considerably lower than the tintometric result. From this observation they came to the conclusion that the vitamin A value obtained by tintometer is not always a true measure. They suggested that the ghol liver oil contained a chromogenic substance which did not promote growth; and this formed nearly a third of the chromogenic material responsible for the blue colour obtained with antimony trichloride.

As some of these liver oils are already in use as substitutes for cod-liver oil, it is of utmost importance to subject them for biological assay in order to see whether the whole of vitamin A estimated with antimony trichloride is available for growth or not. This study is presented in two parts. Part I deals with physical and chemical constants along with Vitamin A values determined by physico-chemical methods in liver oils and Part II deals with biological assay.

Six varieties of fish were taken in this investigation. Every month a sample of fish liver of each variety was collected, the oil extracted and used for the estimation of vitamin A by colorimetric, and physical and chemical constants by standard methods.

EXPERIMENTAL

Niyogi, *et al.* (*loc. cit.*) extracted the oil by the method of Carr and Price (1926) as modified by Coward, *et al.* (1931), for their investigation of the vitamin A content of fish liver oils. The finely minced liver was ground up with anhydrous sodium sulphate and extracted repeatedly with ether in the cold ; the ethereal extract was distilled in presence of nitrogen atmosphere and the oil was recovered. Traces of moisture were completely removed by drying the oil in vacuum over phosphorus pentoxide.

The method adopted for the extraction of the liver oil in this investigation was by steaming. Fresh livers were minced and mixed with twice their volume of water in a tinned copper vessel. The contents were cooked in a water-bath with constant stirring and keeping the temperature at about 80° C. The oil that oozed out was collected, filtered through a muslin cloth and transferred to Winchester bottles having taps at the bottom. The crude oil was allowed to settle for an hour to remove the water layer which was collected at the bottom of the Winchester bottles. To remove traces of moisture the oil was again transferred to the tinned copper vessel, gently warmed in a water-bath and a small quantity of anhydrous sodium sulphate was added. The contents of the vessel were shaken vigorously and kept overnight in presence of nitrogen atmosphere. The clear oil was stored in coloured bottles or in iron drums in presence of an inert gas like nitrogen.

Estimation of Vitamin A

The method of Carr and Price (*loc. cit.*), as modified by Coward, *et al.* (*loc. cit.*), was adopted in this investigation for the estimation of vitamin A. A number of dilutions of oil was prepared in dry redistilled chloroform. The intensity of the colour produced when 0·2 ml. of each diluted solution was mixed with 2 ml. of antimony trichloride reagent in a cell was matched with Lovibond tinted glass the colour being matched within 30 seconds. Yellow and red glasses were frequently used to match the colour. The intensity of the blue colour on the Lovibond tintometer scale was so adjusted that it gave blue values between six and eight. Six or more readings were taken in each case and their mean found.

Since there was a change in the colour of the oil obtained from livers of the same species of fish collected at different seasons of the year, it was thought that there might be some change in some of the physical as well as chemical constants of these oils. The different constants of the liver oils mentioned above therefore were determined by standard methods month by month. The results are given in Table I below :

TABLE I

Showing some of the constants of six liver oils determined month by month

Sp. gr. at 30° C.	Acid value	Sap. value	Non-sap. matter	Acetyl value	Iodine value	Blue Units per gm. of the oil	Month of the year
OIL NO. 1. Local name of the fish : Khada mushi or Kondaicha; Scientific name : *Calcarinus melanopterus*							
0·9200	0·622	166	8·452	26·50	128	12084	July (1944)
0·9206	0·610	166	8·634	26·60	127	13666	August ,
0·9200	0·620	169	8·906	27·30	126	17668	September
0·9196	0·610	171	8·984	27·60	126	21654	October
0·9198	0·620	173	8·063	28·20	125	30608	November
0·9201	0·613	166	8·238	26·60	135	10550	December
0·9200	0·686	165	8·240	26·66	133	10584	January (1945)
0·9201	0·623	165	7·986	26·30	137	9166	February
0·9209	0·706	165	7·989	26·32	136	9986	March
0·9190	0·726	166	8·322	26·40	130	11026	April
0·9198	0·646	166	8·323	26·52	129	11676	May
0·9198	0·658	167	8·587	26·64	129	12502	June
Standard Deviation from Normal							
0·0135	0·0522	5·7	0·386	7·58	6·5		

TABLE I (*Continued*)

Sp. gr. at 30° C.	Acid value	Sap. value	Non-sap. matter	Acetyl value	Iodine value	Blue Units per gm. of the oil	Month of the year
OIL NO. 2. Local name of the fish : Waghbeer or Waghsheer; Scientific name : *Galeocerdo figrinus*							
0·906	0·183	178	1·02	13·28	122	3087	July (1945)
0·913	0·192	182	1·05	13·46	121	3208	August
0·908	0·206	183	1·08	13·63	121	3386	September
0·912	0·186	184	1·12	14·86	119	3948	October
0·914	0·193	180	0·96	14·08	121	3269	November
0·912	0·188	177	0·82	13·09	123	2562	December
0·909	0·192	176	0·80	13·02	123	2286	January (1946)
0·910	0·196	176	0·86	13·42	126	2109	February
0·912	0·203	186	1·14	14·83	120	3846	March
0·912	0·198	183	1·04	14·46	121	3586	April
0·910	0·189	178	0·92	14·06	122	2987	May
0·912	0·193	179	0·92	13·82	123	2808	June
Standard Deviation from Normal							
..	..	7·38	0·097	..	6·09		
OIL NO. 3. Local name of the fish : Kan mushi; Scientific name : *Cestracion Blochii*							
0·908	0·186	176	1·178	41·88	132	8068	July (1945)
0·910	0·230	173	1·173	38·42	135	7606	August
0·916	0·220	174	1·178	39·08	134	7783	September
0·910	0·196	176	1·192	40·08	131	9342	October
0·920	0·206	178	1·192	44·86	122	12626	November
0·918	0·284	176	1·186	42·66	124	10862	December
0·910	0·326	175	1·183	40·87	136	9842	January (1946)
0·918	0·348	176	1·189	41·32	125	10686	February
0·920	0·326	178	1·190	41·86	124	11348	March
0·916	0·296	178	1·196	45·60	123	12746	April
0·920	0·280	178	1·206	45·63	123	12926	May
0·920	0·226	177	1·186	42·32	124	11366	June
Standard Deviation from Normal							
..	0·184	11·45	0·169	8·48	..		
OIL NO. 4. Local name of the fish : Nali, Win, Sondel or Khandere; Scientific name : *Pristis Perrotteti*							
0·908	1·862	186	3·68	13·86	114	4368	July (1945)
0·912	1·932	186	3·63	13·69	114	4308	August
0·915	1·676	188	3·75	13·78	115	4560	September
0·906	1·732	191	3·86	14·06	113	5086	October
0·912	1·286	190	3·84	13·16	115	4986	November
0·910	1·326	186	3·82	13·06	118	4063	December
0·915	1·084	186	3·84	12·96	118	4008	January (1946)
0·913	1·006	187	3·36	13·00	120	3986	February
0·908	1·086	188	3·80	13·68	118	4026	March
0·910	1·832	190	3·86	13·96	116	4580	April
0·913	1·892	190	3·78	14·11	115	4830	May
0·909	1·383	188	3·76	13·84	113	4683	June
Standard Deviation from Normal							
..	0·357	..	0·276	..	18·72		

TABLE I—(*Continued*)

Sp. gr. at 30° C.	Acid value	Sap. value	Non-sap. matter	Acetyl value	Iodine value	Blue Units per gm. of the oil	Month of the year
			OIL NO. 5. Local name of the fish : Wagli; Scientific name : *Rhinoptera Javanica*				
0·912	0·680	183	2·48	22·98	117	1886	July (1945)
0·913	0·628	179	2·39	20·04	120	986	August
0·906	0·726	178	2·36	19·76	121	664	September
0·915	0·770	180	2·41	20·16	120	1096	October
0·912	0·655	181	2·50	22·86	118	1626	November
0·912	0·630	181	2·45	23·04	117	1728	December
0·915	0·710	183	2·45	22·06	118	1806	January (1946)
0·915	0·686	185	2·59	22·86	117	1988	February
0·914	0·686	186	2·72	23·42	116	2506	March
0·914	0·721	188	2·80	24·16	116	2626	April
3·913	0·658	186	2·68	22·06	116	2510	May
0·914	0·662	184	2·56	23·22	117	1968	June
			Standard Deviation from Normal				
..	0·658	..	0·2336	1·47	..		
			OIL NO. 6. Local name of the fish : Surmai; Scientific name : *Cybium Kuhlii*				
0·912	1·263	172	2·45	22·04	127	1347	July (1945)
0·909	0·982	174	2·48	22·18	126	1486	August
0·910	1·246	178	2·80	23·08	124	1792	September
0·908	0·962	178	3·10	23·86	123	1856	October
0·911	0·880	176	2·76	22·40	125	1646	November
0·912	0·906	174	2·54	21·86	126	1436	December
0·912	1·002	173	2·20	20·09	127	1208	January (1946)
0·910	1·182	172	2·12	19·78	128	1106	February
0·910	1·162	166	1·96	17·62	130	988	March
0·912	0·908	172	2·28	21·32	126	1289	April
0·908	1·020	174	2·66	22·48	125	1546	May
0·910	1·012	173	2·52	22·14	127	1354	June
			Standard Deviation from Normal				
..	0·140	..	0·314	2·16	8·28		

DISCUSSION

Slight variation in specific gravity and marked variations in acid value, saponification value, non-saponifiable matter and acetyl value can be observed from the six oils studied (refer Table I above) but in the case of the oils from the same fish studied month by month no such marked variations were observed. In the case of iodine value also it can be seen that there is variation not only in all the six varieties of the oils studied but also with individual oils studied month by month.

No relation was observed between the colour of the oils and their different physical and chemical constants; reference to this observation will be made with quantitative data in Part II. Evers and Smith (1933) and Holmes, *et al.* (1941), state that vitamin A content of liver oils cannot be deduced with any degree of exactitude from mere knowledge of physical and chemical properties. Ganpule and Sarangdhar (1943) working on some of the chemical and physical constants of most of the commercially important sharks landed along the Bombay coast, have come to the conclusion that the physical and chemical constants by themselves furnish no clue whatsoever to the relative vitamin A potency of the respective oils.

From a critical study of the Table I above, in the majority of cases whether in the individual oils studied month by month or in all the six liver oils studied, the following may be observed: An increase in vitamin A content is followed by (*a*) an increase in saponification value, in non-saponifiable matter and in acetyl value, (*b*) no marked change in specific gravity and in acid values and (*c*) decrease in iodine number. The view that the increase in vitamin A is accompanied by decrease in iodine value has been recorded by Molteno and Raspon (1939) while working on seasonal variations in the vitamin A content of certain viscera of the Geelbek. They state that the iodine value varies inversely with the percentage of vitamin A in the oil.

The statements made above will be valid only when these results are found significant when they are treated statistically. Therefore the results were treated statistically and are given below in Table II below :

TABLE II

Constants	Number of the oil					
	1	2	3	4	5	6
	Standard Deviation from the Normal					
1 Specific gravity ..	0·01352	..	..	..	..	..
2 Acid value ..	0·05220	..	0·184	0·357	0·0658	0·140
3 Saponification value ..	5·71480	7·380	11·450	..	..	..
4 Non-saponifiable matter ..	0·38860	0·097	0·169	0·276	0·2336	0·314
5 Acetyl value ..	7·58800	..	8·480	..	1·4770	2·164
6 Iodine value ..	6·51000	6·090	..	18·720	..	8·280

From the above table it was deduced that some constants were found significant and others are not significant. These are shown below in Table III.

TABLE III

Constants		Number of the oil					
		1	2	3	4	5	6
		Statistical treatment of the above result					
1	Specific gravity ..	sig.	..	..	..	..	..
2	Acid value ..	sig.	..	not sig.	sig.	sig.	not sig.
3	Saponification value ..	sig.	sig.	sig.	..	..	..
4	Non-sap. matter ..	sig.	sig.	sig.	sig.	sig.	sig.
5	Acetyl value ..	sig.	..	sig.	..	sig.	sig.
6	Iodine value ..	sig.	sig.	..	sig.	..	sig.

N.B.—The formula used for treating the results statistically was that used by G. W. Snedecor in his book *Statistical Methods*. It was considered that the observed value was significant, if the observed value of t $\left(\text{i.e., } \bar{x}\Big/\frac{\sigma}{n}\right)$ is greater than the theoretical value of t (read on 't' (table) for $(n-1)$ degrees of freedom by 2% (*i.e.*, 2·178).

Besides an attempt has been made to correlate the vitamin A content with the different physical and chemical constants of the oils studied. The vitamin A content was found to be correlated with the logarithm of the (*a*) sap. value, (*b*) non-sap. matter, (*c*) acetyl value and (*d*) iodine value in the case of all the oils studied. Whereas in the case of Surmai relation could be traced between vitamin A content and logarithm of iodine value, saponification value and non-saponifiable matter only. No definite relation existed between vitamin A content and logarithm of specific gravity of oil except in the case of Nali. When these results (*i.e.*, vitamin A in Blue Units in thousands per gram against the logarithm of the different physical and chemical constants of all the liver oils studied) were plotted on a graph paper, a straight line passing through the maximum number of points was obtained. Graphs I to IV have been reproduced as specimen figures. The relationship may be expressed as follows:

Vitamin A (B.U. in thousand per gm. of oil) .. $= \text{K} \log \text{P} + \text{C}.$

Where K and C are constants and P is the physical or chemical constant of the oil.

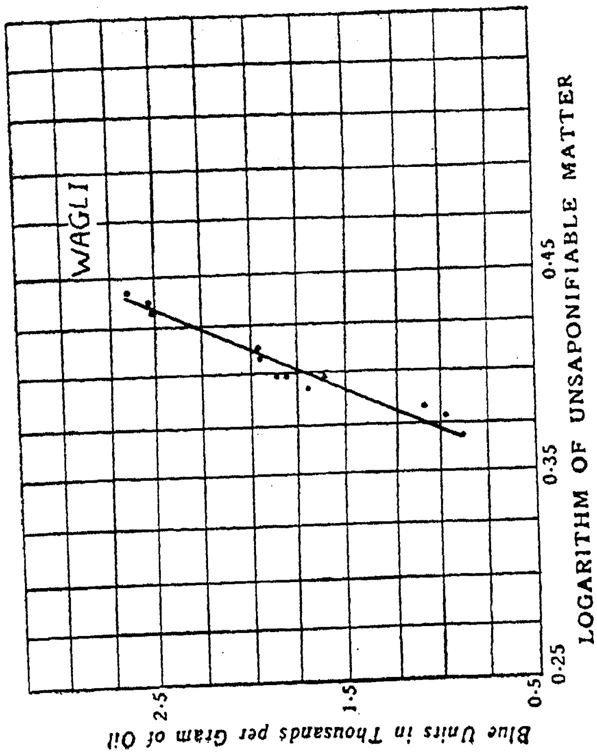

FIG. 3

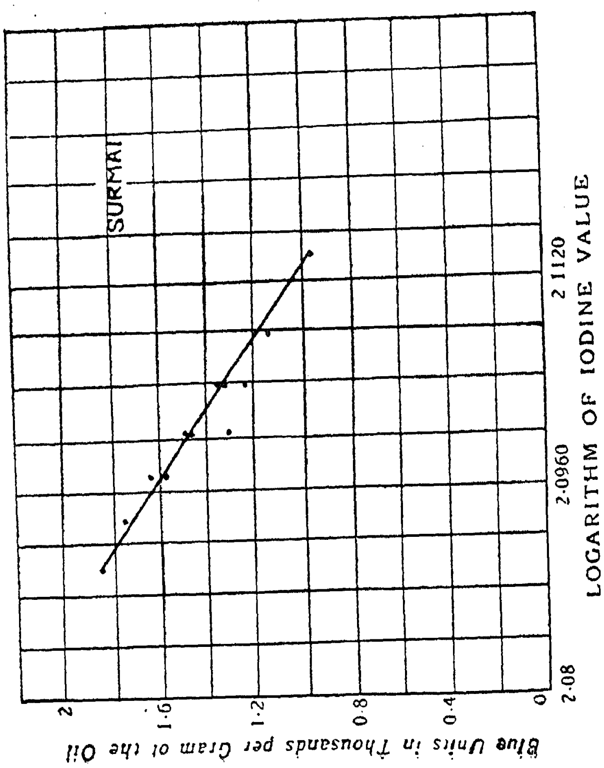

FIG. 4

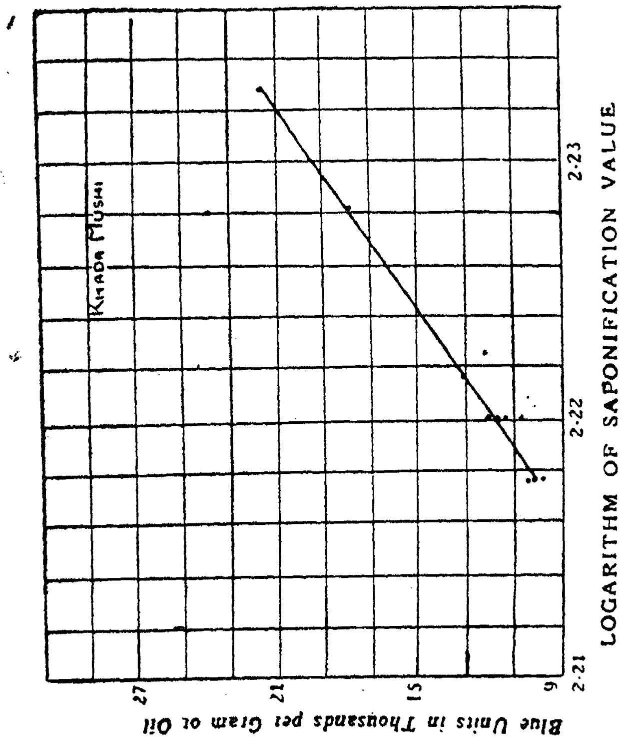

FIG. 1

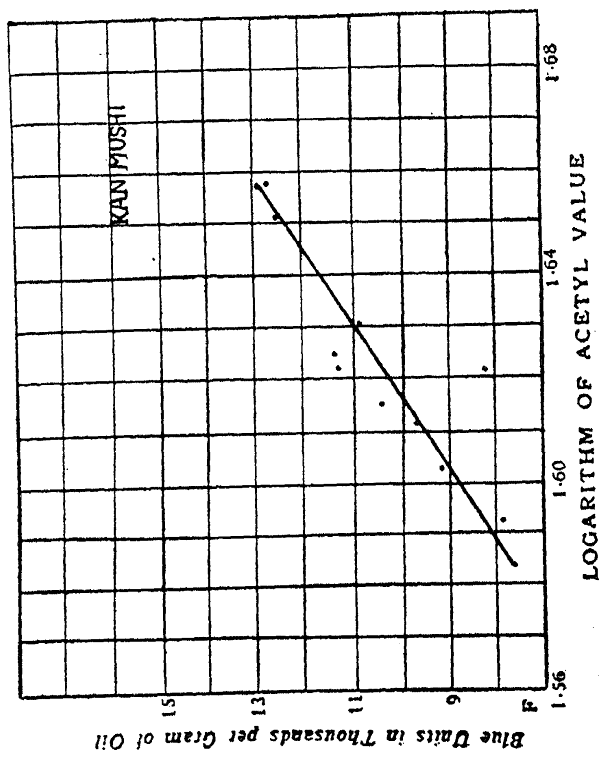

FIG. 2

For all the six liver oils studied the expressions obtained for different constants are given below :

Oil No. 1

Acid value ..	Vit. A (in B.U./gm. of oil)	$= 66\cdot6 \log P - 0\cdot79$
Sap. value ..	Vit. A (,,)	$= 160 \log P - 0\cdot44$
Non-sap. matter	Vit. A (,,)	$= 125 \log P - 0\cdot1$
Acetyl value ..	Vit. A (,,)	$= 600 \log P + 0\cdot6$
Iodine value ..	Vit. A (,,)	$= -136\cdot36 \log P + 6\cdot13$

Oil No. 2

Acid value ..	Vit. A (,,)	$= -4\cdot28 \log P + 1\cdot52$
Sap. value ..	Vit. A (,,)	$= 60\cdot97 \log P + 0\cdot77$
Iodine value ..	Vit. A (,,)	$= 29\cdot43 \log P + 0\cdot23$
Acetyl value ..	Vit. A (,,)	$= -86\cdot2 \log P + 1\cdot94$.

Oil No. 3

Saponification value	Vit. A (,,)	$= 428\cdot6 \log P - 3\cdot05$
Non-sap. matter	Vit. A (,,)	$= 600 \log P - 2$
Acetyl value ..	Vit. A (,,)	$= 71\cdot4 \log P - 0\cdot99$
Iodine value ..	Vit. A (,,)	$= -129 \log P + 8\cdot5$

Oil No. 4

Non-sap. matter	Vit. A (,,)	$= 26\cdot09 \log P - 0\cdot96$
Specific gravity ..	Vit. A (,,)	$= 235\cdot3 \log P - 0\cdot54$
Acetyl value ..	Vit. A (,,)	$= 15\cdot4 \log P + 0\cdot36$

Oil No. 5

Saponification value	Vit. A (,,)	$= 83\cdot3 \log P - 0\cdot49$
Non-sap. matter	Vit. A (,,)	$= 21\cdot43 \log P - 2\cdot07$
Acetyl value ..	Vit. A (,,)	$= 10 \log P + 0\cdot6$
Iodine value ..	Vit. A (,,)	$= -105\cdot26 \log P + 2\cdot55$

Oil No. 6

Saponification value	Vit. A (,,)	$= 35\cdot3 \log P + 0\cdot41$
Non-sap. matter	Vit. A (,,)	$= 4\cdot7 \log P + 1\cdot05$
Iodine value ..	Vit. A (,,)	$= -37\cdot5 \log P + 2\cdot23$

Summary and Conclusion

With a view to finding out whether there exists any relationship among the colour of the oil and its different physical and chemical constants and vitamin A content, liver oils were obtained from six varieties of fish available in the Bombay coastal waters. These fish yielded a large amount of oil

with high vitamin A content. The oil was extracted and analysed month by month and the different physical and chemical constants of the oil along with vitamin A content were investigated. It is found from the table that (1) there is no relation between the colour of the oil and its different constants. (2) An increase in vitamin A content is followed by (*a*) an increase in the saponification value, in non-saponifiable matter and in acetyl value, (*b*) no marked change in specific gravity and in acid values and (*c*) decrease in iodine number. When these results were treated statistically, only the alterations in acid values of Waghbeer and Surmai were found statistically not significant.

An attempt to obtain an expression to correlate the vitamin A content in B.U. and the logarithm of the physical and chemical constants of the liver oils studied has been made. The expression obtained being:

Vitamin A (in Blue Units per gm. of oil) $= K \log P + C$, where K and C are constants and P is the physical or the chemical constant. Values for K and C are different for the different oils studied.

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