REFERENCES

- Adams, E. B. (1954). *Brit. med. J.* **1**, 537.
- Association of Vitamin Chemists (1951). *Methods of Vitamin Assay*, 2nd ed. p. 231. New York: Interscience Publishers Inc.
- Autret, M. & Behar, B. (1954). *Sindrome Policarencial Infantil (Kwashiorkor) and its Prevention in Central America*. FAO Nutritional Studies, no. 13, p. 21. Rome.
- Brock, J. F. & Autret, M. (1952). *Kwashiorkor in Africa*. WHO Monograph Series, no. 8, p. 28. Geneva.
- Broquist, H. P., Stokstad, E. L. R. & Jukes, T. H. (1951). *J. Lab. clin. Med.* **38**, 95.
- Chang, S. C. (1953). *J. biol. Chem.* **200**, 827.
- Cosulich, D. B., Smith, J. M. & Broquist, H. P. (1952). *J. Amer. chem. Soc.* **74**, 4215.
- Dietrich, L. S., Monson, W. J., Gwosh, H. & Elvehjem, C. A. (1952). *J. biol. Chem.* **194**, 549.
- Doctor, V. M., Elam, J. F., Sparks, P., Lyman, C. & Couch, J. R. (1954). *Arch. Biochem. Biophys.* **48**, 249.
- Doctor, V. M. & Trunnell, J. B. (1954). *Proc. Soc. exp. Biol., N.Y.*, **87**, 498.
- Doctor, V. M., Welch, B. E., Perrett, R. W., Brown, C. L., Gabay, S. & Couch, J. R. (1953). *Proc. Soc. exp. Biol., N.Y.*, **84**, 29.
- Gabuzda, G. J., Phillips, G. B., Schilling, R. F. & Davidson, C. S. (1952). *J. clin. Invest.* **31**, 756.
- Guggenheim, K., Leibowitz, J. & Rakower, D. (1953). *Biochem. J.* **55**, 388.
- Joint FAO/WHO Expert Committee on Nutrition. (1953). Third Report, p. 21. Geneva.
- Keresztesy, J. C. & Silverman, M. (1951). *J. Amer. chem. Soc.* **73**, 5510.
- Mindlin, R. L. & Butler, A. M. (1938). *J. biol. Chem.* **122**, 673.
- Nichol, C. A. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 52.
- Nichol, C. A. (1953a). *Proc. Soc. exp. Biol., N.Y.*, **83**, 167.
- Nichol, C. A. (1953b). *J. biol. Chem.* **204**, 469.
- Pieraerts, G. (1949). *Ann. Soc. belge Méd. trop.* **29**, 231, 329.
- Sauberlich, H. E. & Baumann, C. A. (1948). *J. biol. Chem.* **176**, 165.
- Trowell, H. C., Davies, J. N. P. & Dean, R. F. A. (1952). *Brit. med. J.* **2**, 798.
- Walt, F., Wills, L. & Nightingale, R. P. (1950). *S. Afr. med. J.* **24**, 920.
- Welch, A. D. & Nichol, C. A. (1952). *Annu. Rev. Biochem.* **21**, 633.
- Welch, A. D., Nichol, C. A., Anker, R. M. & Boehne, J. W. (1951). *J. Pharmacol.* **103**, 403.

Changes in the Composition of the Fat of *Aspergillus nidulans* with Age of the Culture

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While studying linseed oil Ivanow & Klokow (1933) observed that as ripening of the oil-bearing seeds proceeded the proportion of the linolenic acid component increased, whereas those of the oleic and linoleic acid components decreased. From the results of feeding experiments with rats Boxer & Stetten (1944) formed the opinion that in these animals oleic acid was formed from stearic acid by dehydrogenation and that saturated acids are the primary products in fat biosynthesis. Coleman, Cefola & Nord (1952) studied formation of fat in *Fusarium lini* Bolley, and concluded that a mechanism for the dehydrogenation of fatty acids operates in this mould. Such micro-organisms, which can synthesize relatively large quantities of fat when grown on a simple defined medium of sugar and inorganic salts, may prove useful agents for the study of the reactions which give rise to fat, for both substrate and cultural conditions can be well regulated. Recently, we examined the fat of *Aspergillus nidulans* (Singh, Walker & Meara, 1955), and we have now conducted experiments

which were planned to throw light on the question whether the unsaturated acids that are components of the fat of this organism are formed by dehydrogenation of saturated acids or by some other mechanism.

EXPERIMENTAL

Aspergillus nidulans Eidam was grown as surface cultures from spores sown on a medium which contained (% w/v): NH_4NO_3 , 0.6; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.73; K_2SO_4 , 0.044; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.016; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005; sucrose, 34; the medium was adjusted to pH 3.8 and made up to volume with distilled water. In each experimental series a set of Pyrex penicillin flasks, each containing 175 ml. of this medium, was employed. After sterilization in steam at 10 lb. pressure for 15 min. the flasks were cooled, their contents inoculated and incubated at 25°. Cultures were harvested after 5, 7, 9, 14 and 21 days' incubation. The number of flasks taken at any given time depended upon the growth attained; thus at 5 days it was necessary to combine the contents of twenty flasks in order to obtain sufficient fat for detailed analysis. At the later periods, a smaller number of flasks yielded sufficient fat for this purpose, but the number of flasks withdrawn at any

time was never less than five. After the cultures had been killed by heating in free steam for 10 min. the mycelial felts were removed from the flasks and separated from adhering medium by washing with distilled water on a Büchner funnel. The filtrates from the flasks and the washings were combined, and after suitable dilution (the total metabolism solution from a set of five flasks was made up with distilled water to 2 l.) the residual sucrose was estimated by the method of Lane & Eynon (1923). The felts were dried in air for 3 or 4 days and then in an air-oven at 60° until the weight was constant. The material so obtained was broken down in a small grinding mill and thoroughly extracted with light petroleum (b.p. 40–60°) in a Soxhlet apparatus. The solvent was distilled from the extracted fat, the last traces of petroleum being removed by heating the residue *in vacuo* for 15 min. at 100°. Estimations of acid value, saponification equivalent, iodine value and unsaponifiable matter were then performed on the fat, the procedures being those recommended by Hilditch (1949). The mixed fatty acids were examined spectrophotometrically for their contents of linoleic and linolenic acids, according to the method of Hilditch, Patel & Riley (1951).

RESULTS

The changes in the weight of felt and in its content of fat with time of incubation are recorded in Table 1. The combined contents of a number of flasks, in no case less than five, were used for each

estimation. The results have been calculated on the average contents of one flask and the standard deviation is shown beneath the mean weight of felt. The chemical characteristics of fat samples isolated from felts which were harvested at different stages of the incubation period are given in Table 2. The component fatty acids of the different samples of fat are recorded in Table 3. The weights of the individual fatty acids obtained by saponification of the different samples of fat are given in Table 4. These weights refer in all cases to the contents of one flask.

The rate of synthesis of fat in *A. nidulans* is seen to be slow in the early period of incubation, but it increases rapidly about the seventh day, the maximum weights of felt and fat being attained on the ninth day. Between this stage and the fourteenth day there occurs a slight fall in the weight of the felt and an appreciable fall (approx. 14%) in the weight of fat present in the felt. Thereafter the weight of felt remains almost stationary for the next 7 days, but a further fall occurs in the weight of the fat, to a value about 64% of that attained on the ninth day. The iodine value falls to a minimum on the ninth day and then rises appreciably to its highest value, attained on the twenty-first day. From the proportions of the component acids

Table 1. *Changes in the weight of the mycelium of Aspergillus nidulans and in its content of fat during different periods in the development of the mould in 175 ml. of medium. Sucrose present initially, 59.5 g.*

Time of incubation (days)	Sucrose utilized (g.)	Dry felt		Fat		
		(g.)	(g./100 g. of sucrose utilized)	(g.)	(g./100 g. of dry felt)	(g./100 g. of sucrose utilized)
5	20.70	3.956 (±0.463)	19.1	0.585	14.8	2.8
7	28.25	5.803 (±0.782)	20.5	0.892	15.4	3.2
9	43.45	14.116 (±0.514)	32.5	6.310	44.7	14.5
14	49.06	13.557 (±0.442)	27.6	5.395	39.8	11.0
21	54.90	13.636 (±0.371)	24.8	4.080	29.9	7.4

Table 2. *Chemical characteristics of the fat of Aspergillus nidulans after different periods of incubation*

Time of incubation (days)	Acid value	Iodine value	Saponification equivalent	Unsaponifiable matter (g./100 g. of fat)
5	2.90	85.5	291.2	8.0
7	1.70	83.8	292.9	5.2
9	0.84	73.7	290.2	1.3
14	0.60	80.8	289.1	1.7
21	1.50	88.3	292.5	2.8

shown as percentages in Table 3 it is clear that, during the early period of incubation, oleic acid is the predominant component, and at 7 days its ratio to the percentage of total saturated acids is more than 2:1. By the ninth day, however, the percentage of the total saturated acids has risen appreciably and reached its maximum, while that of oleic acid has fallen to a value above which it rises only slightly in the subsequent period of incubation. Also, after the ninth day the percentage of total saturated acids again falls to an

appreciable extent. Table 4, in which are given the actual weights of the component acids, shows clearly that the quantities of all the component acids, namely the saturated ones together with oleic and linoleic acids, increase in the earlier period of incubation up to the point at which the mould's activity reaches its peak. Then, when incubation is prolonged beyond 9 days, the actual weights of all these components decrease, the fall being relatively small in the case of linoleic acid but much greater in the other cases.

In experiments with three species of mould fungus, one of which was *A. nidulans*, Gregory & Woodbine (1953) found that greater unsaturation in the fat was associated with longer incubation. They indicated, however, that this need not imply that the longer period had enabled synthesis of the unsaturated acid(s) to proceed from the already synthesized fatty acids. They quote a private communication from Dr J. A. Lovern to the effect that 'this whole subject of fatty acid production, however, can only be satisfactorily evaluated on a

Table 3. *Changes in the composition of the fat of Aspergillus nidulans with time of incubation*

Time of incubation (days)	Mixed fatty acids		Component fatty acids as percentages of total component fatty acids			
	Iodine value	$E_{1\text{ cm.}}^{1\%}$ at 234 m μ .	Saturated	Oleic	Linoleic	Linolenic
5	82.2	182	29.1	50.8	20.1	—
7	81.1	147	26.4	57.4	16.2	—
9	73.7	155	35.5	47.4	17.1	—
14	75.0	156	34.1	48.7	17.2	—
21	84.5	196	29.7	48.2	21.0	1.1*

* $E_{1\text{ cm.}}^{1\%}$ at 268 m μ . was 6 and values less than 5 in other cases were neglected.

Table 4. *Actual weights of the individual component fatty acids formed by Aspergillus nidulans during different periods of its development in 175 ml. of medium*

Time of incubation (days)	Acids (g.)			
	Saturated	Oleic	Linoleic	Linolenic
5	0.15	0.25	0.10	—
7	0.21	0.46	0.13	—
9	2.09	2.80	1.01	—
14	1.74	2.48	0.88	—
21	1.13	1.83	0.80	0.04

DISCUSSION

Hilditch (1951) has suggested that syntheses of both saturated and unsaturated fatty acids take place separately, but possibly both are derived from a common precursor. He points out that the 'desaturation' hypothesis is based merely on the interpretation on a percentage basis of the experimental results of workers who were following changes proceeding in ripening seeds, and he shows that if such results are calculated in terms of the actual weights of the fatty acids synthesized at a given time in the same number of seeds, then the quantities of all the acids are seen to increase with the development of the seeds. As an instance, he quotes figures, obtained by Grindley (1950) while studying the development of cotton seed, to show that a decrease observed in the percentage of oleic acid did not necessarily mean that the actual amount of that acid had decreased.

basis of quantitative estimations of particular and individual fatty acids'. The results reported in the present communication provide evidence of the kind indicated by Hilditch and by Lovern as necessary to enable conclusions to be drawn about the question under discussion. It is seen that although the proportions of the component acids of the fat vary during the period of fat synthesis in *A. nidulans*, the actual weight of each component shows at first an increase up to a limit, and then, after the point of maximal synthetic activity has been attained, the actual weight of each acid decreases. The evidence as a whole affords no support for the view that during the biosynthesis of fat the unsaturated acids are formed by dehydrogenation of the saturated acids. This verdict is in agreement with the finding of Bloch (1948), who concluded from the results of experiments made with 'labelled' acetate and pyruvate that saturated and unsaturated acids arise by reactions proceeding along different pathways.

SUMMARY

1. The rate of formation of fat in surface cultures of *Aspergillus nidulans*, when cultivated under the conditions stated, is slow during the first week of incubation, but becomes very rapid during the eighth and ninth days, when the maximal development of the mould is reached. After the ninth day the reserve of fat in the mould decreases in weight.

2. In the early stage of fat synthesis the fat is more acidic and contains a larger percentage of unsaponifiable substances than at any later period.

3. Until the stage of maximal activity of the mould is attained the actual amounts of both saturated and unsaturated component acids continue to increase, but in the subsequent phase of the life of the culture the weight of each of these components diminishes.

4. The results are not in accord with the hypothesis that, for the biosynthesis of fat, unsaturated acids are provided by dehydrogenation of saturated acids.

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REFERENCES

- Bloch, K. (1948). *Cold Spr. Harb. Symp. quant. Biol.* **13**, 29.
 Boxer, G. E. & Stetten, de W. jun. (1944). *J. biol. Chem.* **153**, 607.
 Coleman, R. J., Cefola, M. & Nord, F. F. (1952). *Arch. Biochem. Biophys.* **40**, 102.
 Gregory, M. E. & Woodbine, M. (1953). *J. exp. Bot.* **4**, 314.
 Grindley, D. N. (1950). *J. Sci. Fd Agric.* **1**, 147.
 Hilditch, T. P. (1949). *The Industrial Chemistry of Fats and Waxes*. London: Baillière, Tindall and Cox.
 Hilditch, T. P. (1951). *Nature, Lond.*, **167**, 298.
 Hilditch, T. P., Patel, C. B. & Riley, J. P. (1951). *Analyst*, **76**, 81.
 Ivanow, S. & Klokow, P. (1933). *Allg. Oel- u. Fettztg.* **30**, 149.
 Lane, J. H. & Eynon, L. (1923). *J. Soc. chem. Ind., Lond.*, **42**, 32.
 Singh, J., Walker, T. K. & Meara, M. L. (1955). *Biochem. J.* **61**, 85.

The Composition of the Extracellular Polysaccharides of *Aerobacter-Klebsiella* Strains

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Since the earliest observations on coliform bacteria it has been known that organisms of the *Aerobacter-Klebsiella* group (i.e. *Aerobacter aerogenes*, *Aero. cloacae* and *Klebsiella* species) produce large amounts of slime and capsular material, and thus show mucoid growths. The polysaccharide nature of the slime and capsular material of these organisms was established by Emmerling (1900), Schardinger (1902), Toenniessen (1921) and Kramár (1922), and preliminary analyses of untyped strains showed galactose to be present in each. However, few detailed chemical studies have been made. The capsular polysaccharides of *Klebsiella pneumoniae* types A, B and C (corresponding to *Klebsiella* types 1, 2 and 3 of Edwards & Fife, 1952) have been examined more thoroughly and found to be glucose-containing polyuronides (Heidelberger, Goebel & Avery, 1925; Goebel & Avery, 1927; Goebel, 1927). Wilkinson, Dudman & Aspinall (1955) isolated the slime of *Klebsiella* type 54 (*Aero. aerogenes* A3 (Sl)) from culture supernatants, and found it to be a polyuronic consisting of D-glucose (50%), L-fucose (10%) and an unidentified uronic acid (29%); a small amount of galactose (approx. 1%) detected in the slime was

believed to be present only through contamination with an intracellular galactan. The composition of the slime was shown to be independent of the carbon source on which the organism had been grown. Recently, Levine, Stevenson, Bordner & Edwards (1955) examined the capsular polysaccharides of most known *Klebsiella* types by infrared spectrophotometry and found them all to be polyuronides, in some cases with esterified acetyl groups. No analysis has been reported of polysaccharides of *Aero. cloacae* strains.

Mucoid strains of *Aerobacter-Klebsiella* organisms have been differentiated into a very large number of immunologically distinct types (Edwards & Fife, 1952; Edmunds, 1954; Henriksen, 1954) whose specificities are determined by their capsular polysaccharide antigens (Julianelle, 1926; Edwards, 1929). These results indicate that, as with the pneumococcus, *Aerobacter-Klebsiella* strains of different serological type give rise to immunologically distinct extracellular polysaccharides. Levine *et al.* (1955) found the infrared spectrum of the capsular polysaccharide of each type to be different and distinct.

No attempts appear to have been made in previous studies of bacterial polysaccharides to isolate slime and capsular material separately in

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