We have not been successful in identifying the substance in liver and other tissue homogenates responsible for the oxidizability of glutamic acid by acid $KMnO_4$. The following substances added to a pure solution of glutamic acid had no effect on the oxidizability: $CuSO_4$, $FeSO_4$, $MnSO_4$, haemin, the substances of rabbit bile, the amino-acids contained in casein hydrolysate.

In view of the widespread occurrence of glutamic acid in biological material the direct treatment of deproteinized solutions with permanganate must be deprecated. We separate α-ketoglutaric from glutamic acid by extraction of the strongly acidified solution with ethyl ether, using a continuous extractor of the Kutscher-Steudel type with the dimensions described by Krebs, Smyth & Evans (1940). The deproteinized filtrate is acidified with 0·1 vol. of 50% (v/v) H_2SO_4. The rate of extraction is illustrated by the following data. When 5·84 mg. of α-ketoglutaric acid, dissolved in 15 ml. of 2N-H_2SO_4 were extracted, 71% appeared in the extract within 15 min., 90% within 30 min., 95% within 60 min. These figures depend of course on many factors, especially the design of the apparatus and the rate at which ether passes through the solution; it is therefore necessary to check the rate for each set up. In order to have a safety margin we extract for 2 hr. The ether is then evaporated and the residue is dissolved in a few ml. of water. It is usually divided into 2 parts; in one succinate is determined directly, whilst the other is acidified and treated with an excess of $KMnO_4$ as described by Krebs & Eggleston (1945).

This procedure eliminates interference by glutamic acid, glutamine and arginine, but if α-hydroxyglutarate is present the dinitrophenylhydrazone method (Krebs, 1938) remains the method of choice.

SUMMARY

1. Succinic dehydrogenase is inhibited by phospho-18-tungstate and phospho-24-tungstate, presumably because these reagents oxidize the SH groups of the enzyme.

2. Glutamic acid, whilst stable in the presence of potassium permanganate in pure solution, is oxidized by this reagent to succinic acid in the presence of certain deproteinized tissue extracts.

3. The above reactions may cause errors in the manometric determination of succinate and α-ketoglutarate. Procedures are described which avoid such errors.

REFERENCES

Folin, O. & Marenzi, A. D. (1929). *J. biol. Chem.* **83**, 103.
Hopkins, F. G. & Morgan, E. J. (1938). *Biochem. J.* **32**, 611.
Hopkins, F. G., Morgan, E. J. & Lutwak-Mann, C. (1938). *Biochem. J.* **32**, 1829.
Krebs, H. A. (1937). *Biochem. J.* **31**, 2095.
Krebs, H. A. (1938). *Biochem. J.* **32**, 108.
Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
Krebs, H. A. & Eggleston, L. V. (1945). *Biochem. J.* **39**, 408.
Krebs, H. A., Smyth, D. H. & Evans, E. A. Jr. (1940). *Biochem. J.* **34**, 1041.
Schöberl, A. & Ludwig, E. (1937). *Ber. dtsch. chem. Ges.* **70**, 1422.
Schöberl, A. & Rambacher, P. (1938). *Biochem. Z.* **295**, 377.
Szent-Györgyi, A. & Gözsy, B. (1935). *Hoppe-Seyl. Z.* **236**, 1.
Wu, H. (1920). *J. biol. Chem.* **43**, 195.

New Zealand Fish Oils

5. COMPOSITION OF THE FATS OF THE SCHOOL SHARK (*GALEORHINUS AUSTRALIS*, MACLEAY)

By A. P. OLIVER, *Dominion Laboratory* and F. B. SHORLAND, *Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand*

(*Received* 11 *November* 1947)

The school or snapper shark (*Galeorhinus australis*) occurs in both New Zealand and Australian waters and, in common with related species found elsewhere, such as the North West Pacific soupfin shark (*G. zyopterus*) (Byers, 1940), the South American tollo (*G. mento*) (Pfister, 1936*a*, *b*) and the South African soupfin shark (*G. canis* Rond) (Molteno, Rapson, Roux, Schwartz & van Rensburg, 1945) is an important source of vitamin A-rich liver oil (cf. Davies & Field, 1937; Jowett & Davies, 1938; Cunningham & Slater, 1939). Little, however, is known concerning the composition of oils from these species.

In most work on the fatty acid composition of fish oils from a given species, one sample only has been used so that there is no information as to the extent of the variation between individuals. This may be

large, as in New Zealand groper (*Polyprion oxygeneios*) liver oil (Shorland & Hilditch, 1938), or small as in New Zealand ling (*Genypterus blacodes*) liver oil (Shorland, 1939). In this work, therefore, the fats from eight specimens of school shark selected at random from one day's catch in Cook Strait on 16 February 1944 have been studied separately.

EXPERIMENTAL AND RESULTS

Information about the specimens used in this study is given in Table 1.

The sharks were divided by a transverse cut at the posterior gill slit into head, body, liver and rest of viscera. Each of the tissues was treated with twice its weight of 95% ethanol, held at the boiling point for 15 min. and

Table 1. *Details of school sharks used in this investigation*

Sample (no.)	Locality	Length (cm.)	Weight (kg.)	Weight of liver (g.)	Sexual condition
1	Karori Rock (10 fathoms)	164	15·43	568	♀, commencing development
2	Panario patch (60–80 fathoms)	149	13·63	822	♂, undeveloped
3	„ „	123	6·80	367	♀, undeveloped
4	„ „	136	10·90	908	♀, undeveloped
5	„ „	132	9·99	740	♀, undeveloped
6	Island Bay bank (60–80 fathoms)	151	24·95	1250	♂, commencing development
7	„ „	144	12·27	568	♀, commencing development
8	„ „	138	11·82	1080	♀, commencing development

Table 2. *Proportions of tissues and distribution of fats in New Zealand school shark*

	Head			Body			Viscera other than liver			Liver			
		Oil			Oil			Oil			Oil		
Sample (no.)	% total weight	(%)	% total in fish	% total weight	(%)	% total in fish	% total weight	(%)	% total in fish	% total weight	(%)	% total in fish	% oil in shark
1	21·3	0·2	3·3	67·7	0·5	26·5	7·3	0·6	3·4	3·7	23·1	66·8	1·3
2	21·6	0·2	1·1	66·1	0·5	8·7	6·3	1·0	1·6	6·0	55·9	88·6	3·8
3	21·6	0·3	3·2	66·3	0·5	16·3	6·7	0·6	2·0	5·4	29·6	78·5	2·0
4	17·7	0·1	0·4	69·0	0·5	7·2	5·0	0·7	0·7	8·3	52·7	91·7	4·8
5	18·2	0·4	1·5	69·3	0·7	9·9	5·1	1·2	1·3	7·4	57·6	87·3	4·9
6	10·7	0·1	0·3	78·4	0·4	10·6	5·9	0·8	1·6	5·0	51·8	87·5	3·0
7	23·2	0·2	2·3	66·2	0·3	9·8	6·0	0·9	2·6	4·6	37·6	85·3	2·0
8	19·7	0·2	0·7	65·7	0·5	5·5	5·5	0·9	0·8	9·1	60·7	93·0	5·8
South African soupfin shark	15·3*	0·48	3·5	78·1	0·71	26·8	1·7†	0·7	0·6	4·9	29·2	69·1	2·1

* Excluding foetuses. Recalculated from data given by Molteno *et al.* (1945).
† Intestine only.

Table 3. *Characteristics of New Zealand school shark fats*

	Liver				Body		Head
Sample (no.)	Vitamin A* $\left(\frac{(E^{1\%}_{1\,cm.}\ 328\ m\mu.)}{16}\right)$ (%)	Unsaponifiable matter (%)	Saponification equivalent	Iodine value (Wijs 1 hr.)	Saponification equivalent	Iodine value (Wijs 1 hr.)	Iodine value (Wijs 1 hr.)
1	14·6	31·4	410·0	185·0	366·5	125	112
2	3·6	8·1	317·1	178·0	346·5	146	140
3	0·6	4·5	309·5	183·9	340·5	154	118
4	0·1	2·5	306·5	185·9	328·0	136	103
5	0·4	4·0	307·7	176·8	326·5	136	109
6	2·2	6·4	319·6	170·7	354·0	153	116
7	1·9	8·9	321·0	149·0	301·5	119	137
8	0·6	3·3	308·0	171·7	327·0	127	129

* Spot tests with the Carr-Price reagent showed that some of the body and visceral oils were somewhat richer in vitamin A than cod-liver oil but no positive tests were given by the head oils.

then dried *in vacuo* at a temperature not exceeding 50°. The dried tissues were minced and extracted continuously with light petroleum, b.p. 50–70°, until on replacement with fresh solvent no further lipid was extracted. The final stages of extraction were facilitated by the addition of 5% absolute ethanol to the light petroleum, the ethanolic layer being subsequently withdrawn, evaporated *in vacuo*, re-extracted with light petroleum, and added to the main extract from which the petroleum was distilled, the last traces being removed *in vacuo* on the steam bath.

The proportions of tissues and distribution of fat in the fish are given in Table 2 and the characteristics of liver, body and head fat in Table 3.

As it was not feasible to complete the ester-fractionation analyses of all the samples, a selection was made of the liver oils, while the head and body oils were combined to provide sufficient material for analysis. The head and body fats were further separated into phosphatide and glyceride fractions (cf. Shorland & Hilditch, 1938) yielding 63·6% phosphatide (P = 3·7%) and 36·4% glyceride (P = 0·7%). Qualitative tests showed that the liver oils contained only minor proportions of phosphatide; no separation was therefore made.

The fatty acid composition was determined by the method described by Hilditch (1941) and modified by Shorland & de la Mare (1944). For the purpose of indicating the course of the distillation of the methyl esters the results for school shark liver oil (no. 7) are set out in Table 4 as typical of the general results obtained. The component fatty acids of the fat are listed in Table 5.

DISCUSSION

Rapson, Schwartz & van Rensburg (1945) consider that the composition of the liver fats of marine teleostean fish is largely determined by the extent to which the liver is used as a site for fat storage. They found, in general, that the liver fats of fishes with diffuse oil systems had lower iodine values than the corresponding head, body or intestinal oils, while in the case of fishes with the fat storage localized in the liver the fat from this organ had an iodine value approximating to that of the corresponding body, head and intestinal oil. In the present work it is shown that in common with that of other elasmobranch fish the liver of the school shark is the main site of fat storage (cf. Table 2). Consideration of the iodine values of the liver fats (Table 3) shows that they exceed those of the head and body. Such comparisons of iodine values are complicated by the presence of unsaponifiable matter and by the fact that the head and body lipids are present to a considerable extent as phosphatides. With the methyl esters of the fatty acids, excluding unsaponifiable matter, as basis for comparison, liver oil samples nos. 1, 2, 6 and 7 showed iodine values of 137·3, 152·8, 152·4 and 134·7, respectively, as compared with values of 174·5 and 162·8 for the respective phosphatide and glyceride fractions of the combined head and body oils. The higher iodine value of the head and body oil fatty acids, as

Table 4. *Fractionation of methyl esters of acids from liver fat of school shark no.* 7

	Methyl esters of 'liquid' acids (weight fractionated 39·5 g.)				Methyl esters of 'solid' acids (weight fractionated 11·7 g.)		
No.	(g.)	Saponification equivalent	Iodine value	No.	(g.)	Saponification equivalent	Iodine value
L_1	1·305	246·8	40·1	S_1	0·940	262·9	4·1
L_2	2·929	270·8	78·9	S_2	1·528	255·6	2·1
L_3	3·894	291·8	92·8	S_3	2·647	269·0	2·9
L_4	2·496	295·4	95·7	S_4	2·605	272·0	3·7
L_5	6·549	296·6	99·1	S_5	2·748	297·6	25·4
L_6	4·087	315·0	168·8	S_6	1·088	366·6	61·7
L_7	2·970	322·9	169·6				
L_8	2·953	334·4	257·2				
L_9	2·502	341·6	299·4				
L_{10}	3·241	344·0	308·8				
L_{11}	1·607	347·2	273·7				
L_{12}	1·653	359·3	212·6				
L_{13}	2·971	365·9	183·4				
Total weight of fractions	39·157			Total weight of fractions	11·556		

'Liquid' esters excluding unsaponifiable matter:
L_{12} saponification equivalent 348·2; iodine value 204·0
L_{13} saponification equivalent 359·1; iodine value 177·0

'Solid' esters excluding unsaponifiable matter:
S_6 saponification equivalent 351·6; iodine value 79·2*

* The relatively high iodine value after resaponification is attributed to the removal of traces of residual phosphatides (cf. Hilditch & Shorland, 1937) which would tend to lower the iodine value in the original fraction.

Table 5. *Component fatty acids of the fats of the school shark*

(*a*) Weight percentages

Liver oil sample	Saturated acids C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	Unsaturated acids C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
1	1·3	17·1	6·5	1·5	—	0·6 (−2·0 H)	5·4 (−2·0 H)	25·4 (−2·6 H)	20·7 (−4·5 H)	21·5 (−7·6 H)	—
2	1·7	15·3	3·4	1·1	—	1·2 (−2·0 H)	5·9 (−2·0 H)	26·6 (−2·4 H)	19·5 (−5·8 H)	25·3 (−9·6 H)	—
6	2·4	15·2	3·6	—	—	1·1 (−2·0 H)	6·2 (−2·0 H)	31·7 (−2·4 H)	20·2 (−5·4 H)	19·6 (−8·8 H)	—
7	3·9	16·7	5·3	0·1	1·0	0·7 (−2·0 H)	5·3 (−2·0 H)	26·5 (−2·3 H)	15·5 (−4·5 H)	22·8 (−6·5 H)	2·2 (−4·0 H)
Head and body glycerides	0·2	7·8	19·0	5·2	3·4	0·2 (−2·0 H)	5·2 (−2·0 H)	13·5 (−2·2 H)	22·8 (−6·1 H)	22·7 (−12·0 H)	—
Head and body phosphatides	0·3	9·9	13·3	0·3	1·1	1·1 (−2·0 H)	4·3 (−2·0 H)	22·8 (−2·3 H)	16·1 (−6·5 H)	30·8 (−10·0 H)	—

(*b*) Molecular percentages

1	1·7	19·4	6·6	1·4	—	0·7 (−2·0 H)	6·2 (−2·0 H)	26·3 (−2·6 H)	19·6 (−4·5 H)	18·1 (−7·6 H)	—
2	2·2	17·3	3·5	1·0	—	1·5 (−2·0 H)	6·7 (−2·0 H)	27·3 (−2·4 H)	18·4 (−5·8 H)	22·1 (−9·6 H)	—
6	3·1	17·0	3·7	—	—	1·4 (−2·0 H)	6·9 (−2·0 H)	32·2 (−2·4 H)	18·8 (−5·4 H)	16·9 (−8·8 H)	—
7	4·9	18·8	5·4	0·1	0·8	0·9 (−2·0 H)	6·0 (−2·0 H)	27·1 (−2·3 H)	14·5 (−4·5 H)	19·7 (−6·5 H)	1·8 (−4·0 H)
Head and body glycerides	0·3	9·0	19·8	4·9	3·0	0·2 (−2·0 H)	6·0 (−2·0 H)	14·2 (−2·2 H)	22·1 (−6·1 H)	20·5 (−12·0 H)	—
Head and body phosphatides	0·5	11·4	13·7	0·3	1·0	1·4 (−2·0 H)	5·0 (−2·0 H)	23·8 (−2·3 H)	15·5 (−6·5 H)	27·4 (−10·0 H)	—

The quantities in brackets are the values of the mean unsaturation, thus (−4·0 H) indicates an average unsaturation corresponding with two double bonds, but does not necessarily imply the presence only of a diethylenic acid.

Table 6. *Fatty acid composition of school shark liver oil no. 2 before and after hydrogenation*

	Total groups of acids (mol. %) C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Before hydrogenation	3·7	24·0	30·8	19·4	22·1	—
After hydrogenation	2·8	24·3	33·4	16·7	22·5	0·3
Difference	+0·9	−0·3	−2·6	+2·7	−0·4	−0·3

compared with those of the liver, is in agreement with the views put forward by Rapson *et al.* (1945).

The variation in fatty acid composition between the liver oils (cf. Table 5) reaches a maximum difference in the C_{18} unsaturated acids, where the difference between samples 6 and 1 amounts to 6·3 units weight %. Other similar differences are shown in the C_{20} unsaturated acids, sample 1 containing 5·2 units % more than sample 7, while in the case of the C_{22} unsaturated acids sample 2 is shown to contain 5·7 units % more than sample 6. In assessing differences of this order it is relevant to consider the accuracy of the ester-fractionation method.

Comparison of the original oil with the fully hydrogenated sample (Table 6), shows that by this method in two consecutive analyses differences of up to 2·7 units % may be expected. Using similar comparisons, Hilditch & Houlbrooke (1928), Shorland (1939) and van Rensburg, Rapson & Schwartz (1945) found respectively, differences of up to 1·7, 1·7 and 2·5 units %. Similarly Lovern (1942) found, in duplicate analyses of cod-liver oil, differences between the same fatty acid groups of up to 1·8 units %. By far the most exhaustive and comprehensive analyses carried out on oils, hydrogenated to different iodine values and prepared from the same sample, are those by Hilditch & Terleski (1937) on whale oil and by Harper & Hilditch (1937) on cod-liver oil.

As claimed by these workers (Harper, Hilditch & Terleski, 1937) the final results for the component fatty acids are not necessarily accurate to more than 2 or 3 units % in the case of the higher complex

unsaturated acids. Our interpretation of their claim is that this degree of accuracy has reference to the extent of the deviation from the mean value, so that, when considered from the point of view of the differences likely to be encountered between similar homologous groups of acids in two consecutive analyses, the expected differences are of the order of up to 6 units %. Using the results of the above investigators, the standard deviations of each homologous group of acids have been calculated (Table 7).

Table 7. *Standard deviation of ester fractionation analyses calculated from the data of Hilditch & Terleski* (1937) *and of Harper & Hilditch* (1937)

	Standard deviation from mean of homologous groups of acids (mol. %)					
	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Whale oil (6 analyses)	±0·83	±1·54	±1·65	±1·14	±0·75	—
Cod-liver oil (8 analyses)	±0·62	±1·07	±1·04	±1·81	±1·19	±0·39

It is generally considered that, for the results of a single analysis on two different oils to be significant, their difference, for a first approximation, should differ by more than three times the standard deviation of a single observation. In the present series of analyses, because of the relatively small weight of sample used (*c.* 50 g.), it was not expected that the accuracy would be greater than that obtained in the case of the cod-liver oil and whale oil analyses, where larger weights were taken and the fractionation more detailed. However, because of the use in the present work of a more efficient fractionating column as described by Longenecker (1937), the relatively small weight of oil taken for analysis is compensated to some extent by the relatively greater degree of separation of the esters, as compared with that obtained with the Willstätter bulb used in the cod-liver oil and whale oil analyses. It thus seems that the larger differences in the component fatty acids of the school shark liver oils referred to above are possibly significant, but no conclusions regarding the smaller differences appear to be justified.

Comparing the fatty acid composition of the head and body lipids with that of the liver oil (Table 5), it will be seen that the former have appreciably less C_{18} unsaturated acids and palmitic acid but more stearic acid. Attention should also be drawn to the high mean unsaturation of the C_{20} and C_{22} unsaturated acids of the head and body lipids as compared with those of the liver. The differences normally observed between the fatty acid composition of corresponding phosphatides and glycerides (cf. Hilditch & Shorland, 1937), including increased proportions of stearic acid, and C_{20} and C_{22} unsaturated acids in the former as compared with the latter, are not shown in this work.

van Rensburg *et al.* (1945) extended their earlier view that in fishes with diffuse systems of fat storage there is a direct relationship between the iodine values of the head and body oils and the oil contents of these organs, and put forward the hypothesis that these variations correspond to preferential deposition and utilization of C_{20} and C_{22} unsaturated acids, as well as changes in the degree of unsaturation of the acids when the fish are in fat and thin condition respectively. They suggested further that the same may be true of the liver oils of fish where, as in the present case, the liver is the main site of fat storage. In this connexion they showed that, by comparing the fatty acid composition of the liver oils of *Merluccius merluccius* (50% oil in liver) with that of *M. capensis* (28% oil in liver) and *M. gayi* (23% oil in liver), there was a decrease in the amount of C_{20} and C_{22} unsaturated acids from 45·0 to 31·7% with decreasing oil content.

In the present work the oil content of the liver of the school shark has been shown to vary (Table 2) from 23·1% (sample 1) to 55·9% (sample 2), without any marked effect on the fatty acid composition, suggesting that in the school shark it is not a major factor in determining fatty acid composition. It may also be shown (Table 8) that, in other fish where the liver is the main fat depot and the distribution and amount of oil are similar to that of the school shark, the fatty acid composition differs considerably from that of the latter.

The New Zealand ling and school shark referred to in Table 8 have been taken from similar localities in Cook Strait, but the former is distinguished from the latter by containing highly significantly more C_{18} and C_{20} unsaturated acids and highly significantly less C_{22} unsaturated acids in the liver oil. Thus, although the mode and extent of fat storage in the liver have been shown (cf. Rapson *et al.* 1945) to be associated with wide differences in fatty acid composition, for such fish as the groper and the *Merluccius* species (van Rensburg *et al.* 1945) these factors have not been found important in the school shark. The present comparison of the New Zealand school shark with the similar if not identical Australian species, and with the ling, suggests that in some cases the species factor is of primary importance in determining the fatty acid composition of the liver oil.

It is quite possible that the factors which determine the fatty acid composition of the liver oils of

Table 8. *Comparison between the fatty acid composition of the liver oil of the school shark and of New Zealand ling**

	Fatty acids (% w/w)										Liver oil expressed as percentage of total oil in fish	Oil in fish (%)
	Saturated				Unsaturated							
	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	
New Zealand school shark: Mean value	2·3	16·1	4·7	0·7	0·2	0·9 (−2·0 H)	5·7 (−2·0 H)	27·6 (−2·3 to −2·6 H)	19·0 (−4·5 to −5·8 H)	22·3 (−6·5 to −9·6 H)	0·5 (−4·0 H)	69–89	1·3–3·8
Standard error (4 samples)	±0·55	±0·45	±0·75	±0·40	±0·25	±0·15	±0·20	±1·4	±1·1	±1·3	±0·55		
Australian school shark	2·6	18·7	5·1	0·1	—	0·3 (−2·0 H)	8·4 (−2·0 H)	27·2 (−2·4 H)	17·8 (−6·6 H)	19·8 (−12·0 H)	—		
New Zealand ling (Shorland, 1939): Mean value	1·8	16·4	2·5	—	—	—	7·2 (−2·0 H)	36·1 (−2·1 to −2·6 H)	24·3 (−4·9 to −5·7 H)	11·7 (−6·9 to −9·7 H)	—	*c.* 99	2·9
Standard error (7 samples)	±0·17	±0·30	±0·31	—	—	—	±0·47	±0·64	±0·50	±0·85			

* Shorland (1939).

elasmobranchii differ considerably from those operating in teleostean species. As shown by Hilditch (1941) the former have a much greater range of variation in the nature of the unsaturated acids, which vary from predominantly monoethenoid types to types which are even more unsaturated than those normally encountered in teleostean species. These facts, together with other remarkable differences in the amount and nature of the unsaponifiable matter in the liver oils of elasmobranchii, tend further to emphasize the importance of the species factor in determining the fatty acid composition of this group. Lovern (1942) has classified elasmobranch liver oils into four main groups on the basis of the nature and amount of unsaponifiable matter and the fatty acid composition. Consideration of the school shark-liver oil shows that the higher unsaturated acids are largely polyethenoid, and the fatty acid composition is not very different from that of the average marine fish oil. In this respect the school shark is shown to conform to Lovern's first group from which, however, it must be distinguished by the presence of much more unsaponifiable matter (2·5–31·4%) as compared with usually not more than 1 or 2% considered typical of that group.

SUMMARY

1. Studies of the distribution of oil in eight specimens of school shark (*Galeorhinus australis*) showed that the liver, which varied in oil content from 23·1 to 60·7%, contained from 66·8 to 93·0% of the total oil reserves of the fish. Ester-fractionation analyses of four of the liver oils, and of the phosphatide and glyceride fractions of the combined head and body lipids, showed that the liver fatty acids contained a higher content of palmitic acid and of C_{18} unsaturated acids, but less stearic acid than the head and body lipids.

2. In regard to most groups of fatty acids the liver oils showed great similarity. The wider differences in content of C_{18}, C_{20} and C_{22} unsaturated acids, amounting to as much as 6·3 units % are thought to lie outside the experimental error, but variations in accuracy shown by the results of various investigators are such as not to preclude such differences being due to experimental error.

3. Consideration of the variations in the oil content as a factor in determining the fatty acid composition of *G. australis* liver oil suggest that this fish is not influenced appreciably in this way, and thereby presents a striking contrast to the teleostean species studied by Rapson and collaborators.

The authors wish to thank Mr R. E. R. Grimmett, formerly Chief Agricultural Chemist of the Animal Research Division of the Department of Agriculture, Wellington.

New Zealand, for providing facilities for this work, while the authors were members of that Division. Thanks are also due to Prof. P. W. Robertson, Chemistry Department, Victoria University College, Wellington, New Zealand, for permitting one of the authors (A.P.O.) to carry out part of this work as a thesis for the M.Sc. degree in the University of New Zealand, and to Prof. W. Davies of the University of Melbourne, Australia, who provided a representative sample of Australian school shark-liver oils from a number of recently prepared samples.

REFERENCES

Byers, R. D. (1940). *Calif. Fish. Game*, **26**, 23.
Cunningham, M. M. & Slater, E. C. (1939). *Aust. J. exp. Biol. med. Sci.* **17**, 457.
Davies, W. & Field, D. J. (1937). *Biochem. J.* **31**, 248.
Harper, D. A. & Hilditch, T. P. (1937). *J. Soc. chem. Ind., Lond.*, **56**, 322 T.
Harper, D. A., Hilditch, T. P. & Terleski, J. T. (1937). *J. Soc. chem. Ind., Lond.*, **56**, 310 T.
Hilditch, T. P. (1941). *The Chemical Constitution of Natural Fats*. London: Chapman and Hall.
Hilditch, T. P. & Houlbrooke, A. (1928). *Analyst*, **53**, 246.
Hilditch, T. P. & Shorland, F. B. (1937). *Biochem. J.* **31**, 1499.
Hilditch, T. P. & Terleski, J. T. (1937). *J. Soc. chem. Ind., Lond.*, **56**, 315 T.
Jowett, W. G. & Davies, W. (1938). *Pamphl. Counc. sci. Industr. Res. Aust.* no. 85.
Longenecker, H. E. (1937). *J. Soc. chem. Ind., Lond.*, **56**, 199 T.
Lovern, J. A. (1942). *Spec. Rep. Food Invest. Bd., Lond.*, no. 51.
Molteno, C. J., Rapson, W. S., Roux, E. R., Schwartz, H. M. & van Rensburg, N. J. (1945). *J. Soc. chem. Ind., Lond.*, **64**, 172.
Pfister, A. (1936*a*). *Pharm. Ztg.* **81**, 933.
Pfister, A. (1936*b*). *Chem. Abs.* **30**, 7887.
Rapson, W. S., Schwartz, H. M. & van Rensburg, N. J. (1945). *J. Soc. chem. Ind., Lond.*, **64**, 114.
Shorland, F. B. (1939). *Biochem. J.* **33**, 1935.
Shorland, F. B. & Hilditch, T. P. (1938). *Biochem. J.* **32**, 792.
Shorland, F. B. & de la Mare, P. B. D. (1944). *Analyst*, **69**, 337.
van Rensburg, N. J., Rapson, W. S. & Schwartz, H. M. (1945). *J. Soc. chem. Ind., Lond.*, **64**, 140.

Studies on Suramin (Antrypol, Bayer 205)

6. FURTHER OBSERVATIONS ON THE DETERMINATION OF SURAMIN IN WHOLE BLOOD AND SERUM

By HILARY M. DEWEY and A. WORMALL
Department of Biochemistry and Chemistry, Medical College of St Bartholomew's Hospital, London

(*Received* 1 *December* 1947)

The amount of the trypanocidal drug suramin present in the blood serum or plasma of man, and any other animal which has received an injection of the drug, can readily be determined by a method previously described (Dangerfield, Gaunt & Wormall, 1938; Boursnell, Dangerfield & Wormall, 1939). The technique of this determination has deliberately been made as simple as possible in order that the method might be of use in tropical countries, even in regions where the simplest laboratory facilities are not available. The possibility has always been kept in mind that medical officers in regions well removed from the central hospitals might require blood suramin determinations on some of their treated trypanosomiasis patients. The drug has a very definite prophylactic value, and plasma suramin determinations might be made in future on large groups of people who have been injected with the drug before being exposed to infection.

Serum and plasma from suramin-containing blood contain the same concentration of suramin, and either material serves for the determination of the amount of the drug in the blood. Should the serum or plasma become grossly infected, however, the resulting bacterial changes might produce amines and other compounds which would vitiate the results. Experiments have, therefore, been carried out to determine whether any simple antiseptic can be added to the serum or plasma to preserve it for subsequent suramin determinations without interfering with the colorimetric determination. Experiments have also been carried out to investigate the reliability of suramin determinations on whole blood samples. Although these investigations were primarily designed for the above-mentioned purposes, they have also furnished useful information about the distribution of the blood suramin between the cells and the plasma.