particularly in the muscles, with a corresponding fall in the percentage of protein, water, Na and K salts. The fall in Ca is greater than can be accounted for in this way.

5. The liver of a silver eel may contain over 100 mg. of iron/100 g. wet weight. There may be over 25 % of protein in the skin.

6. The above, and other chemical changes of growth have been correlated with events taking place in the body and with similar and dissimilar changes in the chemistry and growth of the higher vertebrates.

7. 100 g. of the flesh of a silver eel would provide 15 g. of 'first class' protein, and 300 cal. From the point of view of food, elvers must be regarded as a good source of Ca, P and Fe.

The author is indebted to Miss Frost and to other members of the Freshwater Biological Association for supplying the animals, and for their advice about them; to the staff of the Nutrition Laboratory, Cambridge, for the vitamin assays, and to Miss D. N Graves and to Dr E. M. Widdowson for their help with the chemical determinations. The work was the outcome of a request from Dr E. B. Worthington for more information about the food value of the eel.

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Fat Formation in *Torulopsis lipofera*

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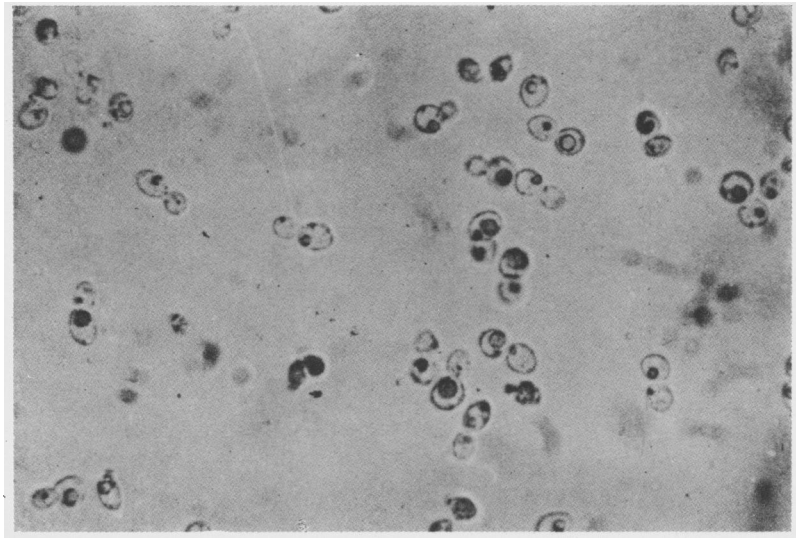
Several micro-organisms are known to be capable of storing considerable quantities of fat in their cells (Fink, Haehn & Hoerburger, 1937; Prescott & Dunn, 1940). The formation of fat in these organisms is either closely connected with growth, or very slow. These organisms therefore are not suitable for an investigation of the mechanism of fat synthesis. In the present paper the metabolism of *Torulopsis lipofera* (den Dooren de Jongh, 1926) was investigated. This organism lends itself particularly well for a study of fat synthesis because of its easy growth and rapid conversion of carbohydrate into fat.

MORPHOLOGY AND GROWTH OF *TORULOPSIS LIPOFERA*

T. lipofera was first described by den Dooren de Jongh (1926) as an asporogenous soil yeast, capable of storing large droplets of fat in its cells. Lodder (1934) investigated the morphological properties and biochemical behaviour of this yeast somewhat more in detail. The strain of *T. lipofera* employed in the present study was obtained from the National Collection of Type Cultures. The yeast grows well on flour-wort-agar, or on simple inorganic medium (Stephenson, 1939) with 0·05 % marmite and carbohydrate, or on molasses containing NH_4, K and Mg salts. Morphologically the cultures consist principally of oval cells containing several droplets of fat and some round cells with a very large fat droplet filling most of the cell volume (see Pl. 4). These two types of cells do not appear to represent different strains, because on single-cell isolation of the oval type of cells round cells also were found in subsequent subcultures.

Growth of T. lipofera. The growth conditions were studied by measuring turbidimetrically the growth rate of the yeast cultivated at room temperature



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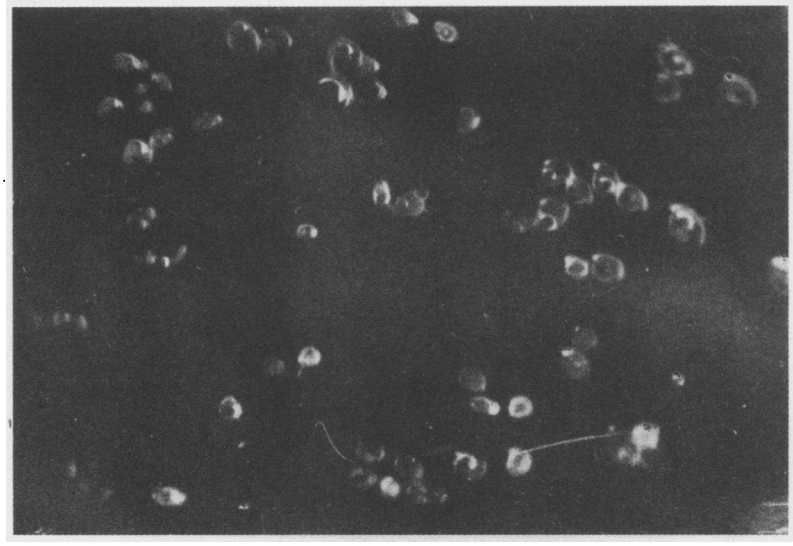

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Microphotographs of *T. lipofera*. A, magnification 500 × ; B, dark-ground illumination, magnification 600 × .

Table 1. *Yield of cells of* T. lipofera

Growth medium	Carbohydrate used; calc. as glucose (g.)	Yeast formed (g.)	Fat (fatty acids and unsaponifiable) (% dry wt.)	Ash (%)	Yeast as % of sugar used
Inorganic medium + marmite (0·05%) + glucose (2%)	9·41	3·92	37·2	—	42·0
Molasses + salts	51·4	24·5	43·0	4·9	47·7

in the inorganic medium (Stephenson, 1939) plus 0·05% marmite and 1–2% carbohydrate.

Approximately 300 ml. of the sterile medium were placed into each of a number of sterile glass tubes 75 cm. long and 3·5 cm. wide, provided with an outlet for withdrawing samples. Thorough aeration was achieved by passing sterile air through porous aerating blocks at a rate of 300 ml./min.; this produces bubbles of about 10μ diameter. The medium was then inoculated and the growth rate at room temperature (16–20°) was measured every 12 hr. for a period of 72–96 hr. After a lag period of about 6 hr. the growth was logarithmic.

Using this method the following different conditions had no effect on the growth rate: variation of pH between 4·0–6·0; addition of tryptic digest of casein (0·05–0·1% N) or asparagine 0·01%; variation of the glucose concentration between 0·5 and 2% and of the NH_4 between 0·03 and 0·12M; replacement of the glucose by an equivalent amount of sucrose or molasses. The generation time of the yeast in these experiments was 11–16 hr.

The optimal temperature for the growth of *T. lipofera* is 23–25°. At 30° the yeast grew for 1–2 days and then died off (Fig. 1). Under best conditions,

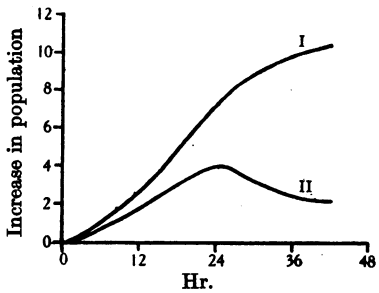

Fig. 1. Growth of *T. lipofera* at 25 and 30°. Medium: molasses containing 2% carbohydrate and NH_4, K, and Mg salts. I, 25°; II, 30°.

i.e. with good aeration and strict control of temperature, pH, N and carbohydrate (molasses) content, the generation time was 5¼–5½ hr.; this growth is relatively slow when compared with that of some other yeasts, e.g. *T. utilis* (Thaysen & Morris, 1943). The dry weight of yeast obtained was 42–48% of the carbohydrate used (Table 1); a remarkably high content of fatty material (unsaponifiable and fatty acids) is obtained.

Nitrogen and vitamin content of T. lipofera. Two samples of freeze-dried yeast were examined for N and vitamin content. Sample I was grown on glucose and contained 28·1% fatty material; Sample II was grown on molasses and contained 18·6% fatty material. Estimations of the vitamins were kindly carried out by Dr Y. L. Wang (aneurin, riboflavin, and nicotinic acid) and by Dr T. Moore (carotene). As shown in Table 2, the content of

Table 2. *Nitrogen and vitamin content of* T. lipofera

	Grown on Glucose I	Grown on Molasses II
Fat (%)	28·1	18·6
Total N (%)	5·43	5·85
Aneurin (μg./g.)	5·8	8·3
Riboflavin (μg./g.)	39	41
Nicotinic acid (μg./g.)	305	230
Carotene (μg./100 g.)	—	10

aneurin, riboflavin and nicotinic acid in *T. lipofera* is of the same order as in other yeasts; the content of carotene is low.

T. lipofera used in the experiments described below was grown at room temperature for 2–3 days in liquid inorganic medium (Stephenson, 1939) plus 0·05% marmite and glucose (1–2%) in the glass tubes described above. The yeast was then spun off, washed twice with 0·1M-NaCl and finally suspended in a small volume of saline. The yeast suspension was kept in an ice-chest and used within 2 days.

EXPERIMENTAL METHODS

1. *The manometric experiments* were carried out in Warburg manometers with conical flasks. A suspension containing 20–25 mg. yeast (dry wt.) in 0·1M-phosphate buffer of varying pH was measured into the main compartment of the flask. The substrates were placed in the side-arm. The volume of the cup contents was brought to 3·0 ml. with 0·1M-phosphate buffer. In experiments where the oxygen uptake was measured, 0·2 ml. 2N-NaOH was placed in the centre well.

The R.Q. was measured by means of the method of Warburg & Yabusoe (1924). As it was subsequently shown that CO_2 inhibits the utilization of glucose and fat formation (see Table 9), the measurements of the R.Q. are of only limited value. In anaerobic experiments nitrogen free from oxygen was used to fill the gas-space and yellow phosphorus was placed in the centre well to absorb traces of oxygen. After thermal equilibrium was reached the oxygen uptake and/or CO_2 output were measured over a period of 2–3 hr.

2. *Glucose* was estimated by the cerimetric method (Miller & Van Slyke, 1936) with Xylene Cyanol FF as indicator. A sample of the suspension was placed in a

25 ml. volumetric flask, 1 ml. of 2N-HCl added to stop any metabolic reactions, and the volume made up to the mark with water. After the yeast had settled a sample of the clear supernatant solution was used directly for the glucose estimation, without deproteinization. Duplicates of glucose estimations agreed within 1%.

3. *Phosphate* was estimated according to Fiske & Subbarow (1925).

Estimation of fat

The method was based on those of Kumagawa & Suto (1908), Lemeland (1923) and Belin (1926). The yeast is saponified with 2·5N-KOH in 98% ethanol, and the soap solution acidified and extracted with ether. The ether extract is dried *in vacuo*, the fatty material redissolved in light petroleum, filtered and estimated either (*a*) by weighing (macro-estimation) or (*b*) by determination of the C content manometrically according to Van Slyke & Folch (1940) (micro-estimation).

(*a*) *Macromethod.* The conditions for the saponification of the yeast were first worked out on a macro scale. Freeze-dried yeast (1 g.) was saponified with 20 ml. ethanolic KOH in a 50 ml. flask provided with an air condenser; the flask was heated on a steam-bath, the ethanolic soap solution diluted with water, the unsaponifiable matter extracted twice with ether, the ether extract washed twice with water, the ether distilled off and the unsaponifiable matter dried and weighed. The soap solution was combined with the washings of the ether extract of the unsaponifiable fraction, acidified with 5N-H_2SO_4, and the fatty acids extracted three or four times with ether. The combined extracts were washed twice with water, the ether distilled off, the dry fatty acids redissolved in light petroleum (b.p. 50–60°), filtered through asbestos, the solvent distilled off and the fatty acids dried *in vacuo* and weighed.

Table 3. *Macro-estimation of fat in* T. lipofera

(2½ hr. saponification.)

	Concentration of KOH (in 80% ethanol)		
	0·8N.	2·5N	5N
Unsaponifiable matter (%)	1·43	2·18	1·91
Fatty acids (%)	26·5	25·9	26·2
Mean mol. wt. of fatty acids	—	273·5	273·5
Carbon content of fatty acids (%)	—	75·84	76·19
Iodine value	—	89·6	83·5

As is shown in Table 3 the yeast is completely saponified by ethanolic-KOH (2·5N) in 2½ hr.; the use of ethanolic-KOH (0·8N) gave somewhat low figures for the unsaponifiable fraction. The mean molecular weight of the fatty acids was 268–282, their C content 75·8–76·2%, and their iodine value (Rosenmund & Kuhnhenn, 1923) 55–82 (see also Table 18).

(*b*) *Micromethod.*

Reagents: (1) 2·5N-KOH in 98% ethanol. The ethanol should be distilled over solid NaOH to remove substances forming resins on boiling with KOH. The reagent must be freshly prepared. (2) Ether, dried over Na, and freshly distilled. (3) Light petroleum, b.p. 50–60°, purified as described by Bloor (1929). (4) 50% H_2SO_4 (by vol.). (5) Reagents for macro- or micro-C-estimation (Van Slyke & Folch, 1940).

Apparatus: (1) 15 ml. centrifuge tubes with glass ground jointed air-condenser 30 cm. long. (2) Kutscher-Steudel liquid extractor of approx. 25 ml. capacity with 100 ml. flask. For details see Krebs, Smyth & Evans (1940). (3) Emich's microfilter with porous glass filter and a layer of washed asbestos. The filter must be dry before use. (4) A B19 standard joint cone with side-arm for suction as shown in Fig. 2A. (5) Van Slyke manometric apparatus equipped for C estimations (Van Slyke & Folch, 1940).

All glass apparatus used for fat estimations must be carefully cleaned with hot chromic-sulphuric acid mixture, washed and dried before use.

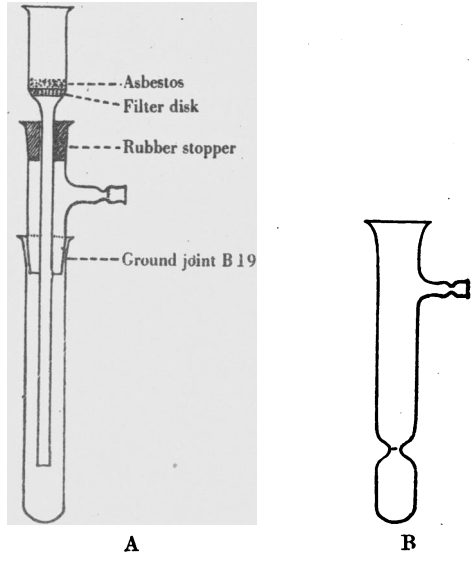

Fig. 2. Filtration of fat. A, filtration into combustion tube; B, calibrated suction tube.

Procedure. The yeast is transferred to the centrifuge tube. In metabolism experiments 10 ml. yeast suspension are run into the tube containing 1 ml. 2N-HCl to stop any metabolic reactions. The suspension is then centrifuged for 5–10 min. at 3000 r.p.m. and the supernatant siphoned off. The yeast is then washed with 4–5 ml. 0·1M-NaCl, centrifuged again and, after removal of the supernatant by suction, ethanolic KOH (4 ml.) and a few grains of clean quartz are placed in the tube, the air condenser fitted, and the yeast saponified by immersing the tube for 2½ hr. in a water-bath at 87–90°. This temperature is sufficient to keep the ethanolic solution boiling gently. After the saponification is completed the condenser is removed, and traces of soap which may have splashed on the joint are washed with 0·5 ml. water into the tube. The ethanol is now carefully evaporated on a water-bath at 85–90°. It was found useful to add a few more grains of quartz before the evaporation in order to prevent undue bumping. The soaps are then quantitatively transferred to the Kutscher-Steudel extractor by pipetting the diluted soap solution and three subsequent washings of the tube and condenser into the inner funnel of the extractor (volume of solution 15–20 ml.). The solution is then acidified with 1 ml. '50% H_2SO_4' and the fatty acids and the unsaponifiable matter are continuously extracted for 1½–2 hr. with a known volume of ether (40–45 ml.). About 12 ml. of ether pass through the

solution per min. The ether in the flask is then distilled off, the residue dried on a vacuum pump at 0·5–1 mm. Hg for 10 min., and 1 ml. light petroleum added. The solution, usually somewhat cloudy, is left standing for 30–60 min. at room temperature in order to allow emulsified resinous material to precipitate (Kumagawa & Suto, 1908). The solution is then filtered through the Emich filter by suction into the combustion tube. Three washings of the flask with 0·5–1 ml. light petroleum ensure quantitative transfer of the fatty material into the combustion tube. The solvent is removed as described by Van Slyke & Folch (1940) and a macro-C-estimation is carried out. The fatty material obtained in this way is generally white or yellowish. It consists of fatty acids and a small amount of unsaponifiable material (see Tables 3 and 18), and can be considered to be free from organic impurities.

If samples of the fatty material solution are to be used for analysis, the light petroleum extract is filtered into calibrated tubes prepared by drawing out suction tubes (conveniently old Thunberg tubes) and calibrating these with Hg after marking them on the narrow part (see Fig. 2B). A sample (1–2 ml. out of 4–6 ml.), after thorough mixing of the contents of the tube, is then taken for micro-C-determination.

As shown in Tables 3 and 18, the unsaponifiable material forms only a small fraction of the fat present (6·8–8·8%). The C content of the fatty acids is 76%. The fatty acid content of the yeast can therefore be obtained with sufficient accuracy by multiplying the C of fat found by the factor 1·32.

A blank analysis on the reagents is carried out, starting from the saponification. In these experiments the blank varied from 0·02 to 0·1 mg. C.

Table 4. *Micro-estimation of fat*

	Recovery of fat	
	(mg.)	(%)
7·65 mg. stearic acid	7·58	99·2
28·68 mg. yeast containing 8·06 mg. fat (macro-estimation)	7·94	98·5

As shown in Table 4, stearic acid is quantitatively recovered after having been treated with the

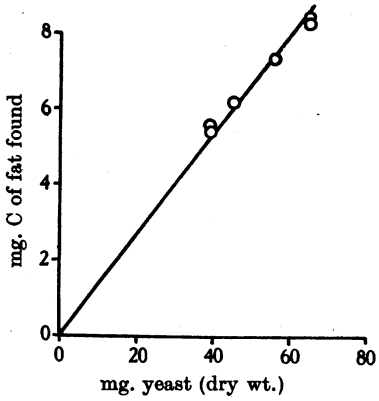

Fig. 3. Micro-estimation of C of fat in *T. lipofera*.

ethanolic KOH, acidified, extracted, filtered, and the C content estimated. The results on yeast agreed with those obtained by the macromethod within 1–3%. Duplicate microdeterminations agreed within 1–2%. The C of fat found increases linearly with increasing amounts of yeast (Fig. 3).

The micromethod described above may be conveniently applied to the determination of fatty material (unsaponifiable and fatty acids) in any tissue after ascertaining the C content of the fatty material, which may vary slightly according to the nature of the fatty acids and the content of unsaponifiable material. If necessary, of course, the unsaponifiable matter and fatty acids could be estimated separately in the usual way.

Procedure for studying fat metabolism

A suspension of yeast in 0·13 M-buffer of the required pH is made up, containing approximately 65 mg. of yeast (dry wt.)/10 ml. Ten ml. of this suspension are then pipetted into 100 ml. conical flasks and 3 ml. additions (substrate and/or water) are added. One ml. of the suspension is—if required—removed immediately for determination of the initial content of substrate. Ten ml. of the contents of one flask are pipetted into a centrifuge tube for the estimation of the initial content of fat. The remaining flasks are then stoppered and shaken in a thermostat at 25° (unless otherwise stated) for 3–9 hr. After removal of a flask from the thermostat, 1 ml. of its contents is taken for analysis of the substrate and 10 ml. for fat determination.

Where the formation of fat in absence of CO_2 was measured, the flasks were provided with a centre-well containing 0·5 ml. 5 N-NaOH and a roll of filter paper to absorb the CO_2.

Under the conditions employed no growth of the yeast occurs in the experimental period, as shown by the cell count (see Table 6).

Terms used (conversion coefficient). In experiments on fat formation, the fat formed is expressed in mg. C of fat formed in 10 ml. of yeast suspension, i.e. in about 50 mg. yeast. The utilization of substrate is expressed in mg. substrate used in 10 ml. suspension. The term *conversion coefficient*, i.e. the percentage of C of substrate converted into C of fat, is used as a measure of the efficiency of the conversion process. For glucose as substrate the conversion coefficient is represented by the expression:

$$\frac{\text{mg. C of fat formed} \times 100}{\text{mg. of glucose used} \times 40} \times 100\,\%.$$

The conversion coefficient is directly related to the R.Q., i.e. the R.Q. increases above 1·0 with increasing conversion coefficient.

Because of the experimental error involved in the estimation of glucose and the C of fat, only differences in the conversion coefficient of more than 2% are significant.

On the assumption that in the course of fat synthesis glucose is first broken down to 2 mol. of C_2 compounds and 2 mol. of CO_2, and that the C_2 compounds are then condensed to fatty acids, the highest conversion coefficient theoretically possible is 66·7 % (Magnus-Levy, 1925). Since *T. lipofera* metabolizes glucose only aerobically, a loss of C in the course of fat formation higher than the above may be expected and the maximal conversion coefficient therefore lower. Terroine & Bonnet (1927), measuring the caloric value of glucose used and fat formed in *Sterigmatocystis nigra*, arrived at a proportion of 4·38 mol. of glucose used per 1 mol. of fatty acid formed (oleic acid); the conversion coefficient according to these experiments would be 71 %. by the constant conversion coefficient. A cell count, carried out at the beginning and at the end of the experimental period, showed that no cell multiplication had taken place. In absence of substrate there was a disappearance of fat, linear with time.

The conversion coefficient found at 25° in the presence of $PO_4^{\equiv}$ (see below) varied between 11 and 30 %, usually between 18 and 25 %. A decrease in the conversion coefficient was observed when the washed yeast suspension was kept in the ice-chest for more than 24 hr.

The observation of Lodder (1934), that *T. lipofera* does not ferment glucose is confirmed. Under anaerobic conditions no CO_2 is formed from glucose by the yeast, and no glucose is used.

Table 5. *Oxygen uptake, CO_2 output and glucose utilization in* T. lipofera

(26·2 mg. yeast (dry wt.), 0·1 M-phosphate buffer pH 5·5; 25°; 3 hr.)

Substrate	Oxygen used (μl.)	CO_2 output (μl.)	Glucose used (μl.)	R.Q.	$\frac{\mu l.\ O_2\ used}{\mu l.\ glucose\ used}$
0	262	202	—	0·77	—
Glucose	1988	2990	1130	1·50	1·76

Table 6. *Time curve of fat formation from glucose in* T. lipofera

(49·4 mg. yeast (dry wt.)/10 ml.; 0·1 M-phosphate buffer pH 6·7; 120 mg. glucose/10 ml.; 25°.)

	Substrate					
	None			Glucose		
Time (hr.)	Fat found (mg. C)	Fat used (mg. C)	Glucose used (mg.)	Fat found (mg. C)	Fat formed (mg. C)	Conversion coefficient (%)
0	8·69	—	—	—	—	—
3	8·50	0·19	21·6	9·65	0·96	11·1
6	8·35	0·34	50·8	10·93	2·25	11·1
9	7·90	0·80	71·5	12·40	3·71	12·9

Cell count (in presence of glucose): initial, 316×10^6 cells/ml.; after 9 hr., 307×10^6 cells/ml.

RESULTS

Oxidation of glucose and formation of fat. Glucose is rapidly oxidized by *T. lipofera*. The Q_{O_2} in presence of glucose varied between 20 and 30, 1·3–2·0 mol. O_2/mol. glucose being used. Since 6 mol. of O_2 are required for the complete combustion of 1 mol. of glucose, the oxidation of glucose by this organism is incomplete. The R.Q. found was always higher than 1·0, varying between 1·15 and 1·5, and indicating a formation of fat from glucose. In the absence of substrate the Q_{O_2} was 3–7, the R.Q. varying from 0·7 to 0·95, indicating an oxidation of stored fat (Table 5).

Table 6 shows that glucose utilization and fat formation increase linearly with time. In the recorded experiment the fat content increased in the course of 9 hr. from 8·69 to 12·4 mg. C/49·4 mg. yeast, i.e. from 23 to 33 %. The C of fat formed remained proportional to the glucose used as shown

Effect of pH *on* R.Q. *and fat formation.* The effect of pH between 5·5–7·5 on the R.Q. and fat formation (Table 7) shows that with increasing pH the O_2 uptake and the R.Q. decrease. At pH 7·5 the R.Q. was 0·74; in other experiments the R.Q. at that pH was about 1·0. As indicated by the measurements of R.Q., the fat formation decreases with increasing pH and was found highest at pH 5·5–6·0 (Fig. 4). The conversion coefficient was also highest at pH 5·5.

Effect of temperature on fat formation. The effect of temperature on fat formation was studied by shaking simultaneously a number of flasks in baths of varying temperatures (15–37°). The utilization of glucose reached a peak at 25–30°, the fat formation was highest at 25°. The conversion of carbohydrate into fat was highest at 20–25°, i.e. at the optimal growth temperature of the organism (Table 8).

Effect of oxygen and CO_2. The effect of oxygen was examined because, under our experimental

Table 7. *Effect of* pH *on* R.Q.

(14·0 mg. cells (dry wt.); 0·1 M-phosphate buffer, glucose; 25°, 3 hr.)

	pH 5·5	6·5	7·0	7·5
O_2 uptake (μl.)	1203	867	555	400
CO_2 output (μl.)	1448	970	637	297
R.Q.	1·20	1·18	1·15	0·74

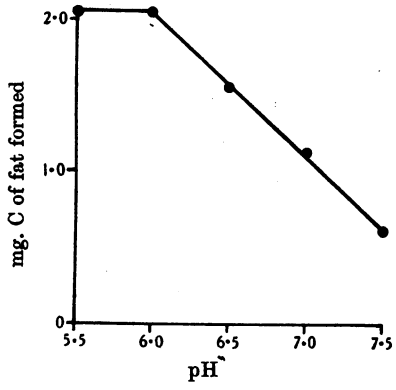

Fig. 4. Effect of pH on fat formation. 53·5 mg. yeast (dry wt.); 0·1 M-phosphate buffer; 100 mg. glucose/10 ml.; 25°; 3 hr.

Table 8. *Effect of temperature on fat formation*

(50·5 mg. yeast (dry wt.); 0·1 M-phosphate buffer pH 5·5; 80 mg. glucose/10 ml.; 5 hr.)

	Temperature 15°	20°	25°	30°	37°
Glucose used (mg.)	16·1	25·6	38·7	35·3	12·9
C content of fat formed (mg.)	0·21	2·29	3·05	2·77	0·30
Conversion coefficient (%)	3·1	22·3	20·1	19·7	5·8

conditions as compared with the conditions for optimal growth, it might be a limiting factor in fat formation. The effect of CO_2 was studied in view of the known role of CO_2 in biological synthesis

Table 9. *Effect of oxygen and* CO_2 *on fat formation*

(50·5 mg. yeast (dry wt.); 0·1 M-phosphate buffer pH 6·0; 100 mg. glucose/10 ml.; 25°; 6 hr.)

Gas mixture	Glucose used (mg.)	C of fat formed (mg.)	Conversion coefficient (%)
Air; NaOH	58·3	5·81	24·9
Air + 5% CO_2	51·3	4·83	23·6
50% O_2; NaOH	59·5	6·21	26·1
50% O_2 + 5% CO_2	49·2	4·83	24·5

(Stephenson & Krebs, 1941). The results recorded in Table 9 show that increased concentration of oxygen has no appreciable effect on the utilization of glucose or on the conversion of carbohydrate to fat. The presence of CO_2 decreases the utilization of carbohydrate and the fat formation, but does not affect the conversion coefficient.

Effect of NH_4^+ *on respiration and fat formation.* It is known from work on *Endomycopsis vernalis* and other yeasts that the formation of fat from carbohydrate is impaired in the presence of nitrogenous substances owing to the use of carbohydrate for growth (Nägeli & Loew, 1878; Smedley MacLean, 1922; Raaf, 1941). The effect of NH_4Cl on the respiration and the fat formation was therefore examined. Since the lag period of the growth of *T. lipofera* was found to be about 6 hr., no appreciable multiplication of the cells could have taken place during the experimental period (3–4 hr.).

Table 10 shows that increasing concentrations of NH_4Cl (0·067–0·2 M) decrease the oxidation of glucose. The formation of fat and the conversion coefficient are decreased by increasing concentrations of NH_4Cl (0·025–0·2 M) (Table 11). Urea, which has been shown by Lodder (1934) to serve as a source of N for the growth of the organism, has an effect similar to that of NH_4Cl on the formation of fat and the conversion coefficient (Table 12).

Table 10. *Effect of* NH_4Cl *on the respiration of* T. lipofera

(26·5 mg. yeast (dry wt.); 0·1 M-phosphate buffer pH 5·5; 15 mg. glucose; 25°; 3 hr.)

NH_4Cl concentration (M)	O_2 uptake (μl.)	Glucose used (mg.)
0	1871	10·76
0·067	1797	9·58
0·134	1600	8·53
0·200	1450	7·80

Table 11. *Effect of* NH_4Cl *on fat formation*

(50·2 mg. yeast (dry wt.)/10 ml.; 0·1 M-phosphate buffer pH 6·0; 50 mg. glucose/10 ml.; 25°; 4 hr.)

NH_4Cl concentration (M)	Glucose used (mg.)	C of fat formed (mg.)	Conversion coefficient (%)
0	44·2	3·14	17·7
0·025	43·5	2·89	16·5
0·05	41·8	2·20	13·1
0·20	35·8	1·39	9·7

Table 12. *Effect of urea on fat formation*

(52·0 mg. yeast (dry wt.)/10 ml.; 0·1 M-phosphate buffer pH 6·0; 100 mg. glucose/10 ml.; 25°; 4 hr.)

Urea concentration (M)	Glucose used (mg.)	C of fat formed (mg.)	Conversion coefficient (%)
0	31·9	2·74	21·4
0·01	30·5	2·78	22·8
0·03	30·1	2·29	19·0
0·10	31·0	1·82	14·6

Effect of $PO_4^{=}$ *on fat formation.* Smedley MacLean & Hoffert (1923, 1924) were the first to show that the phosphate ion is of importance for the conversion of carbohydrate into fat. As shown in Table 13, $PO_4^{=}$ has no effect on the utilization of glucose, but considerably enhances the conversion of carbohydrate into fat. Under the experimental conditions used, 0·001 M-KH_2PO_4 was sufficient to raise the conversion coefficient to the maximum; higher concentrations of $PO_4^{=}$ had no further effect. The experiments confirm the importance of $PO_4^{=}$ in the synthesis of fat from carbohydrate. The formation of some fat in absence of added $PO_4^{=}$ is no doubt due to the phosphate present in the cell.

Table 13. *Effect of* $PO_4^{=}$ *on fat formation*

(0·1 M-phthalate buffer pH 6·0; 100 mg. glucose/10 ml.; 25°; 5 hr.)

KH_2PO_4 concentration (M)	Glucose used (mg.)	C of fat formed (mg.)	Conversion coefficient (%)
Exp. 1, 52·8 mg. yeast:			
0	53·9	2·10	9·8
0·001	51·0	3·87	19·5
0·01	50·6	3·79	18·8
0·05	51·3	3·99	19·5
Exp. 2, 50·0 mg. yeast:			
0	41·7	2·52	15·1
0·001	40·1	3·16	19·6

Effect of Mg^{++} *and* Mn^{++} *etc., on fat formation.* Ohlmeyer & Ochoa (1937) have shown that Mg^{++} is of importance in the carbohydrate breakdown in yeast, and that it can be replaced by Mn^{++}. The effect of additions of Mg^{++} (0·01–0·1 M) or Mn^{++} (0·001–0·01 M) on the formation of fat from glucose by *T. lipofera* was therefore examined. The experiment recorded in Table 14 shows that additional Mg^{++} or Mn^{++} has no effect on the conversion of carbohydrate to fat. This result does not exclude the possible role of Mg, since enough Mg for the metabolism of the organism may be present in its cells.

Table 14. *Effect of* Mg^{++}, Mn^{++}, *aneurin and choline on fat formation*

(49·6 mg. yeast (dry wt.)/10 ml.; 100 mg. glucose/10 ml.; 0·1 M-phosphate buffer pH 6·0; 25°; 6 hr.)

Addition	Glucose used (mg.)	C of fat formed (mg.)	Conversion coefficient (%)
0	46·0	4·49	24·5
0·01 M-$MgSO_4$	44·0	4·32	24·6
0·1 M-$MgSO_4$	45·0	4·46	24·8
0·001 M-$MnSO_4$	43·7	4·54	26·0
0·01 M-$MnSO_4$	42·8	4·35	25·4
Aneurin 1 μg./ml.	44·5	4·62	26·0
Choline 1 mg./ml.	47·5	4·70	24·8

Additions of aneurin or choline chloride had no effect on the fat formation from glucose, though these two substances have been shown to be of importance in the fat metabolism of the animal (McHenry & Gavin, 1941; Perlmann & Chaikoff, 1939).

Effect of iodoacetate on respiration and fat formation. The effect of iodoacetate, which is known as an inhibitor of carbohydrate breakdown, on the oxidation of glucose and formation of fat was examined as it was thought that it might shed some light on the intermediate carbohydrate breakdown products formed in the course of fat synthesis. Table 15 shows that, in absence of substrate, 0·0003 M-iodoacetate has little effect on the respiration or R.Q. of *T. lipofera.* In the presence of glucose iodoacetate considerably inhibits the oxygen uptake and reduces the R.Q. to that of the control without substrate, i.e. below 1·0. A similar effect of iodoacetate, in reducing the R.Q. of frog muscle below 1·0, has been described by Meyerhof & Boyland (1931). It should be noted that whilst in baker's yeast iodoacetate does not inhibit the oxidation of glucose (Lundsgaard, 1930), in *T. lipofera* the oxygen uptake in the presence of glucose is considerably inhibited. Bernheim (1942) also found an inhibition of the oxidation of glucose by iodoacetate in the aerobic yeast *Blastomyces dermatiditis.*

Table 15. *Effect of iodoacetate on the respiration of* T. lipofera

(20·7 mg. yeast; 0·1 M-KH_2PO_4; 25°; 2 hr.)

Substrate	Iodo-acetate	O_2 uptake (μl.)	CO_2 output (μl.)	R.Q.
0	0	284	273·5	0·96
0	0·0003 M	242	225·5	0·93
0·01 M-glucose	0	945	1078	1·14
0·01 M-glucose	0·0003 M	324	306	0·94

Table 16. *Effect of iodoacetate on fat formation*

(Exps. 1 and 2: 0·1 M-phthalate buffer pH 6·0; 0·001 M-KH_2PO_4. Exp. 3: 0·1 M-phosphate buffer pH 5·5; 100 mg. glucose/10 ml.; 25°; 5 hr.)

Exp. no.	Iodo-acetate concen-tration (M)	Glucose used (mg.)	C of fat formed (mg.)	Con-version coeffi-cient (%)
(1) 52·8 mg. yeast	0	50·6	+3·79	18·8
	0·0002	43·6	+3·23	18·5
(2) 50·0 mg. yeast	0	—	−0·03	—
	0	40·1	+3·16	19·65
	0·0005	—	−0·18	—
	0·0005	13·0	−0·05	—
(3) 50·0 mg. yeast	0	43·8	+2·36	13·5
	0·0005	18·4	−0·58	—
	0·00025	30·6	−0·10	—
	0·00017	34·8	+0·98	7·0

As shown in Table 16, iodoacetate inhibits the fat synthesis. At concentrations higher than

0·00025M some glucose is utilized, accompanied by a disappearance of fat; this corroborates the results obtained by R.Q. measurements. Concentrations of iodoacetate below 0·00025M inhibit to some extent both glucose utilization and fat synthesis. Some fat is formed in the presence of such low concentrations of iodoacetate, and, as shown in Exp. 1 (Table 16), the conversion coefficient is in some cases identical with that of the control without iodoacetate. These experiments suggest that the action of iodoacetate is due primarily to a specific inhibition of the carbohydrate breakdown, though it does not appear to influence the synthesis of fat from carbohydrate breakdown products.

Distribution of acid-soluble P *in* T. lipofera *in the presence of iodoacetate and fluoride*

Macfarlane (1936) has shown that considerable changes occur in the distribution of P in the yeast cell during fermentation and that the pyrophosphate-P and organic-P fractions increase in the presence of iodoacetate or fluoride. Since it was shown above that in the presence of 0·0003–0·0005M-iodoacetate *T. lipofera* does not form fat, but can utilize some glucose, the changes of P distribution in this yeast during the oxidation of glucose in the presence of iodoacetate and fluoride were studied.

Equal amounts of a suspension of freshly collected yeast (grown in liquid medium) in 0·1M-NaCl containing 0·002M-KH_2PO_4 were placed into three 1 l. cylinders (1·725 g. yeast (dry wt.)/cylinder). The contents of one cylinder were immediately centrifuged off, the yeast cake washed with saline and then twice extracted with 50 ml. 4% trichloroacetic acid. To the remaining two cylinders iodoacetate (final concentration 0·0003M) and sodium fluoride (0·025M) respectively were added; after 10 min. glucose was added and the contents of the cylinders were well aerated for 6 hr. at room temperature (18°) by passing compressed air through porous blocks. Details of the contents of the cylinders are given in the legend of Table 17. At the end of the experimental period the contents of the cylinders were treated as described for the control above. Samples of the trichloroacetic acid extracts were then taken for estimations of the various P fractions as described by Macfarlane (1939).

The results (Table 17) show that in the presence of both iodoacetate and sodium fluoride there is a disappearance of orthophosphate-P, no appreciable change in the pyrophosphate-P, and a considerable increase in the organic-P. The total acid-soluble P was also increased. The increase of the organic-P in the presence of iodoacetate (1·12 mg. P/1 g. yeast) is nearly accounted for by the increase in hexosediphosphate (0·92 mg. P, i.e. 82% of the organic-P formed). It should be noted that under the experimental conditions described the alkali-labile P (triosephosphate-P) decreased. Iodoacetate is, under anaerobic conditions, or in simple enzymic systems, regarded as a specific inhibitor of the triosephosphate dehydrogenase, and thus brings about an accumulation of alkali-labile-P in the course of carbohydrate breakdown; the present result, therefore, if confirmed by isolation of hexosediphosphate, suggests some differences between oxidative and anaerobic phosphorylation in yeast cells (cf. Mann, 1943). Sodium fluoride brings about an increase of organic phosphate, which is only to a small extent hydrolyzable by N-HCl at 100° in 180 min. If there exists a parallelism between the anaerobic carbohydrate breakdown in other yeasts, and the aerobic phosphorylation in *T. lipofera*, this result would suggest a formation of phosphoglyceric acid.

Carbon balance sheet in T. lipofera

The experiments were carried out in the following way (see Birkinshaw & Raistrick, 1931):

A suspension of yeast (2·5–3·1 g. dry wt./100 ml.) in 0·1M-NaCl containing glucose and 0·01–0·05M-KH_2PO_4 was prepared. One hundred ml. of this suspension were immediately spun off, the yeast cake washed once with 0·1M-NaCl and once with water, and the yeast freeze-dried to constant weight over P_2O_5. The supernatant liquor and the washings were combined and samples of the solution used for the estimation of the various compounds as shown below. One hundred ml. of the yeast suspension were placed in a 3 l. conical flask and CO_2-free air passed at a rate of approximately 100 ml./min. through this suspension whilst it was shaking in a thermostat at 25°. The air passing out of the flask was first dried over $CaCl_2$, and then passed through a potash bulb and three soda-lime U-tubes followed by one of $CaCl_2$. A baryta trap following the U-tubes showed that in this arrangement all the CO_2 was absorbed. After the experimental period (5 hr.) the yeast was spun off and treated in the same way as described above.

In the freeze-dried yeast the C, N, fat and carbohydrate contents were estimated: C according to Van Slyke & Folch

Table 17. P *distribution in* T. lipofera

(Each cylinder contains 450 ml. 0·1M-NaCl and 0·002M-KH_2PO_4, with the following additions:
(1) 1·725 g. yeast.
(2) 1·725 g. yeast; 0·0003M-iodoacetate; 5 g. glucose.
(3) 1·725 g. yeast; 0·025M-NaF; 5 g. glucose.)

	mg. acid-soluble P/g. yeast as					
Cylinder	Orthophosphate	Pyrophosphate	Organic-P	Hexose diphosphate	Alkali labile-P	Total
1	1·15	2·04	2·14	1·75	0·30	5·27
2	0·85	2·06	3·26	2·68	0·14	6·17
3	0·70	2·26	3·18	2·12	0·23	6·15

(1940), N by Kjeldahl, fatty material by the macro-method described above. The iodine value of the fatty acids was estimated according to Rosenmund & Kuhnhenn (1923), the mean molecular weight in the usual way, using an indicator described elsewhere (Kleinzeller & Trim, 1944). The various carbohydrate fractions were estimated (on the advice of Dr D. J. Bell) as follows: A sample of dry yeast (400–500 mg.) was treated with 2 ml. 40% KOH in a boiling water-bath for 2 hr. Ethanol (8 ml.) was then added, the precipitate centrifuged off and washed four times with 80% ethanol, taken up in H_2O and reprecipitated at a concentration of 80% ethanol, until the washings were colourless and no precipitate was formed in the washings on acidification. The precipitate was then extracted four times with small amounts of water, the insoluble matter dried *in vacuo* and weighed. This fraction represents water-insoluble carbohydrate and probably originates from the cell wall of the yeast (carbohydrate 1). The aqueous extract was reprecipitated by adding ethanol (final concentration 60%), the precipitate centrifuged off, redissolved in water, reprecipitated by ethanol, dried *in vacuo* and weighed (carbohydrate 2). This fraction represents carbohydrate similar to or identical with glycogen and mannan. To the combined supernatants of the precipitation of the 'glycogen and mannan fraction' further ethanol was added to a final concentration of 80%. The carbohydrate thus precipitated was dried and weighed (carbohydrate 3).

In the medium the contents of non-volatile C, glucose (Lane & Eynon, 1923), lactic acid (Friedemann & Graeser, 1933), ethanol (Friedemann & Klaas, 1936) were estimated. The pH was measured electrometrically. The nitroprusside reaction was used to test for the presence of keto-acids.

As shown in Table 18, most of the C of glucose used could be accounted for (97%). The conversion coefficient in these experiments was of the same order as in those described above. Very little, if any, unsaponifiable matter was formed. The fatty acids at the end of the experimental period had a markedly lower iodine value, whilst the mean molecular weight did not change appreciably. The decrease in the iodine value may be due either to direct synthesis of saturated fatty acids or to a saturation following the condensation of the short-chain carbohydrate breakdown products to fatty acids. It is of interest to note that in the animal also the fatty acids formed from carbohydrate are of a more saturated character (Longenecker, 1941). Little acid, little, if any, ethanol, and no lactic acid or keto-acids were formed. Since carbohydrate accumulated within the cell at the same time as fatty acids, a more detailed analysis of the stoichiometric propor-

Table 18. *Carbon balance sheet in* T. lipofera

(Exp. 1: Yeast in 0·1 M-NaCl containing 0·01 M-KH_2PO_4; 25°; 5 hr.
Exp. 2: Yeast in 0·1 M-NaCl containing 0·05 M-KH_2PO_4; 25°; 5 hr.)

Exp.		Initial	After 5 hr.	Formed or used (mg.)	C used (mg.)	C formed (mg.)
1	pH	3·55	3·17			
	Glucose	2475 mg.	1699 mg.	− 776	311	—
	CO_2	—	387 mg.	+ 387	—	103
	Yeast (dry wt.)	2616 mg.	3029 mg.	+ 413	—	—
	C in yeast	1366 mg.	1566 mg.	+ 200	—	200
	Unsaponifiable	61 mg.	63 mg.	+ 2	—	(76·5)
	Fatty acids	732 mg.	831 mg.	+ 99		
	Iodine value	66·6	55·4			
	Mean mol. wt.	280	268			
				Total	311	303
					i.e. 97%	
2	pH	3·66	3·21			
	Glucose	3290 mg.	1540 mg.	−1750	700	—
	CO_2	—	1105 mg.	+1105	—	301
	C in medium (non-volatile)	1315 mg.	634 mg.	− 681	(681)	—
	Ethanol	0	10 mg.	+ 10	—	5
	Lactic acid	0	0	—	—	—
	Ketonic acids	0	0	—	—	—
	Yeast (dry wt.)	3146 mg.	3923 mg.	+ 774	—	—
	C in yeast	1533 mg.	1903 mg.	+ 370	—	370
	N in yeast	164 mg.	169 mg.	+ 5	—	—
	Unsaponifiable	56 mg.	62 mg.	+ 6	—	(158)
	Fatty acids	706 mg.	908 mg.	+ 202		
	Iodine value	82·3	77·3			
	Mean mol. wt.	270	275			
	Carbohydrate in yeast					
	1	332 mg.	635 mg.	+ 302	—	(151)
	2	216 mg.	278 mg.	+ 62		
	3	40 mg.	16 mg.	− 24		
				Total	700	676
					i.e. 97%	

The figures in brackets indicate that they have already been included in the balance sheet under a different heading.

tions of glucose converted into fat and CO_2 does not yet appear possible.

Lindner, quoted by Fink *et al.* (1937) in his large-scale test on fat formation in *Endomycopsis vernalis*, found that, out of 12·5 kg. of sugar used, 4·16 kg. were converted into CO_2 and 0·95 kg. fat was formed. These data correspond to a conversion coefficient of 14·25 %; 22·7 % of the C of sugar used was recovered as CO_2. In the above experiments the conversion coefficient was 24·6 and 22·6 % respectively, and 33·1 and 43 % of the C of glucose were oxidized to CO_2.

Oxidation of various substrates

Oxygen uptake in conditions excluding cell-multiplication was measured manometrically in 0·1 M-KH_2PO_4 in order to test which substances are readily oxidized and hence may play a part in fat synthesis. The substrates (0·01–0·05 M final concentration) were placed in the side-arm of the vessels and the contents of the cups were mixed after thermal equilibrium had been reached. The yeast used in these experiments was grown in liquid medium containing glucose as described above. It appears from Table 19 that glucose, fructose, maltose, ethanol are rapidly oxidized; galactose, sucrose, dihydroxyacetone, methylglyoxal, acetate, butyrate and stearate are more slowly oxidized, whilst lactate, pyruvate, propionate, glycerol, glyceraldehyde, acetaldehyde, succinate, fumarate, *dl*-malate, gluconate and citrate are little, if at all, oxidized. The slow oxidation of pyruvate by *T. lipofera* is in contrast with the behaviour of other yeasts, which oxidize pyruvate at a rapid rate (Smythe, 1938). Oleate (0·01 M) decreases the oxygen uptake as compared with the control without substrate. Bernheim (1942) found a similar effect of oleate on the oxygen uptake of *Blastomyces dermatiditis*.

Table 19. *Oxidation of various substrates by* T. lipofera

(0·1 M-KH_2PO_4; 25°; 2 hr.)

Exp.	Substrate	Concentration (M)	Q_{O_2}
1	0	—	3·5
	Glucose	0·025	26·1
	Ethanol	0·05	24·4
	Acetate	0·05	12·6
	Propionate	0·05	8·0
	Lactate	0·05	7·9
	Butyrate	0·05	12·1
	Oleate	0·01	2·6
	Stearate	0·01	9·4
2	0	—	4·8
	Glucose	0·025	22·0
	Fructose	0·025	16·9
	Galactose	0·025	9·2
	Sucrose	0·025	7·4
	Maltose	0·025	14·8
	Ethanol	0·05	19·1
	Acetaldehyde	0·05	6·6
	Pyruvate	0·05	5·7
	Glycerol	0·05	6·2
3	0	—	5·1
	Succinate	0·025	6·2
	Fumarate	0·025	6·7
	dl-Malate	0·025	6·7
4	0	—	7·1
	Glucose	0·01	18·0
	Glyceraldehyde	0·05	8·6
	Methylglyoxal	0·05	10·5
	Dihydroxyacetone	0·05	13·1
5	0	—	7·7
	Glucose	0·01	18·3
	Pyruvate	0·05	9·2
	Dihydroxyacetone	0·05	11·6
	Citrate	0·02	8·4
6	0	—	3·0
	Gluconate	0·02	5·3

Tošić & Krebs (1943) reported that in acetic acid bacteria the oxidation of acetate is enhanced by the presence of small amounts of some easily oxidizable substance, e.g. glucose. The effect of catalytic amounts of glucose on the oxidation of acetic, propionic and butyric acids was therefore examined. Table 20 shows that none of these substrates is more rapidly oxidized in the presence of glucose. Under anaerobic conditions at pH 6·7 or 5·3 *T. lipofera* does not form CO_2 in the presence of pyruvate.

Table 20. *Effect of catalytic amounts of glucose on the oxidation of acetate, propionate and butyrate*

(24·7 mg. yeast (dry wt.); 0·1 M-KH_2PO_4; 25°; 2 hr.)

Substrate	Without glucose (O_2 uptake, μl.)	22 μl. glucose (O_2 uptake, μl.)	Extra O_2 due to glucose (μl.)
0	215	264	49
0·05 M-acetate	565	579	14
0·05 M-propionate	364	437	73
0·05 M-butyrate	504	550	46

Formation of fat from various substrates

The formation of fat from various carbohydrates was examined. As shown in Table 21, *T. lipofera*, grown on glucose, forms fat equally well from glucose or fructose, less from sucrose or maltose, and only little from galactose. The yeast, when adapted to sucrose by cultivation on solid molasses medium and finally in liquid medium containing sucrose, formed fat equally well from glucose and sucrose (Table 21, Exp. 4). It appears, therefore, that invertase is an adaptive enzyme in *T. lipofera*. In *Endomycopsis vernalis* Reichel & Reinmuth (1938) found that fructose and sucrose were more easily converted into fat than glucose.

There is considerable experimental evidence that in a number of micro-organisms both ethanol and acetic acid increase the fat formation from glucose

Table 21. *Formation of fat from various carbohydrates*

(50–52 mg. yeast (dry wt.); 0·1 M-phosphate buffer pH 5·5; 5 hr.; 25°; 100 mg. substrate/10 ml. Yeast in Exps. 1–3 grown on glucose; in Exp. 4 grown on sucrose.)

Exp.	Substrate	C of fat formed (mg.)
1	Glucose	5·80
	Fructose	5·50
	Sucrose	3·68
	Maltose	3·36
2	Glucose	2·33
	Galactose	0·43
3	Glucose	2·12
	Sucrose	1·38
4	Glucose	2·81
	Sucrose	2·96

claimed that acetaldehyde is converted into fat by *Endomycopsis vernalis*; Smedley MacLean & Hoffert (1926) were unable to demonstrate a synthesis of fat from acetaldehyde by baker's yeast.

In the presence of pyruvate, lactate, dihydroxyacetone, gluconate, butyrate, α-ketoglutarate and mixed amino-acids (tryptic casein digest) no fat was formed by *T. lipofera* under the experimental conditions employed. In contrast to this yeast, *Endomycopsis vernalis* and baker's yeast have been shown to form fat from pyruvate and lactate (Haehn & Kintoff, 1926; Smedley MacLean & Hoffert, 1926; Smythe, 1938).

DISCUSSION

The results reported above show that the aerobic yeast *T. lipofera* is capable of storing large amounts

Table 22. *Formation of fat in* T. lipofera *in presence of ethanol and acetic acid*

(Exp. 1: 50·6 mg. yeast/10 ml.; 0·1 M-phosphate buffer pH 6·7; 25°; 4 hr.
Exp. 2: 50·3 mg. yeast/10 ml.; 0·1 M-phosphate buffer pH 5·5; 25°; 5 hr.)

Exp.	Substrate	Glucose used (mg.)	C of fat formed (mg.)	Conversion coefficient (%)
1	0	—	−1·15	—
	0·06 M-glucose	20·2	+2·70	33·4
	0·1 M-ethanol	—	+0·38	—
	0·06 M-glucose + 0·1 M-ethanol	16·6	+1·70	25·6
	0·06 M-acetate	—	+0·02	—
	0·06 M-glucose + 0·06 M-acetate	11·8	+1·58	33·5
2	0·06 M-glucose	36·6	+2·07	14·1
	0·1 M-ethanol	—	−0·46	—
	0·06 M-glucose + 0·1 M-ethanol	31·3	+1·79	14·3
	0·1 M-acetate	—	−0·50	—
	0·06 M-glucose + 0·1 M-acetate	3·0	+0·26	(21·6)*

* On account of the small glucose uptake and fat formation the conversion coefficient is not reliable.

(Lindner, 1922; Stephenson & Whetham, 1922, 1923; Smedley MacLean & Hoffert, 1923, 1924, 1926; Haehn & Kintoff, 1926). The formation of fat from these substrates in absence and in presence of glucose by *T. lipofera* was therefore examined at pH 6·7 and 5·5. As shown in Table 22, no fat is formed from ethanol or acetic acid in absence of glucose, and both substrates inhibit to some extent the formation of fat from glucose. Since ethanol is rapidly oxidized by this organism, but is not converted into fat, this result appears to exclude ethanol as an intermediate in the fat synthesis. Acetic acid is only slowly oxidized; the failure to find fat formation from this substrate, however, cannot be considered to be conclusive evidence that it does not play any role as intermediate in the synthesis of fat. The permeability of the cell membrane to acetic acid may be a limiting factor in the metabolism of this yeast.

In the presence of acetaldehyde, small but definite amounts of fat were formed by *T. lipofera* (Table 23), although acetaldehyde is only very slowly oxidized by this yeast (Table 19). Haehn & Kintoff (1926)

Table 23. *Formation of fat from various substrates*

(50–52 mg. yeast/10 ml.; 0·1 M-phosphate buffer pH 5·5; 25°; 5 hr.)

Exp.	Substrate	C of fat formed (mg.)
1	0·06 M-glucose	+1·86
	0·05 M-gluconate	+0·26
	0·05 M-dihydroxyacetone	−0·19
	0·05 M-pyruvate	−1·24
	0·05 M-lactate	−0·13
2	0·06 M-glucose	+2·33
	0·05 M-gluconate	−0·71
	0·05 M-pyruvate	−1·69
3	0·06 M-glucose	+2·12
	0·02 M-acetaldehyde	+1·28
4	0·06 M-glucose	+1·69
	0·02 M-acetaldehyde (duplicate)	+0·25; +0·51
	Tryptic casein digest, 0·1 mg. N/ml. (duplicate)	−0·48; −0·49
5	0·06 M-glucose	+1·81
	0·04 M-butyrate (duplicate)	−0·06; −0·05
	0·02 M-acetaldehyde (duplicate)	+0·66; +0·60
	0·04 M-α-ketoglutarate	−0·16

of fat in its cells. The conversion of carbohydrate into fat by this organism appears to be more efficient than in *Endomycopsis vernalis*. No detailed information is available about *Nectaromyces reukaufii*, which has been recently shown capable of storing large amounts of fat (Rippel, 1943).

Under anaerobic conditions no glucose is utilized. The carbohydrate breakdown and fat formation in this yeast are therefore both aerobic processes. The fat formation is accompanied by an increase in the carbohydrate of the cell (Table 18). A number of authors have found that other yeasts, e.g. baker's yeast or *Endomycopsis vernalis*, form fat only under aerobic conditions; this observation has, however, not found a sufficient expression in the various schemes put forward for possible pathways of the synthesis of fatty acids from carbohydrate.

The investigation of the effect of additions of Mg^{++}, Mn^{++} and aneurin to the yeast suspension has not disclosed any part played by these compounds in the fat synthesis. Possibly the yeast cell contains sufficient amounts of these compounds to maintain its metabolic activities at a high rate even without further additions, provided sufficient carbohydrate substrate is present. Addition of phosphate appears, however, to be necessary for maximal fat synthesis from carbohydrate.

Acetaldehyde was the only non-carbohydrate substrate of a number tested, in the presence of which fat was formed. A similar observation in other yeasts, as well as that of fat formation in the presence of pyruvate, has hitherto been regarded as evidence that these substrates are intermediates in the synthesis of fat from carbohydrate. It will be noted that the yeast contains a considerable amount of carbohydrate, which might be mobilized in the presence of some substrates and used for synthesis of fat. The result obtained with acetaldehyde cannot, therefore, be considered as conclusive evidence that acetaldehyde is an intermediate in the synthesis of fat from carbohydrate. Such evidence might be provided by the use of acetaldehyde labelled with isotopic C, or by a further investigation of the metabolism of acetaldehyde in this yeast.

SUMMARY

1. The growth and metabolism of *Torulopsis lipofera*, a yeast rich in fat, have been investigated.

2. *T. lipofera* grows well on a medium containing 0·05% marmite, carbohydrate, ammonia and mineral salts, and on molasses containing K, NH_4 and Mg salts. The optimum temperature is 23–25°. The generation time under optimal conditions is $5\frac{1}{4}$–$5\frac{1}{2}$ hr.

3. The content of fatty material (fatty acids and unsaponifiable matter) in the dry yeast varied between 18·6–43%, the unsaponifiable fraction forming 6·8–8·8%.

4. Methods for the macro- and micro-estimation of fat and for the study of fat formation in yeast are described.

5. In the presence of glucose the R.Q. of the yeast is higher than 1·0. The fat content increases linearly with time and is proportional to the glucose used. The conversion coefficient, i.e. the percentage of C of glucose used converted into C of fat, varied between 11 and 33%. In the absence of substrate the R.Q. is lower than 1·0 and fat is utilized.

6. The carbohydrate breakdown and fat formation in *T. lipofera* are aerobic processes. Under anaerobic conditions no glucose is used.

7. The conditions of fat formation from glucose were studied. The fat formation is highest at pH 5·5–6·0; the optimal temperature is 20–25°; increasing concentrations of NH_4Cl and urea inhibit the conversion of carbohydrate into fat.

8. Phosphate is essential for maximal conversion of carbohydrate to fat.

9. Iodoacetate (0·0002–0·0005 M) inhibits the fat formation. In the presence of iodoacetate and glucose there is an increase in the organic-P, the hydrolysis curve of which indicates an accumulation of hexosediphosphate. In the presence of fluoride there is an accumulation of organic-P, which is only to a small extent hydrolyzable.

10. The metabolism of glucose in *T. lipofera* was studied by the C balance sheet method. 97% of the C used was accounted for. Glucose is partly oxidized to CO_2 and partly converted to fatty acids and cell-carbohydrate.

11. The oxidation of various substrates in *T. lipofera* was examined. Glucose, fructose, maltose, ethanol are rapidly oxidized. Saccharose, galactose, dihydroxyacetone, acetate, butyrate, methylglyoxal and stearate are oxidized more slowly, whilst pyruvate, lactate, acetaldehyde, propionate, glycerol, glyceraldehyde, succinate, fumarate, *dl*-malate, citrate and gluconate are little, if at all, oxidized. Oleate decreases the O_2 uptake as compared with the control without substrate.

12. The formation of fat from various non-carbohydrate substrates was studied, acetaldehyde alone of the substrates tested giving a significant increase in fat formation.

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The Microbiological Degradation of Steroids

2. OXIDATION OF CHOLESTEROL BY *PROACTINOMYCES* SPP.

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The identification has been recorded (Turfitt, 1944) of species of the genus *Proactinomyces* as the bacterial agencies concerned in the disappearance of cholesterol from soils. Although many soil samples have been examined, no evidence has yet been adduced that any other bacteria, moulds or actinomycetes are able to accomplish this sterol breakdown; the possibility of the existence of cholesterol-decomposing organisms which do not grow on ordinary solid nutrient media is still being investigated. This apparently unique characteristic of *Proactinomyces* is the more interesting in view of the taxonomic position of the organisms. Classification within the Order Actinomycetales involves a gradation through the unbranched *Mycobacterium* and *Corynebacterium* to the richly branching *Actinomyces*, the genus *Proactinomyces* constituting an intermediate transitional stage. Since bacterial cells are, with few exceptions (Sifferd & Anderson, 1936), entirely devoid of any sterols, whilst the latter are actually synthesized by the yeasts and mould fungi, it is remarkable that one small section of the Natural