required for successful alkyd preparation was found. Under certain conditions the acidolysis reaction occurs simultaneously with esterification; use is made of this fact to improve the process. Commercial use of the acidolysis reaction in alkyd-resin manufacture is economically attractive because it is noncatalytic; therefore no catalyst residues need to be removed by pressure filtration. Another advantage is that glycerol is readily pumped into hot acidolysis-reaction mixtures without opening the kettle, and this eliminates the disagreeable and toxic fumes that escape from hot kettles when they are opened after alcoholysis for addition of solid phthalic anhydride or isophthalic acid.

Applications of acidolysis in conjunction with oil polymerization, fish oil upgrading, and castor oil dehydration were studied briefly, and the results suggest that further work in these fields is desirable.

Acknowledgment

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Conversion of Some C^{14}-Labelled Compounds into the Neutral Lipid of *Neurospora Crassa*

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ALTHOUGH there have been many biochemical investigations of the red bread mold, *Neurospora crassa*, few have been concerned with lipid metabolism. Previous studies have shown that the coloring matter consists of a variety of carotenoids (2,11). Ottke reported the presence of ergosterol in the mycelium (7) and studied its synthesis from acetate (8). Some of the cephalins have been fractionated (1). Lein *et al.* (6) isolated various mutants which required certain 18-carbon unsaturated fatty acids in the medium for normal growth. Information about the fatty acid composition of the fungus lipids also has been published recently (10).

This paper deals with the neutral lipid from conidia-free mycelia (that fraction of the total lipid which is not extracted from hexane by cold aqueous KOH solution), its incorporation of some C^{14}-labelled metabolites, and the nature of its fatty acid components.

Experimental

Neurospora crassa (cross of wild types E 5297a and E 5256A) was grown by the submerged culture technique previously described for the study of carotene biosynthesis (4). Eight C^{14}-labelled compounds were compared as possible precursors of lipids in the 2-day-old fungus under two sets of conditions: a) the culture was given the radio-active substrate and harvested 3½ days later, or b) the mycelial pad was washed gently, transferred to a sucrose-deficient medium complete in all other respects, given the radioactive material, and collected as in the first procedure. Preliminary experiments had shown that about two-thirds of the maximum dry weight of the mat was attained prior to addition of the labelled substrate.

The hexane-soluble fraction prepared as described elsewhere (5) was freed of acidic substances by extraction with the base (methanol-water-potassium hydroxide 90:10:20 v/v/w). Fatty acids esterified to the neutral lipid were then liberated by hot saponification in ethanol and recovered after acidification. Samples of the neutral lipid and its fatty acids were counted at infinite thinness on aluminum planchets in a windowless gas flow counter. Respired carbon dioxide, which was trapped in 40% potassium hydroxide and precipitated as barium carbonate, was also counted.

The fatty acids were determined qualitatively by chromatography on silicone-impregnated paper (9) and qualitatively and quantitatively by gas chromatography. The paper chromatograms were developed with 85% acetic acid-water (v/v) at 25° for 24 hrs. Autoradiograms were prepared by placing the strips on x-ray film for 60 days. Gas chromatography of the methyl esters, prepared by refluxing the unlabelled fatty acids in methanol with sulfuric acid, was performed at 200° with a 5-ft. polyester column (½ in. in diam.) of ethylene glycol and succinic acid (LAC-446)[3] as stationary phase and a helium flow rate of 75 ml./min.

Results and Discussion

Total fat, as crude hexane extract, produced by the mold *Neurospora crassa* in these experiments constituted about 25% of the weight of the dry mycelium. This crude extract was mostly neutral lipid.

Substantial differences were observed in the effi-

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TABLE I

Conversion of Some C^{14}-Labelled Compounds to Carbon Dioxide, Neutral Lipid, and Fatty Acids by *Neurospora crassa*

Compound	Sucrose present	Radio-activity supplied	Radio-activity in respiratory CO_2		Radio-activity in neutral lipid		Radio-activity in fatty acids esterified to neutral lipid	
			Total	% of supplied	Total	% of supplied	Total	% c.p.m. in neutral lipid
		c.p.m.	*c.p.m.*		*c.p.m.*		*c.p.m.*	
Sodium acetate-2-C^{14}	+	305,000	52,500	17.2	48,800	18.0	18,900	38.7
	−	305,000	95,000	31.1	63,000	20.7	7,140	11.3
DL-Mevalonic acid-2-C^{14}	+	1,340,000	118,000	8.8	83,800	6.3	6,080	7.3
	+	1,240,000	88,600	7.1	46,200	3.7	7,920	17.1
	−	1,240,000	27,400	2.2	118,000	9.5	4,800	4.1
Sodium pyruvate-2-C^{14}	+	1,580,000	562,000	35.6	55,400	3.5	32,100	57.9
	−	1,580,000	619,000	39.2	41,600	2.6	19,900	47.8
DL-Alanine-2-C^{14}	+	1,600,000	578,000	36.1	38,800	2.4	19,700	50.8
	−	2,290,000	1,196,000	52.2	37,000	1.6	19,300	52.2
Glycerol-1-C^{14}	+	2,140,000	693,000	32.4	14,400	.7	6,200	43.1
	−	2,140,000	223,000	10.4	18,400	.9	2,510	13.6
Glucose-(UL)	+	1,570,000	237,000	15.1	7,200	.5	5,620	78.1
	−	1,570,000	195,000	12.4	8,200	.5	4,280	52.2
DL-Phenylalanine-3-C^{14}	+	106,000	19,760	18.6	1,060	1.0	.....	.....
Choline-1-C^{14}	+	138,500	12,600	9.1	0	0	.....	.....

ciency with which the fungus utilized different isotopic substrates in the production of carbon dioxide and neutral lipid (Table I). The presence or absence of sucrose in the medium did not markedly affect the quantity of radio-activity incorporated into respiratory carbon dioxide and neutral lipid despite growth reduction in a sucrose-deficient medium. Substantial radio-activity in the respiratory carbon dioxide from all experiments showed that each of the compounds tested participated in cell metabolism. Acetate with a value of 18% gave the highest percentage incorporation into neutral lipid. Mevalonic acid with 6.3% incorporation served as a fair substrate for production of neutral lipid, but relatively little of it appeared in the form of esterified fatty acid. Alanine, pyruvate, and glucose, though used inefficiently for neutral lipid production, were found to contribute more to the fatty acids than to other components of the neutral lipid. No labelling from choline was perceptible.

Distribution of radio-activity in some components of the neutral lipid was studied in an experiment in which the N,N′-dibenzylethylenediamine salt of 2-C^{14}-mevalonic acid was supplied to the 2-day-old fungus for 12 hours. This salt was incorporated to the extent of 1.7%, twice that of the sodium salt. Of the 616,000 counts in the crude lipid, the acidic material removed by extraction with base contained 148,000 (24%); the sterols precipitable by digitonin, 152,000 (25%); the carotenes separated as a group on a silicic acid column, 52,000 (8%); and the fatty acids, 101,000 (16%). No attempt was made to recover or count the dark residue left on the silicic acid column or the water-soluble products after the saponification treatment. Weights of these different fractions were not determined.

Paper chromatography of the fatty acid fraction from the neutral lipid of parallel cultures grown on labelled and unlabelled sucrose medium, when compared with authentic samples, showed four acids to be present: palmitic, stearic, oleic, and linoleic. Autoradiograms showed the radio-activity to be confined to those acids. Gas chromatography of the esters prepared from the neutral lipid of a culture grown on unlabelled sucrose confirmed the presence of these four as well as a small amount of palmitoleic and some unidentified acids. Emergence of the latter from the column before palmitic ester suggests that they were of shorter chain length than C_{16}. Peak areas were used to estimate the fatty acid composition on a weight percentage basis (Table II).

Linolenic acid was not detected in the present investigation. This finding differs from the data of Todd *et al.* (8), who reported that linolenic acid comprised one-third of the component fatty acids of the fungus grown on surface culture. It is apparent that wide differences in fatty acid composition of *Neurospora crassa* lipids are obtained, depending upon the strain and the cultural methods employed.

TABLE II

Fatty Acids Esterified to the Neutral Lipid of *Neurospora crassa*

Acid	Percentage of total fatty acids
Palmitic	31
Palmitoleic	2
Stearic	4
Oleic	32
Linoleic	30
Unidentified	1

Summary

Conidia-free mycelia of *Neurospora crassa* converted C^{14}-acetate into neutral lipid more efficiently than C^{14}-labelled mevalonic acid or six other compounds tested. Pyruvate, glucose, and alanine contributed little to the neutral lipid fraction, but that contribution was mainly as fatty acids. Substantial losses of C^{14} as carbon dioxide indicated that all of the labelled compounds participated in cell metabolism.

Analysis of the fatty acids esterified to the neutral lipid of unlabelled mycelia showed that palmitic, oleic, and linoleic acids each comprised nearly one-third of the total while stearic, palmitoleic, and a small amount of unidentified acids contributed the remainder.

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Polymerization of Linseed Oil in an Electric Discharge

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WHEN MINERAL OILS or fatty oils are subjected to the action of glow discharges in a hydrogen or a nitrogen atmosphere at low pressure a considerable increase in viscosity is effected, probably because of polymerization of the starting material. In the "Elektrion" process of De Hemptinne (1), also known as "Voltol" process (2), this reaction was applied, especially during the first World War, on a commercial scale for the preparation of high-grade lubricating oils.

The phenomena in an electric discharge are very complicated. In a low-pressure hydrogen atmosphere the action of hydrogen atoms, in all probability, is of primary importance (3). Polymerization of unsaturated as well as saturated compounds takes place by the combination of radicals which have been formed primarily by the action of the hydrogen atoms; in addition, hydrogenation and dehydrogenation reactions have been reported (4).

Several authors (5, 6) investigated the applicability of the Voltol-process for the preparation of polymerized oils in the paint industry. According to Goldenstein (6), the properties of voltolized linseed oils are better in several respects than thermally polymerized oils, especially in regard to the hardness and resistance of the films after drying. This might be due to the completely different chemical structure of the polymerized oils. The thermal polymerization, which can be considered as a Diels-Alder condensation of conjugated double bonds in the fatty acid chains, results in the formation of six-membered rings in the polymerized oils (7, 8); polymerization in a glow discharge at low temperature, *e.g.*, 70°C., is a result of a combination of radicals into compounds in which the occurrence of cyclic structures is questionable (9).

In 1952 Boelhouwer, Jol, and Waterman (8) published a general scheme for analysis of polymerized fatty oils, which allows for a reliable determination of the contribution of intramolecular reactions (combination of fatty acid chains in the original glyceride molecules) and intermolecular reactions (combination of fatty acid chains of different glyceride molecules), also for a proper study of the coupling of the fatty acid chains in the polymerization process, particularly in regard to the occurrence of rings in the polymerized oils.

According to this scheme the polymerized oil to be investigated is first stabilized by hydrogenation (150°C., 100 atm. hydrogen, 5% of a nickel on kieselguhr catalyst) to saturate the olefinic double bonds, then separated quantitatively into monomeric and polymeric glycerides by repeated molecular distillation in a falling film still (10). Both the monomeric and the polymeric glycerides are saponified and the fatty acids, after transformation into their methyl esters, are submitted to ordinary vacuum fractionation. Monomeric esters distill at 150°–200°C. at 1 mm. Hg and dimeric esters remain as a residue (boiling point >250°C. at 1 mm. Hg). The degree of intramolecular polymerization is indicated directly by the amount of residual methyl esters obtained from the monomeric glycerides. To investigate the presence of rings, the methyl ester fractions are transformed into saturated hydrocarbon mixtures, *e.g.*, by direct hydrogenation (11) (300°–350°C., 300 atm. hydrogen, and 20% of a nickel copper on kieselguhr catalyst). The average number of rings per molecule in the hydrocarbon mixtures follows from their physical properties according to ring analysis methods (13) and can also be calculated directly from ultimate analysis and molecular weight.

This scheme was applied in earlier work to the study of thermally polymerized linseed oils (8) and tung oils (12). For comparison the analysis of some voltolized linseed oils is described in this paper.

Description of Apparatus

For the treatment of linseed oil a laboratory "Voltol" reactor was constructed (Figure 1) which resembles commercial equipment described in the literature (1, 2) and allows voltolization of oils under corresponding conditions.

The experiments are conducted in a rotating cylindrical Pyrex tube (500 × 90 mm.) composed of two parts, which are held together by glass flanges, tightened with oil-resistant rubber rings. The narrow ends of the rotating reactor are connected with fixed inlet and outlet tubes with spherical joints, lubricated with Apiezon grease.

Two concentric aluminum cylinders serve as electrodes. They are separated by a glass cylinder, protruding 60 mm. on both ends of the electrodes. The contact wires, which connect the electrodes with sliding contacts on both ends of the reactor, are laid on in such a way that there is no danger of sparks. Tungsten wires connect with the outside of the reactor.

In the reactor approximately 600 ml. of oil can be treated. The surfaces of the electrodes and the separating glass cylinder are wetted continuously by an

[1] Compare T. Hoekstra, Thesis, Delft 1958 (in Dutch).