REFERENCES

- Abraham E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A. & Florey, H. W. (1941). *Lancet*, **241**, 177.
- Akasi, S. (1939). *J. Biochem., Tokyo*, **29**, 21.
- Black, M. M. & Schwartz, H. M. (1950). *Analyst*, **75**, 185.
- Blix, G. (1948). *Acta chem. scand.* **2**, 467.
- Bohonos, N., Woolley, D. W. & Peterson, W. H. (1942). *Arch. Biochem.* **1**, 319.
- Brian, P. W. (1949). *Ann. Bot., Lond.*, **13**, 59.
- Brown, F. (1950). *Biochem. J.* **47**, 598.
- Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, 2nd ed. London: Crosby Lockwood.
- Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
- Flood, A. E., Hirst, E. L. & Jones, J. K. N. (1948). *J. chem. Soc.* p. 1679.
- Foster, J. W. (1949). *Chemical Activities of Fungi*. New York: Academic Press.
- Gorgica, H. J., Peterson, W. H. & Steenback, H. (1934). *Biochem. J.* **28**, 504.
- Holden, M., Pirie, N. W. & Tracey, M. V. (1950). *Biochem. J.* **47**, 399.
- Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
- Immers, J. & Vasseur, E. (1950). *Nature, Lond.*, **165**, 898.
- Irving, J. C. (1909). *J. chem. Soc.* p. 564.
- Krüger, D. & Tschirch, E. (1930). *Ber. dtsh. chem. Ges.* **63**, 826.
- Kuttner, T. & Lichtenstein, L. (1932). *J. biol. chem.* **95**, 661.
- Markham, R. (1942). *Biochem. J.* **36**, 790.
- Nelson, N. (1944). *J. biol. chem.* **153**, 375.
- Norman, A. G. & Peterson, W. H. (1932). *Biochem. J.* **26**, 1946.
- Norman, A. G., Peterson, W. H. & Houtz, R. C. (1932). *Biochem. J.* **26**, 1934.
- Olmsted, W. A., Whitaker, W. M. & Duden, C. W. (1929). *J. biol. Chem.* **85**, 109.
- Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
- Phillips, M. (1938). *J. Ass. off. agric. Chem., Wash.*, **21**, 678.
- Pinck, L. A. & Allison, F. E. (1944). *Soil Sci.* **57**, 155.
- Pirie, N. W. (1936). *Brit. J. exp. Path.* **17**, 269.
- Porges, N. (1932). *Bot. Gaz.* **94**, 197.
- Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
- Schulz, G. (1937). *Planta*, **27**, 196.
- Takata, R. (1929). *J. Soc. chem. Ind. Japan*, **32**, 245 B.
- Thom, C. & Phillips, M. (1932). *J. Wash. Acad. Sci.* **22**, 230.
- Tracey, M. V. (1951). *Biochem. J.* **49**, xx.
- Van Slyke, D. D., Dillon, R. T., Macfadyen, D. A. & Hamilton, P. (1941). *J. biol. Chem.* **141**, 671.
- Waksman, S. A. (1938). *Humus*. 2nd ed. London: Baillière, Tindall and Cox.

The Component Acids of the Fats of some Indian Fresh-water Fish

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The fats studied were from the body, viscera and liver of bhakur fish (*Catla buehanani*) and from the liver of pahuna fish (*Wallago attu*). These fish were from the River Ganges at Benares.

METHODS

The separated tissues (body, viscera or liver) were cut up into small pieces and extracted exhaustively with hot acetone. The extracted material was then taken up in light petroleum (b.p. 40–60°), the watery portion separated and the lipids recovered and dried at 100° under reduced pressure. The body and liver tissues were also treated by alkali digestion as recommended by Rapson, Schwartz & Van Rensburg (1943), but little fat could be extracted by this treatment. The fats were kept in 10 vol. of acetone at 0° to separate the phosphatides, if present, from the glycerides.

The fats were hydrolysed, and the mixed fatty acids recovered were resolved into groups of simpler mixtures of acids by the lithium salt-acetone (Tsujimoto, 1920; Tsujimoto & Kimura, 1923) and lead salt-ethanol methods. Each group of the acids was separately converted into methyl

esters, taking precautions recommended by Bjarnason & Meara (1944), and fractionated through an electrically heated and packed column. From the iodine values and equivalents of the ester fractions the composition of the latter were calculated by the method of Hilditch (1947). The mean unsaturation, expressed by the fractional number of hydrogen atoms short of saturation, e.g. –2.0 (monoethenoid), was determined as usual by interpolation or extrapolation from the respective ester fractions in each group from which the mean equivalent of each of the homologous ester groups (C₁₆, C₁₈, C₂₀, C₂₂ and C₂₄) follows. From the composition of each of the separated groups of fatty acids, that of the original fat was obtained.

RESULTS

The lipids and phosphatide contents of the tissues and the iodine values of the fats are given in Table 1.

Bhakur fats. The mixed fatty acids obtained by the hydrolysis of fats, as described earlier, from body (159.6 g.), viscera (202.8 g.) and liver (29.3 g.) were

Table 1. *Components of tissues of bhakur and pahuna*

	Bhakur			Pahuna liver
	Body	Viscera	Liver	
Water content (% on wet tissues)	67.5	40.2	72.7	68.3
Lipid content (% on wet tissues)	6.3	44.8	8.5	5.1
Lipid content (% on dry tissues)	19.0	75.0	31.3	16.1
Phosphatides in lipids (%)	0	0	5.4	12.5
Iodine value of the fat	97.9	87.2	90.5	122.9

Table 2. *Fractionation of tissue fats of bhakur*

Group description	Body		Viscera		Liver	
	Weight (%)	Iodine value	Weight (%)	Iodine value	Weight (%)	Iodine value
A Lead salt ethanol-insoluble	31.8	1.4	36.8	5.3	32.8	22.4
B Lithium salt acetone-insoluble but lead salt ethanol-soluble	47.8	111.8	49.9	105.0	67.2	138.0
C Lithium salt acetone-soluble	20.4	248.2	13.3	237.6	—	—

Table 3. *Fractionation of methyl esters of bhakur body-fat acids A, B and C*

Fraction	Weight (g.)	Saponification equivalent	Iodine value
Methyl esters of acids A			
A1	3.05	254.6	0.2
A2	3.29	265.7	0.4
A3	3.86	268.0	0.6
A4	3.93	270.7	0.6
A5	4.01	271.2	0.6
A6	4.80	271.5	1.1
A7	3.26	272.2	1.1
A8	3.67	274.8	1.6
A9	5.37	284.3	2.4
A10	4.24	289.2*	11.6
	39.48		
Methyl esters of acids B			
B1	2.38	253.7	51.6
B2	3.16	267.1	72.0
B3	4.53	270.0	83.1
B4	4.97	272.7	83.8
B5	5.22	273.3	90.1
B6	3.57	273.8	93.0
B7	4.65	277.6	93.6
B8	4.75	278.0	99.9
B9	2.33	278.5	100.2
B10	5.02	278.7	102.6
B11	3.68	279.4	107.4
B12	4.20	281.9	112.3
B13	4.46	285.9	134.8
B14	2.04	299.6	169.7
B15	4.25	327.3*	183.1
	59.66		
Methyl esters of acids C			
C1	1.38	271.5	128.5
C2	2.84	281.2	153.3
C3	2.66	299.3	176.0
C4	3.67	308.4	256.7
C5	2.95	325.5	292.0
C6	3.05	335.6	332.7
C7	5.38	344.8	336.7
C8	3.90	345.8	340.9
C9	2.98	358.0*	143.6
	28.81		

* Equivalents of esters (freed from unsaponifiable matter): A10, 285.5; B15, 317.6; C9, 350.1.

separated into simpler groups of acids by the methods indicated in Table 2.

Each group of the acids was separately converted into methyl esters, which were fractionally distilled under reduced pressure through the column mentioned. The fractionation data for the methyl esters of the three groups of the body fat are summarized in Table 3.

The percentage composition, calculated from iodine values, and the equivalents and the mean unsaturation determined for each group of acids, and thence the component acids of the body fat, are recorded in Table 4.

Similarly, the component acids of the visceral and liver fats of bhakur were studied and their fatty acid compositions are given Tables 5 and 6, respectively.

Pahuna liver fat. 37.5 g. of the lipid, obtained in the usual manner from 736 g. of wet tissues, deposited 4.7 g. of crude phosphatides from a 10% acetone solution at 0°. As the quantity of the acids available was small it was decided, unlike the bhakur liver fatty acids, to convert them as such into methyl esters and fractionate. The fractionation data are given in Table 7 and the figures for component fatty acids in the whole fat in Table 8.

DISCUSSION

Bhakur body, viscera and liver fats. It is unfortunate that not much work has so far been done on the fats of fresh-water fish, specially on those of Indian origin. Analyses of fats from different parts of the same animal have rarely been recorded, but such data as are available indicate that significant differences in composition may occur. This view is confirmed by the present studies.

In Tables 4-6 are given the component acids of bhakur body, viscera and liver fats. The saturated acid contents of body, viscera and liver fats are about 38, 37 and 30%, respectively, and the contents of

the individual acids are somewhat similar—the chief difference being the much lower content of palmitic acid in the liver fat. It is also interesting to note the gradual increase in the relative proportions of saturated acids of higher molecular weights from body to viscera and to liver fats. The differences

(25 %), of viscera C_{18} (37 %) and of liver C_{20} (26 %), although the C_{18} acid content (25 %) of the liver fat is also almost equally high. Unsaturated C_{14} and C_{24} acids are minor components of these fats. In the visceral and the liver fats the unsaturated C_{16} acid group is much lower than in the body fat, but these

Table 4. *Component acids of bhakur body fat*

Acids	A (31.8%)	B (47.8%)	C (20.4%)	Total	Whole fat excluding unsaponifiable	
					(%, w/w)	Moles %
Myristic	1.8	1.1	—	2.9	2.9	3.5
Palmitic	24.0	4.9	—	28.9	29.0	30.6
Stearic	5.2	1.3	—	6.5	6.5	6.2
Unsaturated:						
C_{14}	Trace	0.2	—	0.2	0.2	0.2
C_{16}	0.4	23.2	1.7	25.3	25.3	26.9
C_{18}	0.4	13.7	3.8	17.9	17.9	17.3
C_{20}	—	3.3	4.2	7.5	7.5	6.6
C_{22}	—	—	10.1	10.1	10.1	8.3
C_{24}	—	—	0.5	0.5	0.6	0.4
Unsaponifiable	Trace	0.1	0.1	0.2	—	—
Mean unsaturation of:						
C_{14}	-2.0	-2.0	-2.0	-2.0	—	—
C_{16}	-2.0	-2.0	-4.0	-2.7	—	—
C_{18}	-2.0	-2.3	-5.0	-2.5	—	—
C_{20}	-2.0	-5.0	-8.0	-5.7	—	—
C_{22}	—	—	-9.0	-9.0	—	—

Table 5. *Component acids in groups A, B, C and in the whole bhakur visceral fat*

Acids	Component acids in groups			Component acids in whole fat	
	A (36.8%)	B (49.9%)	C (13.3%)	(%, w/w)	Moles %
Myristic	1.3	1.5	—	2.8	3.4
Palmitic	24.7	0.6	—	25.3	26.9
Stearic	7.4	0.5	—	7.9	7.6
Arachidic	1.4	—	—	1.4	1.2
Unsaturated:					
C_{14}	Trace	0.4	1.0	1.4	1.6
C_{16}	0.7	6.0	3.3	10.0	10.8
C_{18}	0.7	33.2	3.3	37.2	36.1
C_{20}	0.6	7.7	3.6	11.9	10.6
C_{22}	—	—	2.1	2.1	1.8
Unsaponifiable	Trace	Trace	Trace	—	—
Mean unsaturation of:					
C_{14}	-2.0	-2.0	-2.0	-2.0	—
C_{16}	-2.0	-2.0	-4.0	-2.7	—
C_{18}	-2.0	-2.3	-5.0	-2.5	—
C_{20}	-2.0	-5.0	-8.0	-5.7	—
C_{22}	—	—	-9.0	-9.0	—

become marked in the unsaturated portions. Here also, in a general way, the relative proportions of acids of higher molecular weights increase in the same order accompanied by a general decrease in the mean unsaturation—the mean unsaturation of C_{16} and C_{18} acids in the visceral fat deviating slightly from the rule. In the unsaturated acid portions the chief component acid in the fat of body is C_{16}

fats are richer in unsaturated C_{18} acids. Liver fat is rich in C_{20} acids. Visceral fat has a small amount of C_{22} acids, but body and liver fats have larger and almost equal amounts (10 %) of this group.

The analyses show large differences in the contents of the fatty acids in the body, visceral and liver fats of bhakur, specially in the unsaturated acid portions. These results are somewhat different from

those for jacobever (*Sebastichthys capensis*) (Van Rensburg, Rapson & Schwartz, 1945), inasmuch as that the jacobever visceral fat resembles the body fat; but in the liver fat the proportion of C_{18} acids is

differences in the unsaturated acids of the body and liver fats of turbot and halibut. Hilditch & Pathak (1949) found that blubber and liver fats of the common seal showed distinct differences in their

Table 6. *Component acids of bhakur liver fat*

Acids	A (32.8%)	B (67.2%)	Total	Acids excluding unsaponifiable	
				(%, w/w)	Moles %
Myristic	0.6	—	0.6	0.6	0.7
Palmitic	16.5	2.4	18.9	19.0	21.3
Stearic	4.1	2.3	6.4	6.4	6.5
Arachidic	3.1	—	3.1	3.1	2.9
Unsaturated:					
C_{14}	Trace	—	Trace	Trace	Trace
C_{16}	1.4	5.5	6.9	7.0	7.9
C_{18}	0.9	24.3	25.2	25.4	25.8
C_{20}	5.8	20.5	26.3	26.5	24.8
C_{22}	—	9.3	9.3	9.4	8.1
C_{24}	—	2.5	2.5	2.6	2.0
Unsaponifiable	0.4	0.4	0.8	—	—
Mean unsaturation of:					
C_{16}	-2.0	-2.0	-2.0	—	—
C_{18}	-2.0	-2.7	-2.7	—	—
C_{20}	-2.0	-5.1	-4.8	—	—
C_{22}	—	-6.5?	-6.5?	—	—
C_{24}	—	-8.0?	-8.0?	—	—

Table 7. *Fractionation data of methyl esters of pahuna liver fat acids*

Fraction no.	Weight (g.)	Saponification equivalent	Iodine value
1	1.82	262.0	27.9
2	2.07	270.3	28.9
3	1.84	278.0	41.1
4	2.07	288.7	79.1
5	2.37	293.6	92.7
6	2.05	295.6	98.9
7	2.37	301.3	120.0
8	2.27	318.6	207.6
9	1.97	333.7	259.0
10	2.10	338.3	283.2
11	2.32	353.7*	104.9

* Equivalent of ester (freed from unsaponifiable matter): 327.0.

Table 8. *Component acids of pahuna liver fat*

Acids	%	(%, w/w)	Moles %
Myristic	1.44	1.5	1.8
Palmitic	14.15	14.2	15.9
Stearic	4.00	4.0	4.1
Unsaturated: C_{14}	0.42 (-2.0)	0.4	0.5
C_{16}	8.42 (-2.0)	8.4	9.5
C_{18}	32.45 (-2.4)	32.6	33.2
C_{20}	19.76 (-5.3)	19.8	18.5
C_{22}	19.00 (-8.0)	19.1	16.5
Unsaponifiable	0.36	—	—

about 46 as compared with 28% for the body whilst the C_{20} and C_{22} acids are 13 and 8% as compared with 22 and 17% for the body fat. Other small noticeable differences (of 2–3%) in the body and the liver fats are in palmitic, stearic and unsaturated C_{24} acids. Lovern (1932, 1937) found similar

saturated acids and still greater differences in their unsaturated acids.

Unfortunately not many visceral fats have been studied so far. But some of the analyses of visceral fats have been made in cases in which data is also available for the blubber fat of the same animal, even if it has not been studied by the same person. The composition of herring visceral fat found by Hilditch & Pathak (1948) is widely different from that of herring body fat given by Lovern (1938) with respect to unsaturated acids and there are smaller differences in the saturated acids. The ling visceral and liver fat analyses of Shorland (1939) show specific differences in their C_{18} , C_{20} and C_{22} acids.

Pahuna liver fat. In comparison with bhakur, the pahuna has more phosphatide in the liver lipids, and the liver fat is less rich in saturated acids. Palmitic acid is the chief saturated acid in both. The C_{16} unsaturated acid content is similar. The chief unsaturated components of pahuna liver fat are C_{18} unsaturated acids (33%, which is higher than in the bhakur), but the C_{20} acid group (20%) and C_{22} acid group are major components. Comparison with published figures for fats of fresh-water fish from other parts of the world gives the impression that the Indian fish livers are low in C_{16} and high in C_{22} unsaturated acids, but the comparison may not be valid, for the published figures are generally not for liver fats, known to be richer in acids of higher molecular weight.

It is worth while to draw attention to the potential source of fat for industrial purposes in the viscera of

bhakur. The wet tissue, now discarded, contains 45% of fat, the recovery of which should be remunerative.

SUMMARY

1. The compositions of body, viscera and liver lipids from bhakur (*Catla buehanani*), and of liver fat of pahuna (*Wallago attu*) have been studied. Preliminary separations of the fatty acids into groups differing in unsaturation have been carried out with the help of lithium and lead salts in acetone and ethanol, respectively, and the compositions studied by the ester fractionation procedure.

2. The component acids of bhakur body, viscera and liver fats show significant differences in their proportions and degree of unsaturation. The fat content of viscera is remarkably high, 45%, as compared to 6.3% in the body and 8.5% in the liver on wet tissues, and industrial exploitation of the fat has been suggested.

3. The liver lipids of pahuna contain more phosphatides than bhakur liver fat, and there are minor differences in the acid components.

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REFERENCES

- Bjarnason, O. B. & Meara, M. L. (1944). *J. Soc. chem. Ind., Lond.*, **63**, 61.
 Hilditch, T. P. (1947). *The Chemical Constitution of Natural Fats*, 2nd ed. pp. 505-9. London: Chapman and Hall.
 Hilditch, T. P. & Pathak, S. P. (1948). *Biochem. J.* **42**, 316.
 Hilditch, T. P. & Pathak, S. P. (1949). *Biochem. J.* **44**, 218.
 Lovern, J. A. (1932). *Biochem. J.* **26**, 1978.
 Lovern, J. A. (1934). *Biochem. J.* **28**, 394.
 Lovern, J. A. (1937). *Biochem. J.* **31**, 755.
 Lovern, J. A. (1938). *Biochem. J.* **32**, 1214.
 Rapson, W. M., Schwartz, H. M. & Van Rensburg, N. J. (1943). *J. Soc. chem. Ind., Lond.*, **62**, 221.
 Shorland, F. B. (1939). *Biochem. J.* **32**, 1935.
 Tsujimoto, M. (1920). *J. Soc. chem. Ind. Japan*, **23**, 1007.
 Tsujimoto, M. & Kimura, K. (1923). *J. Soc. chem. Ind. Japan*, **26**, 891.
 Van Rensburg, N. J., Rapson, W. S. & Schwartz, H. M. (1945). *J. Soc. chem. Ind., Lond.*, **64**, 139.

The Formation of Hydrogen Peroxide by α -Radiation in the Presence of the Enzyme Carboxypeptidase

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The enzyme carboxypeptidase is inactivated by exposure to either α -radiation or X-radiation in aqueous solution. In all except the most concentrated solutions, the inactivation is due almost entirely to energy dissipated as ionization and excitation, in the water, leading to the formation of radicals or other active chemical intermediates which enter into the reaction with the enzyme. Whilst this is true of both types of radiation, the number of enzyme molecules inactivated per unit energy dissipated in the water by α -radiation is only about one-twentieth of the number inactivated by an equal dose of X-radiation. When these experimental observations were reported (Dale, Gray & Meredith, 1949), it was pointed out that the small amount of inactivation brought about by α -radiation could, in fact, be reasonably ascribed to the very low energy electrons, or δ -rays, which branch off as spurs along an α -particle track, in which case it would

appear that the main part of the α -ray energy which is dissipated within a narrow column is almost without effect on the enzyme. It was natural to associate the absence of effect on the enzyme with the well established fact that α -ray energy, unlike X-ray energy, gives rise to hydrogen peroxide and hydrogen when dissipated in pure air-free water, and to suppose that at all enzyme concentrations used in our experiments the radicals formed at very high concentration within the α -ray column combine together to form hydrogen peroxide and hydrogen before diffusion has brought them into effective collision with enzyme molecules. In this case, the presence of the enzyme should have little or no effect on the hydrogen peroxide yield, since the enzyme does not react with hydrogen peroxide. An alternative interpretation might be to assume that, owing to the very high concentration of radicals, interaction with enzyme molecules is confined to