Lipid Formation in *Cryptococcus terricolus*

I. Nitrogen Nutrition and Lipid Formation

T. A. PEDERSEN*

Royal Institute of Technology, Division of Food Chemistry, Stockholm 70, Sweden

The yeast *Cryptococcus terricolus* was found to contain up to 70 % lipids on a dry weight basis. The lipid production was found to be independent of the quantities of nitrogen supplied. Even with 40 g $(\text{NH}_4)_2\text{SO}_4$ per litre of solution, corresponding to a carbon/nitrogen ratio of 1.9, the cells contained 55 % of lipids. Lipid production was also independent of the nature of the nitrogen source. The protein content amounted to 10–18 %.

Lipid production was closely connected with growth. In the logarithmic phase of growth, the production of lipids was at its maximum. Lipid respiration started when the carbohydrate of the medium had been consumed. This was indicated by the low R.Q. values found for starving yeast cells. The addition of glucose to starving cells caused a sudden increase in the R.Q. value.

The inverse relationship between lipids and proteins, usual in "fat" yeasts, does not occur in this organism. The protein content remains almost constant during growth, while the lipid content increases up to 65–70 %.

Numerous microorganisms have been shown to be capable of synthesizing large amounts of lipids from carbohydrates. As far as yeasts are concerned, many different species and strains have been tested for lipid production. As examples can be listed *Rhodotorula glutinis*, *R. gracilis*, *Lipomyces starkeyi*, and *Torulopsis lipofera*. These organisms are capable of accumulating 50 % or more of their dry weights as lipids. The conservative term "fat" yeast has been retained for these yeasts, but it is pointed out that "lipid" yeast would be the correct name.

The first isolations of *Cryptococcus terricolus*¹ revealed that the cells contained large globules, which after staining with Sudan III, could be said to contain lipids. The cells contained this lipid globule in all isolations and plate controls, independent of the medium used.

* Permanent address: Department of Microbiology, Agricultural College of Norway, Vollebakk, Norway.

Working with another soil yeast, now called *Lipomyces starkeyi*, Starkey² found that lipid formation occurred only on a nitrogen-deficient agar. His results were in accordance with the generally accepted view, that insufficient nitrogen supply leads to accumulation of lipids in "fat" yeast cells. The above-mentioned observations on *Cryptococcus terricolus* seemed to indicate that lipid formation was independent of the composition of the medium. An investigation of the influence of different sources of nitrogen in varying concentrations on the lipid synthesis of *C. terricolus* was therefore considered to be of interest. The results of this investigation are presented in this paper.

Microscopical examinations indicated that the lipid globule constituted as much as $\frac{1}{2}$ — $\frac{3}{4}$ of the cell volume. Exact determinations of the lipid content should therefore be of interest.

EXPERIMENTAL

Strain No. 1 of *C. terricolus*, kept as a stock culture on vitamin-free synthetic agar¹, was used in this work. The strain was transferred to new slants at least fortnightly. In physiological experiments a two- or three-day-old culture was used as inoculum.

To obtain lipid formation the following substrate, used by Lundin and collaborators³ in their work on *Rhodotorula gracilis*, was employed: glucose 40.0 g, $(\text{NH}_4)_2\text{SO}_4$ 1.0 g, KH_2PO_4 1.0 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.0 g, NaCl 0.5 g, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 0.5 g, and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0.005 g to one litre of distilled water. Beer wort was not added in these experiments, as it had been established previously⁴ that optimal growth occurred in a synthetic, vitamin-free medium. Analytical grade chemicals were used.

The organism was grown in 750 ml conical E-flasks, containing 250 ml of nutrient solution. The flasks were placed at 25°C after inoculation and continuously shaken during the incubation period. The pH was controlled daily in all experiments by taking out aliquots. When necessary the culture fluid was adjusted to pH 4.5 — 6.0. All series were run at least in duplicate, and all experiments repeated.

The growth of the organism was determined as dry matter by centrifuging of 10 ml samples, followed by two washings of the precipitate, which was then dried over night at 80°C.

The ash content was determined by heating samples of dry matter in an electric muffle furnace at 550 to 650°C to constant weight.

The nitrogen contents of cells and culture solutions were determined according to the standard Kjeldahl procedure. The weight of yeast protein was calculated by multiplying the weight of the cell nitrogen by the factor 6.25.

The yeast carbohydrate content was determined by means of Dreywood's anthrone reagent⁵. The best reproducibility between the duplicates in these experiments was obtained by using the former method of Trevelyan and Harrison⁶. The determination was carried out as follows: 100 ml of yeast suspension were mixed with 10 ml of saturated mercuric chloride⁷, and centrifuged at 4 000 r.p.m. for 15 min. The precipitate was washed twice and diluted with distilled water to a turbidity between 0.1 and 0.3, as measured by a Beckman spectrophotometer at 560 m μ . After colour development, the solutions were always clear, and the optical density was measured with the same spectrophotometer at 620 m μ . Only total carbohydrates were determined, and the results were referred to a glucose standard.

The decrease in the sugar content of the nutrient solutions during growth was followed by sugar determinations according to the modified Schoorl-method⁸.

The determination of the lipid content was carried out according to the method of Steinberg and Ordal⁹. While these authors used 1.0 N hydrochloric acid for autoclaving the yeast material before extraction with petroleum ether, 1.2 N hydrochloric acid was used in this investigation. It should be mentioned that the lipid obtained by this procedure was a crude material. Possible contaminations were not removed, but re-extraction proved, in some cases, the amount of non-lipid components to be insignificant.

Fig. 1. Yield of yeasts, lipids, and proteins by *Cryptococcus terricolus* as functions of time. 1.0 g $(\text{NH}_4)_2\text{SO}_4$ added per litre. $\circ$ yeast dry matter, $\bullet$ lipids, $\square$ proteins.

Fig. 2. Endogenous respiration of 5 day old *Cryptococcus terricolus* cells. Each vessel contained 11.7 mg of yeast material. Calculated R.Q. = 0.63. $\square$ O_2 consumption, $\circ$ CO_2 evolution.

The yeast material obtained after centrifuging followed by two washings was frozen and kept at -20°C . All analyses of the cell materials could then be performed within a few days.

Oxygen consumption was measured by the conventional Warburg technique. The washed cell suspension was added to the main compartment of the Warburg vessels, and the glucose solution, when used, to the side arm. The center well of the flasks contained 0.2 ml of 10 % KOH, absorbed on filter paper. The CO_2 evolution was measured according to the direct method of Warburg. All respiration measurements were made at 25°C and pH 4.8.

RESULTS

The ability of *Cryptococcus terricolus* cells to produce lipids was tested by means of the *Rhodotorula* medium mentioned previously. The high production of lipids shown by *Rhodotorula gracilis* in this medium was due to the low contents of nitrogen and phosphorus¹⁰. As these reductions are generally accepted to be necessary for obtaining yeasts with high lipid contents, this medium should also be expected to favour lipid production in *Cryptococcus terricolus*.

Fig. 1 shows the results for *C. terricolus* when grown in this medium. The amount of yeast increased rapidly between the first and the sixth day after which a weak autolysis set in. Maximum yield of dry matter was nearly 14 g per litre of solution; an amount comparable to that obtained for *Rhodotorula*

gracilis in solutions with the same sugar content. The production of lipids and proteins was closely associated with the growth of the organism. Maximum production of dry matter, lipids, and proteins occurred after six days. After the autolysis had started, the decrease in lipid content was evident. As, at that time, the sugar was shown to be completely consumed, this greater loss could perhaps be accounted for by lipid respiration. No unpleasant odours were produced during the relatively short autolysis period in this experiment.

The economic coefficient (g of yeast yield/100 g of sugar, in this case glucose) was about 35, and the "fat" coefficient (g of newly formed lipids/100 g of sugar consumed)¹¹ approximately 23. Consequently, the production of cells was of the order usually found in other "fat" yeast species, but the ability to convert carbohydrates to lipids was unusually high. After 4 days the protein constituted only *ca.* 12 % of the dry weight of the cells. This inverse proportionality between lipid and protein contents is always found in "fat" yeasts.

To verify that lipid respiration really occurred after the energy source had been consumed, investigations were made, using the Warburg technique. The R.Q. (Respiratory Quotient = CO_2 produced/ O_2 consumed) indicates the nature of the metabolism taking place. In the oxidation of fatty acids, the amount of oxygen consumed must exceed that of CO_2 liberated, and the R.Q. will consequently be less than 1. Determinations of the endogenous respiration at different growth intervals of the organism always gave an R.Q. less than 0.9. Fig. 2 shows the endogenous respiration of a five-day-old culture. There

Fig. 3. Effect of addition of glucose on the respiration of starving 6-day-old *Cryptococcus terricolus* cells. Each vessel contained 39.9 mg of yeast material. Endogenous respiration, R.Q. = 0.85, respiration after addition of 5 mg of glucose per vessel, R.Q. = 1.20. □ endogenous O_2 consumption, ○ endogenous CO_2 evolution, ▲ O_2 consumption after glucose addition, ● CO_2 evolution after glucose addition.

Fig. 4. Yield of yeasts, lipids, and proteins by *Cryptococcus terricolus* as functions of time. 10.0 g $(\text{NH}_4)_2\text{SO}_4$ added per litre. ○ yeast dry matter, ● lipids, □ proteins.

Fig. 5. Contents of glucose and nitrogen in a nitrogen-poor (I) and a nitrogen-rich (II) medium as functions of the growth time of *Cryptococcus terricolus*. I: 1.0 g $(\text{NH}_4)_2\text{SO}_4$ per litre, II: 10.0 g $(\text{NH}_4)_2\text{SO}_4$ per litre. ○ glucose content, both series I and II, □ nitrogen content in series I, ■ nitrogen content in series II.

is a significant difference between the volume of O_2 taken up and that of CO_2 given off. The R.Q. value of 0.63 can be explained only if lipids be the respiratory material.

A starving yeast culture, oxidizing lipids endogenously, was supplied with an energy source, in this case glucose. The results are shown in Fig. 3. After one hour of endogenous respiration, glucose was added to the yeast suspension in two flasks, while the endogenous respiration was going on in two other vessels. The respiratory activity increased rapidly after glucose addition. A higher CO_2 evolution than O_2 consumption was now established. The R.Q. increased from 0.85 to 1.20. This might indicate that lipid production started when glucose became available to the organism.

Effect of varying nitrogen concentrations on the lipid formation

As mentioned above, the isolated strains always contained a large lipid globule, regardless of the medium used for isolation. The different media, of course, contained varying amounts of nitrogen. An investigation of the effect

Table 1. Effect of varying concentrations of nitrogen on growth, lipid and protein formation in *Cryptococcus terricolus*. Results after 5 days' growth. Nitrogen contributed by the inoculum 0.04 g/l in all series. This amount is included in the C/N ratio.

Series	Nitrogen supply (NH ₄) ₂ SO ₄ N		Ratio C/N	Yeast dry matter g/l	Lipid production		Protein production		Nitrogen content in protein g/l
	g/l	g/l			g/l	%	g/l	%	
1	40.00	8.48	1.9	0.48	0.26	55.0	0.09	18.2	0.01
2	20.00	4.24	3.7	6.04	3.40	56.3	0.98	16.2	0.16
3	10.00	2.12	7.4	6.86	3.88	56.6	1.04	15.1	0.17
4	5.00	1.06	14.5	7.07	4.16	58.8	1.07	15.1	0.17
5	2.50	0.53	28.1	7.41	4.47	60.3	1.17	15.8	0.19
6	1.25	0.27	51.6	7.86	4.77	60.7	1.27	16.1	0.20
7	0.63	0.13	94.1	7.74	4.74	61.3	1.17	15.1	0.19
8	0.32	0.07	145.4	7.00	4.59	65.6	0.87	12.4	0.14
9	0.16	0.03	228.6	5.71	3.88	67.9	0.88	15.4	0.14
10	0.00	0.00	400.0	5.03	3.49	69.3	0.73	14.6	0.12

of varying nitrogen concentrations on the lipid production therefore seemed to be of interest.

Fig. 4 shows the results obtained when the organism was grown in the usual Rhodotorula-medium, in which 10.0 g (NH₄)₂SO₄ were used instead of the original 1.0 g. A comparison of Figs. 1 and 4 shows the curves to be almost identical with respect to growth, lipid production, and protein production.

Fig. 5 gives the explanation to this conformity. The consumption of glucose followed the same pattern in both experiments. In the solution containing the least amount of nitrogen, all available nitrogen was assimilated. In the solution rich in nitrogen, however, only nitrogen from a little more than 1.0 g (NH₄)₂SO₄ was assimilated, the rest being left unassimilated in the medium.

As these results were highly unexpected, it was found worth while to investigate the effect of varying amounts of nitrogen on *C. terricolus*. The results are shown in Table 1. The concentrations of glucose and salts, except (NH₄)₂SO₄, were as before. From the table it will be seen that the yeast yield reached its maximum value between 0.32 and 10.0 g (NH₄)₂SO₄ per litre, with the best output at 0.63 and 1.25 g per litre. If the growth was prolonged to seven days, it was found that less than 0.63 g (NH₄)₂SO₄ per litre restricted the growth seriously. More than 10.0 g (NH₄)₂SO₄ per litre inhibited the growth, but with prolonged cultivation a certain adaptation occurred. Even with 40.0 g (NH₄)₂SO₄ per litre of nutrient solution, 55 % of lipids were found in the cells. With decreasing nitrogen supplies a slightly increased lipid content was found. A "fat" yeast, however, was produced in all cases. At optimal growth, the cells contained approximately 60 % of lipids. It was also found that the total lipid production reached its maximum under these conditions, whereas the lipid content of the cells increased to approximately 70 % with further decreasing nitrogen concentrations. The protein content constituted from 12 to 18 % of the dry weight. The highest protein value, however, was obtained under conditions causing a serious decrease in the growth of the organism. In series 7–10 the nitrogen values for the protein formed were higher than

expected from the amount of nitrogen supplied to the culture. This was probably due to the presence of assimilable nitrogen compounds in the air or in the chemicals used. As shown earlier⁴ nitrogen fixation does not occur.

Effect of the nature of the nitrogen source on lipid formation

It was found earlier that *C. terricolus* could utilize ammonium-, nitrate-, amino-, and amido-nitrogen as sources of nitrogen⁴. In stationary cultures, the yeast yields were found to be almost the same with these various sources of nitrogen. A suboptimal growth found in the case of urea was perhaps due to a shift in pH during growth. In continuously shaken cultures with daily pH regulation, it was found that urea caused the most rapid growth. Concentrations of glucose and salts in the medium, except the nitrogen compounds, were as before. The yield of dry matter after 5 days was approximately 40 % higher with urea than with equivalent nitrogen quantities, supplied as $(\text{NH}_4)_2\text{SO}_4$, KNO_3 , NH_4NO_3 , or asparagine (Table 2). The lipid contents lay within the range of 58–64 % with the various nitrogen sources, except in the KNO_3 series where the lipid content was only 41 %. In preliminary experiments it was found that an additional supply of the quantity of potassium used here, had no effect on the lipid production. The results presented in Table 2 indicate a decrease in lipid production, possibly due to the presence of nitrate ions in the medium, without a corresponding increase in the protein content. The highest lipid output was found with urea as nitrogen source. This is in accordance with Kleinzeller¹² who found for *Torulopsis lipofera* that the conversion coefficient (percentage of carbon in substrate converted to carbon in lipids) was higher with urea than with NH_4Cl as the nitrogen source. Protein production was not promoted by any of the compounds, and nitrogen was left in all solutions.

Total cell composition

The composition of the *Cryptococcus terricolus* cells was primarily determined by analyzing the dry matter on lipids, proteins, and ash. A dry matter rest

Table 2. Influence of various sources of nitrogen (0.318 g N per litre) on growth, lipid and protein production in continuously shaken cultures of *Cryptococcus terricolus*. Results after 5 days' growth.

Addition per litre	Yeast dry matter g/l	Lipid production		Protein production		Nitrogen in solution	
		g/l	%	g/l	%	Start g/l	Rest g/l
1.500 g $(\text{NH}_4)_2\text{SO}_4$	7.93	4.91	61.9	1.17	14.7	0.32	0.15
2.288 g KNO_3	7.92	3.29	41.6	0.93	11.8	0.32	0.18
0.909 g NH_4NO_3	7.47	4.33	58.0	0.73	9.8	0.32	0.22
0.683 g urea	11.23	7.22	64.3	1.20	10.7	0.32	0.13
1.704 g asparagine	8.04	4.82	60.0	1.08	13.4	0.32	0.16

Fig. 6. Total composition of *Cryptococcus terricolus* cells as function of time. Grown in nitrogen-poor solution; 1.0 g $(\text{NH}_4)_2\text{SO}_4$ per litre. $\circ$ lipids, $\bullet$ proteins, $\triangle$ ash, $\square$ dry matter rest.

Fig. 7. Total composition of *Cryptococcus terricolus* cells as function of time. Grown in nitrogen-rich solution; 10.0 g $(\text{NH}_4)_2\text{SO}_4$ per litre. $\circ$ lipids, $\bullet$ proteins, $\triangle$ ash, $\square$ dry matter rest.

was calculated as the difference between the total cell content and the analyzed components.

Fig. 6 shows the total composition of the cells grown in nitrogen-poor solutions (1.0 g $(\text{NH}_4)_2\text{SO}_4$ per litre), and Fig. 7 the results in nitrogen-rich cultures (10.0 g $(\text{NH}_4)_2\text{SO}_4$ per litre). The ash content of the cells showed only minor variations, and 4 % was taken as the mean value throughout the experiment. The variations in lipid and protein amounts were almost identical in the two experiments. There were some indications that during the first days of growth, the lipid production was slightly increased by a low nitrogen content in the medium. From the fifth day the lipid content was about the same in both series. The protein content decreased from approximately 18 % in the start material to 10 % after more than five days' growth. Instead of the usual

Table 3. The content of carbohydrates, expressed as glucose, in *Cryptococcus terricolus* cells. Cells grown in a nitrogen-poor solution.

Days after incubation	Carbohydrates, as glucose mg/g	Days after incubation	Carbohydrates, as glucose mg/g
0	207	6	163
1	212	7	151
2	216	8	165
3	206	9	184
4	207	10	186
5	201		

inverse relationship between lipids and proteins in "fat" yeast cells, it seems in this case more correct to speak of an inverse relationship between the lipids and what so far can be called the dry matter rest.

As this rest includes the cell carbohydrates, analyses were performed to determine quantitatively the carbohydrate fraction. The results are shown in Table 3.

The carbohydrate content, as shown in the table, remained at a relatively constant level during the growth of the organism. If these results are compared with the curves for the dry matter rest in Figs. 6 and 7, the conclusion may be drawn that the carbohydrate content is possibly identical with the dry matter rest, with one distinct exception. The first day after inoculation a decrease in lipid content occurred in the cells. Simultaneously, a rapid increase in the dry matter rest without simultaneous increase in the carbohydrate content was noted. It seems very likely that an intermediary metabolite accumulated in the cells, as the lipid content on the second day showed an extremely rapid increase.

DISCUSSION

It has repeatedly been demonstrated that, in most "fat" yeasts and other lipid-forming organisms, growth and lipid production occur in the following order: When a medium, containing a small amount of assimilable nitrogen and a relatively great amount of sugar, is inoculated with a "fat" yeast, two cultural phases can be observed. During the first phase, *i.e.* the "protein" phase, the cells proliferate at logarithmic rate, and have a low lipid content. During the second phase, *i.e.* the "fattening" phase, beginning after the medium has been depleted of nitrogen, the cell population remains nearly constant, but the lipid content increases. This sequence has been clearly demonstrated by Enebo, Anderson and Lundin¹⁰ in *Rhodotorula gracilis*. Later Steinberg and Ordal⁹ separated the phase of fattening from that of growth or multiplication in this *Rhodotorula* yeast. This made it possible to study the lipid synthesis independent of the growth. Kleinzeller¹² found *Torulopsis lipofera* to be a suitable organism for a study of lipid synthesis, because of its rapid conversion of carbohydrates into lipids.

In *Cryptococcus terricolus* it seems impossible to separate growth and lipid production into two phases. Here, growth and lipid formation are closely connected. When the growth of the organism is in the logarithmic phase, the rate of lipid formation is simultaneously at its maximum. The fraction of lipids that forms part of the cell structure, follows, of course, the increase in cell number, but this fraction usually amounts to only a few per cent of the dry matter. In *C. terricolus*, the capacity for nitrogen assimilation seems to be very restricted, and this may be one of the reasons why this organism produces large quantities of lipids already in the early stage of its development. At present it is very difficult to account for this; however, one explanation may be found in connection with the ecology of the isolated strains. Living in carbohydrate-rich and nitrogen-poor spruce litter soil localities¹, the organism might have lost the power of high protein production. In any case, additional nitrogen, supplied as the compounds used in this investigation, does not

result in increased protein production. Investigations on the lipid-protein balance in this yeast are in progress.

The successful biotechnical production of yeasts depends on a rapid growth. For *Rhodotorula* yeast¹⁰, three days were found to suffice for maximum yeast and lipid production, whereas in the experiments presented in this paper, the growth time had to be prolonged to six days. However, the great difference between the inocula used should be kept in mind. Enebo *et al.*¹⁰ inoculated with an amount corresponding to ca. 1 g of dry substance per litre of final culture solution, while the inoculum in this work amounted to only 0.02 g. The generation time is a measure of growth; for *Rhodotorula gracilis* it was found to be 18.4 and 16.5 h, in two experiments. The calculated generation time for *Cryptococcus terricolus* was 15.9 h. In physiological experiments, it was found best to work with poor inocula, but, for biotechnical purposes, the inoculum should be of the magnitude used by Enebo *et al.*

When *Rhodotorula gracilis* was grown in solutions with a generous supply of nutrient salts, including nitrogen, for obtaining rapid growth, the generation time was shortened to only 2.8 h. In this case, however, the lipid content after 42 h was only 9.35 %. When *R. gracilis* was grown by continuous cultivation in a nitrogen-deficient medium to obtain high lipid formation, the generation time became 51 h¹⁰. In *Cryptococcus terricolus*, in which lipid formation is closely associated with growth, 15.9 h was the actual generation time for obtaining lipid-rich yeasts. For practical purposes it is essential to try to shorten this generation time. It should therefore be of interest to make a systematic investigation on the effect of varying growth conditions on the relationship between growth rate and lipid production.

The economic coefficient for *C. terricolus* was found to be 35. While this value is of the same order as that found in other "fat" yeasts^{2,10}, higher values have been found for protein yeasts¹³. The production of a certain amount of lipids, however, is a process requiring more energy than the production of the same quantity of proteins. One of the best food and fodder yeasts, *Torulopsis utilis*, has been found to contain maximally 60 % of protein, when grown in beet molasses¹³. Supposing the economic coefficient to be about 60 in this substrate, the calculated protein coefficient would be 36 (the economic coefficient based on the total sugar content was found to vary between 53.5 and 65.3¹⁴). This means that *T. utilis*, under conditions favourable to protein production, would produce 36 g of protein from 100 g of sugar. However, if lipids are the desired product, *Cryptococcus terricolus* would produce 23 g of lipids from the same quantity of sugar. If the higher calorimetric value of lipids is taken into consideration, the calorific value of proteins produced by *Torulopsis utilis* under optimal conditions, is of the same order as the energy stored in the lipids produced by *Cryptococcus terricolus*.

In a recent review on microbiological lipids (Woodbine¹⁵), the lipid percentages and "fat" coefficients found in earlier experiments have been summarized. Of the different yeast species only *Endomyces vernalis*, *Lipomyces starkeyi*, and *Rhodotorula gracilis* were found to contain more than 50 % of lipids on a dry weight basis. *Lipomyces starkeyi* was reported to contain more than 60 %, and only *Rhodotorula gracilis* was found to contain more than 70 %. The 65–70 % content of lipids found in *Cryptococcus terricolus*

is, therefore, very high. It should be mentioned that this value refers to crude lipids. The content of purified lipids, however, is maximum only a few per cents lower. As far as could be controlled, the lipid values in the other publications mentioned also refer to crude lipids.

Blinč and Hočevár¹⁶ found 74 % lipids in *Rhodotorula gracilis*. The economic coefficient was 28, and the "fat" coefficient consequently 21. If, as a mean value, it is calculated with 66 % lipids in *Cryptococcus terricolus*, the "fat" coefficient becomes 23. This high lipid content and effective lipid production is only possible if the protein content is very low. Rippel¹¹, in his theoretical calculations on lipid production, postulated 30 % proteins in the "fat" yeast. In fact, the protein content in *C. terricolus* was found to be only 12 %. Blinč and Hočevár¹⁶ found 13.8 % proteins in *Rhodotorula gracilis*. With these low protein values the presence of reasonable amounts of carbohydrates and ash in the cell, can be explained.

The ash content of *Cryptococcus terricolus* lies within the limits usually found for yeasts¹⁷. The fraction of the cell content gathered under the name "dry matter rest" ranges from 17 to 50 % of the dry matter. The structural carbohydrates of brewer's and baker's yeast are a glucan and a mannan, constituting about 10 % of the dry weight¹⁷. The carbohydrate fraction of *C. terricolus*, at different stages of growth, was found to vary between 15 and 21 %, expressed as glucose.

The 50 % value for the dry matter rest after one day's growth, of which the carbohydrates constitute only *ca.* 2/5, is difficult to explain. The present picture of the fatty acid biosynthesis is represented by a cycle in which all steps are reversible, so that both a synthesis and a degradation of fatty-acid chains is possible, depending on the nutritional status of the cells. This reversibility between lipid production and oxidation is indicated also in *C. terricolus*. A lipid-oxidizing culture seems to start synthesizing lipids approximately 20 min after glucose has been added. The fatty acid composition of the lipids in a one-day-old culture will be investigated in a future work. By qualitative changes in the lipid composition in a young culture, which may lead to losses of lipids during the preparations of the extracts, the irregularities of the lipid and dry matter rest curves in Figs. 6 and 7 might be explained.

Our knowledge today of the direct link between the carbohydrate and the fatty acid metabolism, on the one hand, and the carbohydrate and the amino acid metabolism, on the other, both through the action of the citric acid cycle, might help us to explain the relationship between lipid and protein production. A protein yeast, supplied with nitrogen compounds, incorporates the nitrogen and forms amino acids and proteins. In a usual "fat" yeast, the products of the citric acid cycle, in the absence of nitrogen, are transformed into fatty acids. With regard to *C. terricolus*, a rich nitrogen supply does not result in an increased formation of amino acids and proteins. A supplement of different organic nitrogen compounds, such as peptone, does not alter this fact. The possible reason of this may be a block in the amino acid synthesis of the organism, causing the formation of fatty acids from the products of the citric acid cycle.

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