XXVII. THE CONDITIONS INFLUENCING THE FORMATION OF FAT BY THE YEAST CELL.

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Very little is known of the story of fat metabolism in the lower organisms although a considerable amount of work has been carried out on the conditions attending the formation of fat in yeast. Yeast offers a particularly favourable field of study as it can readily be grown in large quantity and considerable variations can be effected in the conditions of its growth. In spite of the work that has been done but little progress has been made since the work of Naegeli and Loew [1878, 1879] carried out more than forty years ago.

The conclusions arrived at by Naegeli and Loew were briefly as follows:

(1) The fatty acid of the yeast cell consisted chiefly of oleic acid.

(2) The amount of fat obtainable from yeast was about 1 to 2 % if the dried yeast was directly extracted by ether; this figure might be raised to about 5 % by first evaporating the yeast with concentrated HCl several times on a water-bath. The acid destroyed the cell wall and the ether then was no longer prevented from extracting the fat by the impermeability of the cell wall.

(3) The more vigorous the growth of the yeast cell, the greater was the amount of fat formed. Both the total amount of dry substance formed and the percentage of fat it contained were raised.

(4) Other conditions being similar, the percentage of fat formed increased with the supply of oxygen. They found that the fat content of a yeast grown in a solution of sugar containing ammonium tartrate (2 %) which was aerated throughout the time of growth was 12·5 %, whereas a yeast grown on peptone and sugar at a low temperature with scanty respiration contained only 5 % of fat.

(5) Naegeli discussed the question of the origin of the fat formed and concluded that under different conditions both carbohydrate and protein might act as sources of fat. He drew attention to the rape seed which, before maturity, is filled with starch grains and from which, when ripe, the oil is pressed and to the case of fungi in which, when put into water, the plasma diminishes with the appearance of fat, the cellulose membrane also increasing during fat formation.

(6) Naegeli recognised also that the fat contained a sterol which he termed cholesterol and further that the conditions which led to an increase of fat led also to an increase of sterol.

The nature of yeast fat.

This has since been elucidated by the work of Hinsberg and Roos [1903, 1904], Neville [1913], Gérard [1895], and Smedley MacLean and Thomas [1920]. As the result of these investigations it has been shown that the fatty acids present are palmitic, oleic and linoleic with a small quantity of lauric. The fat is chiefly remarkable for the large proportion of sterol which it contains, the sterol being apparently identical with ergosterol, a characteristic constituent of the fats of all the lower plant world.

The amount of fat present in yeast.

In the normal yeast cell the percentage of fat present has been described as from 1 to 5 % of the dried yeast. Naegeli pointed out that the extraction of the air-dried yeast with ether only removed part of the fat, and that if the cells were first treated with concentrated HCl from two to three times as much fat could be extracted, this being however hydrolysed to the free fatty acids. Naegeli's results have been criticised by later observers who consider that prolonged treatment with the strong acid may give rise to ether-soluble decomposition products of the yeast cell and that the higher figures obtained do not represent the true percentage of fat.

Hinsberg and Roos [1903] and Bokorny [1916, 3] therefore retain the ether extraction method in determining the fat percentage of dried yeast. Bokorny [1916, 2] indeed did not confirm Naegeli's results; he treated the air-dried yeast for 24 hours with concentrated HCl and found that the percentage of fat was only 0·66 % of the weight of dried yeast compared with 2·66 % of fat obtained by ether extraction without the preliminary treatment with acid: the sticky mess obtained by treating the yeast with acid, he found unsuitable for ether extraction.

In a series of experiments carried out with the object of determining the proportion of fat to carbohydrate in the yeast cell under different conditions, I adopted the method of hydrolysing the yeast by boiling with *N* HCl for two hours, filtering and washing the residue with water until the washings no longer reduced Fehling's solution; the residue was then air-dried overnight at the laboratory temperature and extracted with ether in a Soxhlet apparatus and the filtrate and washings used for the estimation of carbohydrate. After evaporating off the ether, the residual fat was taken up with dry ether and dried to constant weight in a vacuum desiccator at the laboratory temperature.

I found that the amount of fat found in this way might be several times as great as the amount obtained by the direct extraction of the dried yeast with ether. In the latter method the yeast was dried by treating it with a large volume of absolute alcohol, the alcoholic extract was evaporated and the residue added to the dried yeast before extracting it with ether.

A comparison of the fat obtained by the two methods showed that the two specimens were similar in appearance and from both sterol separated on

standing; the iodine values of both varied considerably in different experiments but no consistent difference could be detected: the difference between the Wijs and Hubl numbers which may be taken as an indication of the amount of sterol present [Smedley MacLean and Thomas, 1921] also showed no constant variation between the two series of experiments.

Table I. *Showing the amount of fat extracted by ether before and after hydrolysis of the yeast.*

Sample of yeast	Weight of dried yeast g.	Amount of fat: (*a*) Without hydrolysis: Weight g.	(*a*) Without hydrolysis: % on dry yeast	(*b*) After hydrolysis: Weight g.	(*b*) After hydrolysis: % on dry yeast
Pressed yeast (12·5 g.)	2·99	0·1118	3·74	—	—
	3·07	—	—	0·1948	6·35
	3·05	—	—	0·1892	6·20
12·5 g. above sample incubated 48 hours at 26° in glucose solution					
Oxygenated	4·17	0·0960	2·29	—	—
,,	4·80	—	—	0·5402	11·24
Not oxygenated	4·18	0·1114	2·67	—	—
,, ,,	4·80	—	—	0·2434	5·07
A pure culture of yeast grown on wort containing lactic acid (*N*/10) at 30°					
Oxygenated	8·05	0·1076	1·34	—	—
,,	8·05	—	—	0·1975	2·45
Not oxygenated	6·49	0·0996	1·53	—	—
,, ,,	6·49	—	—	0·1524	2·30

It must be admitted therefore that ether extraction of the yeast dried either in air or by means of alcohol, does not remove all the fat from the cells. The criticisms brought forward against Naegeli and Loew's method of repeated evaporation of the yeast with concentrated HCl cannot be urged against the much less drastic treatment of boiling for two hours with 3·6 % or even with 1·8 % HCl, a method by which the fat is not hydrolysed, its acid value being barely affected.

The state in which the fat occurs in the yeast cell.

Naegeli and Loew apparently regarded the hydrochloric acid as acting by impairing the cell membrane and thus permitting the entrance of the solvent into the cell containing the fat. They considered it probable that continued treatment with alcohol or ether would completely remove the fat, an expectation which does not appear to be realised even when the extraction is continued for a very long time. Two views present themselves: (1) a proportion of the fat may exist in the free state in the cell, being probably formed as a decomposition product of the cell-plasma. The remainder of the fat may be in combination in the plasma of the cell and may only be liberated on hydrolysis when some complex substance in the plasma is itself decomposed. (2) The sub-microscopic fat particles may be retained in a protein meshwork, only the larger fat particles being extracted by the ether. The smaller fat particles would then only

be liberated by the breaking down of the protein when they would be extracted by the ether.

All the known facts as to the extraction of fat from yeast are in agreement with the hypothesis that the fat is closely associated with the sterol and protein and possibly with the carbohydrate of the cell; this association may be of the nature of a chemical combination.

The evidence upon which this view is based, is as follows:

(*a*) Extraction with alcohol and ether removed readily from 1 to 3 % of yeast fat, calculated on the dried yeast, after which only traces of fat were obtained by long continued extraction.

(*b*) As stated above about twice as much fat may be removed from the yeast cell after boiling with normal or semi-normal acid as is obtained by direct ether extraction of the dried yeast. The greater part of the fat which is obtainable from dried yeast by direct extraction with ether is removed in a comparatively short time; further prolonged extraction only results in the separation of traces of fat.

Thus in an experiment carried out by Miss D. Hoffert, 12·5 g. yeast were soaked overnight in alcohol, the alcohol evaporated, the residue added to the yeast, the whole dried overnight and extracted with ether in 14 hours; the amounts of fat extracted varied from 0·0822 to 0·1060 g. 12·5 g. of the same sample of yeast were hydrolysed with *N* HCl for two hours, the solid residue dried overnight and extracted for 14 hours with ether; 0·2111 g. fat was obtained.

The amounts of fat obtained per hour by direct extraction with ether are shown in the following table.

Table II.

Time in hours...	1st	2nd	3rd	4th	5th	6th	7th	7th to 14th	Total in 14 hours
(1) Weight of fat from 12·5 g. yeast	0·0334	0·0216	0·0146	0·0090	0·0082	0·0079	0·0055	0·0058	0·1060
(2) „ „	0·0652	0·0077	0·0023	0·0013	0·0014	0·0013	0·0011	0·0019	0·0822
(3) „ „	—	—	—	—	—	—	—	—	0·0888

(*c*) Old yeast cells or cells grown under unfavourable conditions, *e.g.* an abnormally low or high temperature, give considerably higher fat percentages than normal cells when extracted directly with alcohol and ether. Such cells when examined microscopically show small globules of fat staining with osmic acid. In experiments where the period of incubation is long, partial autolysis of the yeast probably takes place—the amount of yeast formed is much reduced and the proportion of fat extracted by ether is greater. The total amount of fat obtained is decreased, but since the total amount of yeast is proportionately still less, the percentage of fat is raised.

Tubes containing 10 cc. wort were inoculated with a pure culture of brewer's yeast and after 48 hours the contents added to 1500 cc. sterilised wort and incubated. The yeast was centrifuged, filtered and dried with

alcohol; the residue from the alcohol was added to the dried yeast and the product extracted with ether in a Soxhlet apparatus for 14 hours. From the figures given below it will be noted that (1) the quantity of yeast formed is in inverse ratio to the fat percentage, (2) the percentage of fat tends to be higher when the time of incubation is long and (3) incubation in the presence of 1 % lactic acid at 35° is particularly unfavourable and leads to the production of the smallest amount of yeast and the highest percentage of fat.

Table III. *Showing that a higher percentage of fat is extracted by ether from yeast which has been grown under unfavourable conditions.*

			Temperature 25–26°				
	(*a*) Reaction neutral				(*b*) 1 % lactic acid added		
No. days	Weight fat. g.	Weight dry yeast. g.	Fat %	No. days	Weight fat. g.	Weight dry yeast. g.	Fat %
2	0·105	7·4	1·4	2	0·131	6·5	2·0
2	0·205	8·9	2·3	2	0·117	5·3	2·2
3	0·143	7·7	1·8	2	0·110	5·0	2·2
3	0·152	7·4	2·0	3	0·168	5·8	2·8
3	0·155	6·5	2·4	3	0·130	5·8	2·2
6	0·155	4·6	3·3	5	0·149	4·2	3·6
				6	0·153	3·7	3·3
Mean...	0·153	7·1	2·2		0·134	5·2	2·6
			Temperature 35–36°				
4	0·127	4·1	3·1	4	0·148	3·1	4·6
4	0·101	2·1	4·7	4	0·063	2·6	2·4
4	0·105	2·9	3·5	4	0·076	1·3	5·2
6	0·083	2·4	3·4	8	0·087	0·96	9·0
8	0·051	2·9	1·7	12	0·032	0·49	6·5
12	0·058	1·5	3·8	12	0·028	0·69	4·0
Mean...	0·087	2·65	3·4		0·072	1·52	5·3

(*d*) Bokorny [1916, 3] found that when yeast is submitted to the action of protoplasmic poisons the amount of fat obtained by extraction was very considerably increased. He soaked pressed yeast for some hours in such solutions as phenol (5 %), formaldehyde, mercuric chloride, etc. While it seems unlikely that living processes would continue to be carried on by yeast soaked in 5 % phenol solution, it is certainly conceivable that such treatment might decompose the complex substance in the plasma and liberate fat from it, if fat be indeed one of its constituents.

Bokorny's experiments were carried out on small amounts of yeast and the quantities of fat weighed were small. It is known that protein readily absorbs phenol [Cooper, 1912] and it is possible that when the phenol-treated yeast was extracted with ether, the small amount of fat present may have been augmented by traces of phenol which contributed to the 12 % of fat obtained. In repeating this work it was found very difficult completely to remove the phenol, but I think there is no doubt that after the soaking with phenol the proportion of fat extracted by ether is appreciably increased. Bokorny regarded the increase as being caused by an abnormal secretion of fat deposited as a protection against unfavourable conditions of growth. More probably it is to be regarded as fat liberated from combination in the cell

contents by the action of the poison. It is interesting to note that a German patent (D.R.P. 309,266) recommends the auto-digestion of the yeast to ensure the liberation of the fat globules from the cells before extracting with solvents.

Conditions affecting the amount of fat in the cell.

During the Great War the question of using the lower organisms as sources of fat became one of practical importance especially in Germany where a good deal of work was carried out on these lines. Lindner's [1916, 1919] "mineral yeast" (*Endomyces vernalis*) was cultivated as a source of both fat and protein and in this organism a fat percentage of 18 % was claimed. The work of Bokorny and other observers was directed to producing a similar result with yeast; Bokorny [1916, 1] found that by using peptone as his nitrogenous food and repeatedly adding sugar, the fat content of yeast could be raised. In one German patent (D.R.P. 320,560) it is claimed that by applying the methods used by Lindner to increase the fat content of mineral yeast, the fat content of beer yeast and of pressed yeast may be raised to from 20 to 50 % viz. by growing a surface culture on a non-nitrogenous medium. The method of estimating the fat is not given and only the microscopic appearance is described. Another patent (D.R.P. 307,789) describes the application of hydrogen peroxide and of violent aeration of the glucose solution to increase the fat content of yeasts which do not form surface cultures. Here the increase of fat is not stated nor is the method of extraction indicated.

In the first series of experiments I carried out, the air-dried yeast was extracted with alcohol and ether. Pure cultures of yeast were grown on wort with and without aeration of the medium; pressed yeast was added to glucose solutions and to wort and incubated with and without aeration of the medium. No marked differences were produced in the amount of fat extracted and the oxygenation of the medium appeared to be without result. If however the yeast was first hydrolysed with normal HCl in the manner already described and the solid residue extracted with ether, very marked variations were observed and the fat content appeared to be largely raised by the aeration of the medium.

Pressed yeast incubated for 44 hours in glucose solution without oxygenation showed a marked increase in the total weight of the dried yeast, and the total weight of fat present was increased although the percentage of fat calculated on the dry weight was decreased. But in the oxygenated glucose solution, not only was the total dry weight of the yeast increased, but the percentage of fat was sometimes more than doubled. Thus in one experiment the fat percentage rose from 6·0 to 11·6 %; but while 11·6 % of fat was extracted after hydrolysis, part of the same material treated with alcohol and then extracted with ether showed only 3 % of fat. The increased amount of fat formed when yeast is grown in a glucose solution which is oxygenated throughout the experiment appears to be held in combination in the cell

plasma and is not extracted by ether until by hydrolysis it is set free from the cell complex. The ether-soluble material which was weighed as fat contained both fat and sterol.

Yeast incubated in a solution of glucose to which nitrogenous material has not been added is characterised by a high percentage of carbohydrate. If a given quantity of yeast be incubated for 44 hours in (1) a solution of glucose and in (2) wort containing the same percentage of carbohydrate, the total amount of yeast formed in the wort is from two to three times as much as in the carbohydrate solution. The total amounts of carbohydrate contained in the yeasts after incubation in (1) and in (2) respectively are approximately the same, though, since much more yeast is formed during the incubation in the wort, the *percentage* of carbohydrate in the latter specimen is much less. The total amount of fat as well as the percentage are considerably higher in the yeast incubated in the glucose solution.

It is not clear whether the decreased amount of fat in the yeast incubated in the medium rich in nitrogen is due to a lessened synthesis of fat or to an increased breaking down of the fat after it has been formed. The amount of fat is however markedly greater in the yeast from the oxygenated wort than in that from the wort which has not been aerated.

The part played by the oxygen requires further elucidation and is at present being further studied; it is uncertain whether, as Slator [1921] suggested in his study of the conditions affecting the growth of yeast, it acts by removing the detrimental influence of the carbon dioxide or by some specific action of its own. In all the experiments which I have so far carried out an increase in the total amount of fat has been associated with a high percentage of carbohydrate in the cell.

The observation of Naegeli that the more vigorous the growth of the yeast cell (as is the case in the aerated medium) the greater the amount of fat formed is therefore confirmed. It will be remembered that Naegeli extracted the fat after previously warming the yeast with concentrated HCl. Bokorny's observation that in strongly growing yeast cells there is a diminished fat content probably depends on the fact that he extracted the dried yeast directly with ether, for when this method of extraction is used his results are in agreement with those quoted above [Bokorny, 1916, 2].

In the following experiments 12·5 g. of pressed yeast were incubated for 44 hours at 26° in 1500 cc. of the sterilised medium, in some of the experiments a current of oxygen being passed through the medium during the experiment. The yeast was then filtered and the total reducing substance determined after hydrolysis by Bertrand's method, the result being calculated as glucose. The figures given for fat refer to the total amount of material soluble in dry ether.

The nature of the substance from which fat is formed.

Naegeli and Loew [1879] appear to have recognised clearly that fat is formed in moulds and yeasts from substances existing in the plasma of the

cell; they noticed that as the fat globules appeared the plasma diminished, and argued therefore that the fat could not be derived from the nitrogen-free carbon compounds since these were only present in very small quantity in the cell contents. In this case therefore they claimed that the fat must have been formed from protein. When peptone was used as the source of nitrogen, they regarded the sugar or tartaric acid in the medium as the source of fat.

Table IV. *Showing the effect of oxygenation.*

Medium	Oxygen	Yeast dry weight. g.	Fat weight. g.	Fat %	Carbohydrate weight. g.	Carbohydrate %	Nitrogen %
Original yeast		3·17	0·1643	5·2	0·9	28·2	—
		3·19	0·1812	5·7	—	—	—
In water	−	2·64	0·1734	6·2	0·55	20·8	—
„ „	+	2·65	0·1343	5·1	0·55	20·6	—
„ glucose	−	3·72	0·1892	5·1	2·0	53·8	—
„ „	+	4·17	0·4532	10·8	1·85	44·1	—
Original yeast		3·40	0·2277	6·7	0·92	27·2	—
		3·48	0·2158	6·4	0·88	26·0	—
In water	−	2·87	0·1542	5·4	0·66	23·0	—
„ „	+	3·03	0·1812	6·0	0·64	21·2	—
„ glucose	−	4·98	0·2824	5·7	2·50	50·1	—
„ „	+	5·69	0·6623	11·6	2·64	46·3	—
Original yeast		3·13	0·1892	6·05	0·49	15·7	7·87
		3·07	0·1948	6·37	0·55	17·9	7·67
In glucose	−	4·80	0·2440	5·07	2·40	50·0	5·10
„ „	+	4·80	0·5402	11·24	2·21	46·1	4·73
Original yeast	−	2·98	0·2183	7·32	0·51	17·2	8·78
		2·90	0·2193	7·56	0·52	18·1	8·69
In glucose	+	4·65	0·6267	12·43	2·00	23·3	4·77
Original yeast		3·93	0·2481	6·30	0·84	21·5	7·96
In wort	−	11·26	0·1637	1·45	2·42	21·5	8·55
„ „	−	12·27	0·2153	1·76	2·56	20·9	8·72
„ „	+	11·73	0·2846	2·43	2·63	21·1	8·51
Original yeast							
(1) In wort	−	6·44	0·1487	2·31	1·72	26·7	8·70
(2) „ „	−	3·29	0·1169	3·51	1·05	28·4	8·97
(1) „ „	+	10·04	0·4165	4·15	2·61	26·0	8·67
(2) „ „	+	9·27	0·3535	3·65	2·30	23·72	—

(1) Instead of 12·5 g., 2·5 g. of yeast containing 0·0505 g. fat was added to the solution.
(2) The inoculating dose was here 0·25 g. containing 0·005 g. fat.

They thought it probable that all materials for fat formation were first built into the protoplasm protein and in this way utilised for the formation of fat, the fat being subsequently split off and stored as reserve material.

The figures already quoted (Table IV) show conclusively that when yeast is grown in oxygenated glucose solution, where no external supply of nitrogen is available, the loss in protein even if the protein were wholly converted to fat could account only for a small portion of the fat formed. As it is extremely unlikely that all the amino-groups present would be converted into fatty chains, it is clear that some other source must be found for the manufacture of the fat. When yeast is grown in glucose solution there is a large accumulation of carbohydrate in the cell and since the protein which has disappeared is insufficient to account for the increase in fat, either the carbohydrate of the

external medium or that of the yeast cell itself must have acted as the starting material for the formation of fat. The following figures will make this apparent.

	Dry yeast g.	Carbohydrate g.	Fat g.	Nitrogen g.	Protein g.
1. 12·5 g. of the original sample of yeast contained	3·1	0·52	0·192	0·2409	1·50
12·5 g. yeast incubated 44 hours in oxygenated glucose solution at 26° and filtered. The residue contained	4·80	2·21	0·540	0·2270	1·42
Gain or loss	+1·7	+1·69	+0·348		−0·08
2. 12·5 g. of the original sample of yeast contained	2·94	0·52	0·219	0·257	1·61
After incubating 44 hours at 26° in glucose solution and filtering. The residue contained ...	4·65	2·00	0·627	0·222	1·39
Gain or loss	+1·71	+1·48	+0·408		−0·22

I endeavoured to find out whether the carbohydrate stored in the yeast cell was converted in the presence of a free supply of oxygen into fat; a sample of yeast which had been grown in glucose solution for 44 hours and contained a high carbohydrate percentage (46·7 %) was then transferred to water and a rapid current of oxygen passed through for about 20 hours. The carbohydrate and fat were determined in the yeast before and after it was submitted to the action of the oxygen.

3·84 g. dry yeast contained before being submitted to the action of oxygen 0·1932 g. fat and 1·79 g. carbohydrate; afterwards 0·2146 g. fat and 0·96 g. carbohydrate were detected. In another experiment 3·32 g. dry yeast contained 0·1477 g. fat and 1·446 g. carbohydrate, after growing in the glucose solution. After oxygenation in a solution containing 1 % of potassium and magnesium phosphates 0·1736 g. fat and 0·88 g. carbohydrate were found. Though the differences in the amounts of fat are not very large the increase suggests that the fat is formed from carbohydrate material stored inside the yeast cell.

Conclusions.

Free fat exists in the normal yeast cell in small amount; the percentage of fat is increased in old and degenerating cells, in cells grown under unfavourable conditions and in cells exposed to the action of protoplasmic poisons. A large part of the fat normally present in yeast and of the sterol associated with it, is in some form of combination in the plasma of the cell and is not extracted from it by treatment with alcohol and ether. This complex is decomposed by boiling with dilute mineral acids and the fat can then be readily extracted from it by ether. It is possible that part of the protein and carbohydrate which are hydrolysed by the dilute acid are also combined with the sterol and fat.

A free supply of oxygen and a non-nitrogenous medium rich in carbohydrate are conditions producing an increased amount of fat in yeast but

the fat formed in this way appears to be entirely held in combination in the yeast cell, and is only set free by hydrolysis.

The fat and sterol formed in this way are derived from carbohydrate.

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REFERENCES.

Bokorny (1916, 1). *Allgemein. Brauer- und Hopfen-Zeitung*, **56**, 603.
—— (1916, 2). *Allgemein. Brauer- und Hopfen-Zeitung*, **56**, 1479.
—— (1916, 3). *Biochem. Zeitsch.* **75**, 346.
Cooper (1912). *Biochem. J.* **6**, 362.
Gérard (1895). *Compt. Rend.* **126**, 909.
Hinsberg and Roos (1903). *Zeitsch. physiol. Chem.* **38**, 1.
—— —— (1904). *Zeitsch. physiol. Chem.* **42**, 189.
Lindner (1916). *Wochsch. Brauerei*, **33**, 193.
—— (1919). *Zeitsch. techn. Biol.* **7**, 79.
Naegeli and Loew (1878). *Annalen*, **193**, 322.
—— —— (1879). *München Akad. Sitzber.* 289.
Neville (1913). *Biochem. J.* **7**, 347.
Slator (1921). *J. Chem. Soc.* **119**, 115.
Smedley MacLean and Thomas (1920). *Biochem. J.* **14**, 483.
—— —— (1921). *Biochem. J.* **15**, 319.