4. The rate or amount of glycogen resynthesis was not affected by wide variation in phosphate concentration.

5. Of agents stimulating respiratory rate, 2:4-dinitrophenol and electrical pulses reduced or eliminated glycogen resynthesis while increased potassium-ion concentration had no effect.

The author is very grateful to Dr H. McIlwain, at whose suggestion this problem was undertaken, for guidance, advice and criticism throughout the work. He also wishes to express his gratitude to Mr R. Rodnight for valuable advice and criticism, to Dr P. J. Heald for help and advice with the quick-transfer technique, and to Mr T. B. P. Claypole for technical assistance.

REFERENCES

- Albaum, H. G., Tepperman, J. & Bodansky, O. (1946). *J. biol. Chem.* **164**, 45.
 Brante, G. (1954). *1st Int. Neurochem. Symp.* Oxford (in the Press).
 Buchanan, J. M., Hastings, A. B. & Nesbitt, F. B. (1949). *J. biol. Chem.* **180**, 435.
 Chance, M. R. A. & Yaxley, D. C. (1950). *J. exp. Biol.* **27**, 311.
 Chesler, A. & Himwich, H. E. (1943). *Arch. Biochem.* **2**, 175.

- Gaevskaya, M. S. (1953). *Zhur. Vysshei Nervnoi Deyatel.* **3**, 617. (From *Chem. Abstr.* 1954, **48**, 12 273.)
 Gore, M. B. R. & McIlwain, H. (1952). *J. Physiol.* **117**, 471.
 Heald, P. J., McIlwain, H. & Nelligan, D. (1954). *Biochem. J.* **56**, xlviii.
 Kahan, J. (1953). *Arch. Biochem. Biophys.* **47**, 408.
 Kerly, M. & Ottaway, J. H. (1954). *J. Physiol.* **123**, 516, 534.
 Kerr, S. E. (1936). *J. biol. Chem.* **116**, 1.
 Kerr, S. E. (1938). *J. biol. Chem.* **123**, 443.
 Kerr, S. E. & Ghantus, M. (1936). *J. biol. Chem.* **116**, 9.
 Klein, J. R. & Olsen, N. S. (1947). *J. biol. Chem.* **167**, 747.
 McIlwain, H. (1951*a*). *Biochem. J.* **49**, 382.
 McIlwain, H. (1951*b*). *Brit. J. Pharmacol.* **6**, 531.
 McIlwain, H. (1952). *Biochem. J.* **52**, 289.
 McIlwain, H., Buchel, L. & Cheshire, J. D. (1951). *Biochem. J.* **48**, 12.
 McIlwain, H. & Gore, M. B. R. (1951). *Biochem. J.* **50**, 24.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Ostern, P., Herbert, D. & Holmes, E. (1939). *Biochem. J.* **33**, 1858.
 Peiss, C. N. & Field, J. (1948). *J. biol. Chem.* **175**, 49.
 Raymond, A. L. & Blanco, J. G. (1928). *J. biol. Chem.* **79**, 649.
 Rodnight, R. & McIlwain, H. (1954). *Biochem. J.* **57**, 649.
 Stadie, W. C. & Zapp, J. A. (1947). *J. biol. Chem.* **170**, 55.
 Stetten, M. R. & Stetten, D. (1954). *J. biol. Chem.* **207**, 331.

The Component Fatty Acids of the Fat of *Aspergillus nidulans*

By J. SINGH AND T. K. WALKER

College of Technology, University of Manchester,

AND M. L. MEARA

Department of Organic Chemistry, University of Liverpool

(Received 11 November 1954)

Considerable interest has been shown from time to time in the production of fats on an industrial scale by micro-organisms, with the object of alleviating the possible world shortage of fats produced by the more usual agricultural methods. It is now well known that a considerable number of micro-organisms when cultured on suitable media exhibit high carbohydrate into fat-conversion rates. It is also known that in many cases the percentage of free fatty acid (expressed as oleic acid) present in the fat produced is of an order too high to permit of economical refining.

The mould fungus *Aspergillus nidulans*, grown under suitable conditions, gives rise to a high yield of fat, the free acidity of which is low, and of the order of that usual in a commercial seed fat. The results of a preliminary nutritional investigation of this fat, which was carried out at the Dunn Nutritional Laboratory, Cambridge (private com-

munication from Dr T. Moore and Mr R. J. Ward), were deemed sufficiently promising to warrant more extensive nutritional trials. At the same time it was considered highly desirable to investigate the component fatty acids and glycerides of *A. nidulans* fat.

EXPERIMENTAL

The fat was produced by culturing *A. nidulans* at 25° under static conditions on a medium of the composition (g./100 ml.): sucrose, 34; NH_4NO_3 , 0.6; K_2SO_4 , 0.044; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.016; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.73; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.50; made up with distilled water, the pH adjusted to 3.8. After 8 days, when fat accumulation had reached its maximum, the mycelial felts were removed, sterilized in free steam for 15 min., washed and pressed on a pad of filter paper. The felts were then dried in air for 2 days at room temperature and finally in an air-oven at 70° for 3 hr. The dried material was broken up, ground in a coffee-mill and extracted thoroughly (Soxhlet apparatus)

Table 1. *Low-temperature crystallization of mixed fatty acids*For explanation of symbols *A*, *B* and *C*, see text.

Group	Properties	Acids		
		Wt. (g.)	% (w/w)	Iodine value
<i>A</i>	Insoluble in 10 vol. ether at -35°	23.85	42.3	14.6
<i>B</i>	Insoluble in 10 vol. acetone at -70°	21.10	37.5	90.2
<i>C</i>	Soluble in 10 vol. acetone at -70°	11.37	20.2	158.1

with light petroleum (b.p. $40-60^{\circ}$). Removal of the solvent by distillation (last traces removed by heating *in vacuo* for 15 min. at 100°) yielded 45% (calculated on the dry felt weight) of a pale yellow semi-solid fat. This, when examined by the procedures given in the publication of British Standards Institution (1950), had the following characteristics: saponification equivalent 290.8, iodine value 73.4, free fatty acids (as oleic) 0.8%, unsaponifiable matter 1.4%.

Spectroscopic examination of the mixed fatty acids isomerized under standard conditions (Hilditch, Riley & Patel, 1951) after removal of unsaponifiable matter gave:

$E_{1\text{ cm.}}^{1\%}$ at 268 μ . ($170^{\circ}/15$ min.), 4;

$E_{1\text{ cm.}}^{1\%}$ at 234 μ . ($180^{\circ}/60$ min.), 155;

whence triethenoid acids, trace; diethenoid, 17.1; monoethenoid, 47.1; saturated acids, 35.8; the assumption being that the only unsaturated acids present are of the C_{18} series.

Determination of component fatty acids

A portion of the fat was saponified; the fatty acids were collected and resolved by low-temperature crystallization, using the technique elaborated by Hilditch (1947, p. 471). A solution (10%, w/v) of mixed fatty acids in acetone was crystallized at -70° , the material remaining in solution at this temperature constituting group *C*. Recrystallization of the acetone-insoluble material from solution in ether (10%, w/v) at -35° yielded two further fractions, group *B*, recovered from the soluble portion, and those acids insoluble in ether at this temperature (group *A*). The original mixed fatty acids were thereby resolved into three groups, *A*, *B* and *C*, consisting of mainly saturated, mainly monoethenoid and mainly polyethenoid acids, respectively, the proportions and iodine values of which are given in Table 1. Each group of acids was methylated (Bjarnason & Meara, 1944) and fractionally distilled (Table 2). Spectroscopic data on the acids recovered from *A* 6–*A* 8, *B*, and *C* mixed fatty acids, respectively, from which the distribution of the unsaturated acids in each group of acids is deduced, is recorded in Table 3. From the analytical data obtained for each ester fraction and from the appropriate spectroscopic data, its composition and thence that of each acid group and finally that of the whole fat is deduced (Table 4) according to the general principles enunciated by Hilditch (1947, pp. 495–509).

Identification of fatty acids

Acids recovered from *A* 2–*A* 4: acids crystallized from ethanol, m.p. 62.7° , unchanged on admixture with pure palmitic acid.

Acids recovered from *A* 6–*A* 8: saturated acids obtained after Lapworth–Mottram oxidation, on crystallization from ethanol gave m.p. 69.3° , mixed m.p. with stearic acid $69.4-69.6^{\circ}$.

Table 2. *Fractionation of methyl esters of acids of groups A, B and C*

Fraction	Wt. (g.)	Saponification equivalent	Iodine value
Methyl esters of acids of group <i>A</i>			
<i>A</i> 1	0.90	265.8	0.0
<i>A</i> 2	1.45	269.9	0.2
<i>A</i> 3	2.02	269.5	0.4
<i>A</i> 4	2.85	269.8	0.5
<i>A</i> 5	2.90	278.3	24.5
<i>A</i> 6	2.90	295.9	28.7
<i>A</i> 7	2.65	295.9	30.2
<i>A</i> 8	2.60	295.9	15.9
<i>A</i> 9	2.26	299.0	7.9
<i>A</i> 10	0.75	361.2*	11.0
Methyl esters of acids of group <i>B</i>			
<i>B</i> 1	2.24	289.4	79.2
<i>B</i> 2	3.22	289.7	83.2
<i>B</i> 3	3.27	293.3	88.7
<i>B</i> 4	2.67	292.4	88.5
<i>B</i> 5	2.34	291.7	89.8
<i>B</i> 6	2.09	293.3	88.6
<i>B</i> 7	0.73	306.7*	79.5
Methyl esters of acids of group <i>C</i>			
<i>C</i> 1	0.87	267.8	118.4
<i>C</i> 2	1.20	286.4	147.0
<i>C</i> 3	1.37	291.4	157.3
<i>C</i> 4	1.38	291.6	156.9
<i>C</i> 5	1.35	293.6	154.5
<i>C</i> 6	1.38	293.2	154.8
<i>C</i> 7	0.86	293.7	152.0
<i>C</i> 8	1.11	317.4*	137.5

* Equivalents of esters (freed from unsaponifiable matter) *A* 10, 361.5; *B* 7, 306.5; *C* 8, 311.8.

Table 3. *Spectrophotometric data for selected fractions*

Acids	Iodine value	$E_{1\text{ cm.}}^{1\%}$ after alkali isomerization	
		268 μ .	234 μ .
<i>A</i> 6– <i>A</i> 8	27.1	—	21.5
Mixed fatty acids of group <i>B</i>	90.2	2	66.8
Mixed fatty acids of group <i>C</i>	158.1	6	671.0

Acids recovered from *B* 3–*B* 4: dihydroxy acids soluble in hot ethyl acetate after Lapworth–Mottram oxidation, m.p. 130° , mixed m.p. with 9:10-dihydroxystearic acid 131° .

Acids recovered from *C* 3–*C* 5: bromination in light petroleum (b.p. $40-60^{\circ}$) gave tetrabromostearic acid, m.p. 114° , unchanged on admixture with tetrabromostearic acid from seed-fat linoleic acid.

Table 4. *Component fatty acids of Aspergillus nidulans fat*

	Acid groups			Total fatty acids excluding unsaponifiable material	
	A	B	C	% (w/w)	% (mol.)
	(42.3%)* (%)†	(37.5%)* (%)†	(20.2%)* (%)†		
Myristic	0.6	—	2.2	0.7	0.8
Palmitic	43.7	6.6	0.6	20.9	22.5
Stearic	36.8	1.0	—	15.9	15.5
Arachidic	3.4	—	—	1.4	1.3
Behenic					
Lignoceric					
Hexadecenoic	0.1	3.1	—	1.2	1.3
Oleic	13.6	81.0	20.3	40.3	39.5
Linoleic	1.3	7.1	68.3	17.0	16.8
Linolenic	—	—	1.0	0.2	0.2
Unsaturated C ₂₀	0.5	1.8	7.4	2.4	2.1
Unsaponifiable	—	—	0.2	—	—

* Groups as % (w/w) of total acids.

† Component acids as % (w/w) of group.

Table 5. *Component fatty acids of micro-organism fats*

Micro-organism ...	<i>Aspergillus nidulans</i>	<i>Penicillium sp.</i>	Yeast strain no. 72	<i>Rhodotorula sp.</i>	<i>Torulopsis sp.</i>	Baker's yeast
Investigator ...	Present investigation	Hilditch & Meara (1944)	Hilditch & Shrivastava (1948)	Holmberg (1948)	Reichert (1945)	Newman & Anderson (1933)
Total lipids %*	45	—	25-33	49.8	6.4	6.0
Free acidity (% oleic)	0.8	76†	33	18	51.2	14.3
Myristic	0.7	3.5	0.1	1.1	0.3	—
Palmitic	20.9	40.5	25.6	29.8	7.9	21
Stearic	15.9	7.9	5.9	8.8	3.8	7
Arachidic	1.4	1.1	5.1	1.4	0.2	—
Behenic						
Lignoceric						
Hexadecenoic	1.2	7.9	1.3	1.8	7.6	18
Oleic	40.3	19.0	54.5	40.1	21.5	—
Linoleic	17.0	20.1	5.7	11.2	49.7	54
Linolenic	0.2	—	0.7	4.8	4.4	—
C ₂₀₋₂₂	2.4	—	1.1	1.0	—	—

Other mould fats have had the following free acidities: *Aspergillus niger*, 71.2 (Bernhauer & Posselt, 1937); *A. sydowi*, 43.4 (Strong & Peterson, 1934); *Penicillium aurantio-brunneum*, 11.6 (Kroeker, Strong & Peterson, 1935); *P. javanicum*, 10.6 (Ward & Jamieson, 1934).

* Total lipid (acetone-soluble fat + insol. phosphatides) content is given as g./100 g. dry. wt. of cells.

† Unpublished observation of M. L. Meara.

DISCUSSION

The component fatty acids of the present specimen of fat, together with those of the fats elaborated by a number of micro-organisms are correlated in Table 5. It can be seen from Table 5 that the fat produced by *A. nidulans* is marked by the very low free acidity as compared with that produced by other moulds and yeasts so far studied. Some doubt exists as to whether the high free acidities are inherent in certain cases of fat synthesis or whether it is due to degradative reactions occurring due to cultural conditions. However, while certain moulds are able to produce fats of low free acidities, it would appear from the literature available that the free acidity of the fats elaborated by yeasts is of a relatively higher order.

The most outstanding feature of *A. nidulans* fat resides in the composition of the saturated acids. In the triglyceride portion of all fats of micro-biological origin so far reported the palmitic acid content, while being somewhat variable, constitutes about 70 % of the saturated acids present, the remaining saturated acids consisting of stearic acid with smaller amounts of myristic, and the higher arachidic, behenic and lignoceric acids. In *A. nidulans* fat, however, the stearic acid content is only of the order of 5 units per cent lower than that of palmitic acid. This feature is of considerable interest since in this respect the fat resembles to some extent the seed fats elaborated by certain tropical plants, e.g. *Madhuca latifolia* (Hilditch & Ichaporia, 1938) and also that of the depot fat produced by certain (ruminant) animals. While the

glyceride constitution of the former fats conforms to the general rule of even distribution, the stearic-rich animal depot fats contain a higher proportion of fully saturated glycerides. The glyceride structure of *A. nidulans* fat, now under investigation, will form the subject of a further communication.

Hilditch & Lovern (1936) showed that natural fats could be classified into biological groupings according to their component fatty acids. Thus the fats of the most primitive organisms contain a large number of acids, the number of major component acids decreasing with biological development. The content of hexadecenoic acid in fats appeared to run in conformity with this principle but a number of apparent exceptions are now known. Thus while Newman & Anderson (1933) reported 18% of hexadecenoic acid in the fat from baker's yeast, Hilditch & Meara (1944) detected 8% in the fat elaborated by a *Penicillium* sp., and Reichert (1945) 7.6% in the fat synthesized by *Torulopsis utilis*. On the other hand, Hilditch & Shrivastava (1948) and Holmberg (1948) reported the presence of 1.3 and 1.8%, respectively, in certain yeast fats which gave a high carbohydrate into fat conversion. Holmberg therefore suggested that an increase in the reserve fat content was accompanied by a decrease in the hexadecenoic acid content of the fat. It is seen from Table 5 that the low hexadecenoic acid content of *A. nidulans* fat is in harmony with this concept.

The reported linoleic acid content of the triglyceride portion of fats produced by micro-organisms ranges from 5 to 50%, and no correlation can yet be made even between the linoleic acid contents of the yeast fats. The linoleic acid content of *A. nidulans* fat (17%) falls well within the above range. This value is somewhat greater than that in the more unsaturated olive oils (12–15%), but is somewhat less than that in groundnut oils (20–35%). It is probable therefore that the fat elaborated by *A. nidulans* will be no more susceptible to oxidative rancidity than these commercially valuable vegetable oils.

SUMMARY

1. The fat elaborated by *A. nidulans* grown on a sucrose medium is remarkably low in free acidity.

2. The component acids of the fat have been shown to be: myristic, 0.7; palmitic, 20.9; stearic, 15.9; higher saturated acid, 1.4; hexadecenoic, 1.2; oleic, 40.3; linoleic, 17.0; linolenic, 0.2; C_{20–22} unsaturated 2.4% (w/w).

3. The fat appears to be somewhat anomalous compared with the fats of other micro-organisms by virtue of its relatively high stearic acid content.

4. The low hexadecenoic acid content is in harmony with the suggestion by Holmberg (1948) that high fat formers produce fats of low hexadecenoic acid content.

5. The linoleic acid content of this fat is somewhat higher than that of olive oil but lower than that of groundnut oil.

Our thanks are due to the Committee on Chemical Microbiology of the Medical Research Council for financial assistance.

REFERENCES

- Bernhauer, K. & Posselt, G. (1937). *Biochem. Z.* **294**, 215.
 Bjarnason, O. B. & Meara, M. L. (1944). *J. Soc. chem. Ind., Lond.*, **63**, 61.
 British Standards Institution (1950). *British Standard Methods of Analysis of Oils and Fats*, B.S. 684: 1950.
 Hilditch, T. P. (1947). *The Chemical Constitution of Natural Fats*, 2nd ed. London: Chapman and Hall Ltd.
 Hilditch, T. P. & Ichaporia, M. B. (1938). *J. Soc. chem. Ind., Lond.*, **57**, 44.
 Hilditch, T. P. & Lovern, J. A. (1936). *Nature, Lond.*, **137**, 478.
 Hilditch, T. P. & Meara, M. L. (1944). *J. Soc. chem. Ind., Lond.*, **63**, 112.
 Hilditch, T. P., Riley, J. P. & Patel, C. B. (1951). *Analyst*, **76**, 81.
 Hilditch, T. P. & Shrivastava, R. K. (1948). *Biochim. biophys. Acta*, **2**, 80.
 Holmberg, J. (1948). *Svensk kem. Tidskr.* **60**, 14.
 Kroeker, E. H., Strong, F. M. & Peterson, W. H. (1935). *J. Amer. chem. Soc.* **57**, 354.
 Newman, M. S. & Anderson, R. J. (1933). *J. biol. Chem.* **102**, 219.
 Reichert, R. (1945). *Helv. chim. acta*, **28**, 484.
 Strong, F. M. & Peterson, W. H. (1934). *J. Amer. chem. Soc.* **56**, 952.
 Ward, G. E. & Jamieson, G. S. (1934). *J. Amer. chem. Soc.* **56**, 973.