XLVI. THE CARBOHYDRATE AND FAT METABOLISM OF YEAST.

PART III. THE NATURE OF THE INTERMEDIATE STAGES.

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Various hypotheses have been put forward to explain the steps by which carbohydrate is converted into fatty acids in the living organism. No direct evidence has hitherto been available from the study of the organism itself, and the theories which have been formulated have been based, on the one hand, on a study of laboratory methods of formation of fatty acids, starting from materials which are believed to occur as decomposition products of carbohydrate in the organism; and, on the other hand, on the nature of the fatty acids known to occur in the natural fats. Many years ago Emil Fischer suggested that the carbohydrate molecules directly condense with one another, joining together by the terminal carbon atoms and thus forming straight chains of carbon atoms from which by subsequent oxidation and reduction fatty acids may be formed. Two circumstances have prevented this hypothesis from finding general acceptance. In the first place we are quite unable to make the hexose molecules condense in this way in the laboratory; in the next place the fats which occur naturally contain all the even-numbered carbon atoms from two to twenty and if these indeed be formed by a process of synthesis it is extremely difficult to account for their formation by the method proposed by Fischer. E. F. Armstrong [1924] has suggested that perhaps the unit attacked is the C_{18} unit, the nucleus of most polysaccharides consisting of three C_6 units united through oxygen atoms. Magnus Levy [1902] proposed acetaldehyde as the intermediate substance which could be derived from sugar and which by undergoing the aldol condensation might build up successive acids containing even numbers of carbon atoms linked together in straight chains. Attempts to synthesise the acids by this method in the laboratory lead however to the formation of acids containing not straight but branched chains. Raper [1907] showed, however, that the aldol molecule will condense with itself to form a straight chain of eight carbon atoms and one of us [Smedley, 1911] showed that the same holds good for crotonaldehyde. To explain the formation of straight chains increasing by two carbon atoms

it was suggested by Smedley and Lubrzynska [1913] that pyruvic acid was the required intermediate and they showed that *in vitro* straight chains of even-numbered carbon atoms can be built up from pyruvic acid, in a series increasing by two carbon atoms. More recently, as the result of investigations on yeast, Lindner [1921] has put forward the view that alcohol may condense with itself in the living organism forming straight chain compounds which by subsequent oxidation form the series of even-numbered fatty acids. This hypothesis is built up on the observation that when yeast is incubated in a solution of ethyl alcohol with a free supply of oxygen, an increased storage of fat takes place in the yeast.

For some years past we have been investigating the action of yeast when incubated in solutions of various simple carbon compounds with the object of throwing some light on this subject. Yeast, if given sufficient oxygen, will utilise various simple carbon compounds and form fat and carbohydrate from them, whilst other closely related substances appear to be entirely unassimilated, and we hoped that some information might be gained from a classification of these substances. The experiments can be carried out quantitatively and the complete balance of the carbon worked out, under a variety of conditions. We made therefore a systematic investigation, choosing substances containing two, three and four carbon atoms respectively, and comparing their effect on the storage of fat and carbohydrate by yeast.

Method of Experiment.

This was similar to that already described by us in an earlier paper [1923]. A quantity of yeast was incubated for 22 hours in the solution to be tested, the medium being well oxygenated throughout the whole period. The yeast was then filtered, weighed and examined as previously described. After the yeast had been hydrolysed with normal hydrochloric acid, the residue was filtered, well washed, dried and extracted with ether; the reducing sugar present was determined in the filtrate by Bertrand's method and calculated as glucose. The ether-soluble substance was dried *in vacuo* to constant weight, hydrolysed with alcoholic potash ($N/2$) and the saponification value determined: the soap solution was extracted with ether three times to remove unsaponifiable matter, and the ether solution of the latter washed with dilute alkali and water. The washings were added to the soap solution, the fatty acids liberated, extracted with ether, dried over anhydrous sodium sulphate and finally the ether evaporated and the acids weighed. The ether was evaporated from the solution of unsaponifiable matter, the residue taken up with pure dry ether and both acids and unsaponifiable matter were dried to constant weight at the laboratory temperature in a vacuum desiccator. The experiments were carried out at the temperature of the laboratory but in the cold weather they were left near the radiator where the temperature recorded was about 25°.

The Carbohydrate Content of Yeast.

In our earlier experiments the yeast obtained from the brewery was washed several times, filtered and used directly for the experiment; the carbohydrate of such a yeast was usually from 25 to 40 % of the dry weight. In such a case there is nearly always a fall in the carbohydrate after the yeast has been incubated in the acetate or other solution, but the loss of stored carbohydrate in the yeast which has been incubated in a solution of acetate is much less than in a yeast which has been incubated in water. When yeast is incubated in water to which oxygen is freely supplied carbohydrate is being continually decomposed during the period of incubation and carbon dioxide given off. Although at the end of the incubation period in the medium less carbohydrate may be present than in the original yeast, carbohydrate may have been formed and again decomposed: it is better therefore to start with a yeast with a low carbohydrate content, because on subsequently incubating this in the solution to be tested any increase of stored carbohydrate is more readily detected. Such a yeast may be obtained by incubating it in water for some hours, generally overnight, and passing a fairly rapid current of oxygen through the water. Part of the carbohydrate is thus oxidised and part fermented and a yeast with a low carbohydrate content is obtained. It is clear, however, that the final amount of carbohydrate represents a balance and it will be less than the sum of the initial amount of carbohydrate together with any new carbohydrate formed during the experiment by the amount of carbohydrate oxidised or fermented during the course of the experiment. It is probable that some of the substances tested may have been directly oxidised by the yeast and have thus acted as carbohydrate sparers but there is no evidence which will enable us to decide whether this is the case or whether the substance was first converted into carbohydrate and then burnt as such.

Compounds containing Two Carbon Atoms.

The two-carbon atom compounds investigated were ethyl alcohol, acetaldehyde, glycol and the sodium salts of acetic, glycollic, glyoxylic and oxalic acids. The only two of these which were readily used by the yeast and which led to the storage of notable quantities of both fat and carbohydrate were ethyl alcohol and sodium acetate. The amounts stored depend largely on the rate of the oxygen supply: at first we found that the acetate produced more storage of carbohydrate than the alcohol, but when the oxygen supply was increased in both cases the difference was removed; the oxidation is aided by shaking, but if the oxygen is bubbled through at the rate of about 6 litres per hour the supply is sufficient and the results obtained are in close agreement whether alcohol or sodium acetate be contained in the medium.

Acetaldehyde tends to act injuriously on the yeast cells, for the effects produced by incubating yeast in a solution of acetaldehyde were never very successful. The yeast only seems able to tolerate the aldehyde at very low

concentrations: it is probable that conditions of administering the aldehyde can be found which may give better results but, although at low concentrations of aldehyde some storage of fat took place, the results were never so favourable as when the medium contained acetic acid or alcohol. Lieben [1923] also found that acetaldehyde was very little used by the yeast cells, most of it remaining in the yeast filtrate.

Table I. *The effect of incubating yeast in solutions of two-carbon compounds.*

12·5 g. yeast (after incubating in oxygenated water) contain			12·5 g. yeast incubated in 1500 cc. of a solution of		Change in		
Carbohydrate g.	Fat g.	Protein g.	%		Carbohydrate g.	Fat g.	Protein g.
Oxygen rate slow.							
0·76	0·13	1·46	0·15	Ethyl alcohol	−0·10	+0·11	−0·16
0·69	0·12	1·28	0·15	,, ,,	−0·19	+0·14	−0·02
0·53	0·16	1·42	0·17	,, ,,	−0·13	+0·14	−0·10
0·69	0·12	1·28	0·30	,, ,,	−0·29	+0·14	−0·12
0·48	0·15	1·31	0·17	,, ,,	+0·04	+0·12	−0·20
0·43	0·07	1·33	0·17	,, ,,	+0·02	+0·24	−0·06
Oxygen rate 13 cc. per min.							
0·49	0·13	1·57	0·17	,, ,,	−0·19	+0·08	−0·20
Oxygen rate 70 cc. per min.					−0·11	+0·17	−0·14
Oxygen rate about 100 cc. per min.							
0·75	0·12	1·71	0·17	,, ,,	−0·15	+0·13	−0·13
0·52	0·12	1·79	0·25	,, ,,	+0·04	+0·34	−0·18
0·30	0·14	1·71	0·25	,, ,,	+0·19	+0·26	−0·22
0·43	0·11	1·47	0·30	,, ,,	+0·12	+0·27	−0·00
0·46	0·09	1·60	0·30	,, ,,	+0·18	+0·36	−0·07
Oxygen rate slow.							
0·76	0·13	1·46	0·20	Sodium acetate	−0·12	+0·14	−0·16
0·53	0·16	1·42	—		−0·03	+0·06	−0·16
0·48	0·15	1·31	—		−0·04	+0·12	−0·20
0·46	0·14	1·39	0·20	,, ,,	+0·14	+0·13	−0·02
,,	,,	,,	0·60	,, ,,	+0·07	+0·16	−0·04
,,	,,	,,	0·80	,, ,,	+0·03	+0·17	−0·02
0·40	0·11	1·31	0·20	,, ,,	+0·07	+0·20	−0·11
Oxygen rate about 10 cc. per min.							
0·30	0·10	1·71	0·17	,, ,,	−0·04	+0·04	−0·05
Oxygen rate about 100 cc. per min.					+0·20	+0·14	−0·04
0·52	0·12	1·79	0·21	,, ,,	+0·03	+0·24	−0·15
0·30	0·14	1·71	0·21	,, ,,	+0·17	+0·13	−0·26
0·76	0·10	1·60	0·21	,, ,,	−0·17	+0·29	−0·12
0·44	0·08	1·63	0·21	,, ,,	+0·00	+0·22	−0·18
0·70	0·12	1·56	0·21	,, ,,	−0·02	+0·27	−0·03
0·43	0·07	1·33	0·17	Ethyl alcohol	+0·02	+0·28	−0·06
,,	,,	,,	0·20	Glycol	−0·09	+0·05	−0·26
,,	,,	,,	0·10	Glycollic aldehyde	−0·24	+0·03	−0·07
,,	,,	,,	0·25	Na glycollate	−0·17	+0·05	−0·12
1·36	0·13	1·20	0·20	Na acetate	+0·07	+0·19	−0·11
,,	,,	,,	0·25	Na glycollate	−0·17	+0·01	−0·12
,,	,,	,,	0·20	Na glyoxylate	−0·16	+0·01	−0·12
1·36	0·13	1·20	0·15*	Acetaldehyde	−0·81	+0·06	−0·04
,,	,,	,,	0·27	,,	−0·80	+0·02	−0·06
0·73	0·09	1·33	—		−0·59	+0·03	−0·03

* The initial concentration of aldehyde was 0·06 %; acetaldehyde was then bubbled in during the course of the experiment.

Compounds containing Three Carbon Atoms.

The three-carbon atom compounds chosen for examination were acetone, glycerol and the sodium salts of lactic and pyruvic acids. Earlier experiments [Smedley MacLean and Hoffert, 1923] had indicated that fat could be formed from lactate and pyruvate and the effects of these two substances were now compared with those of alcohol and acetate. Fürth and Lieben [1922] have shown that lactate is readily used by yeast and have deduced the formation of a non-hydrolysable carbohydrate in yeast after its incubation in the oxygenated lactate solution. We found that the lactic acid is readily used by the yeast, carbon dioxide is given off and both acetic and pyruvic acids can be detected in the medium. Kayser [1924] also detected both these acids in a solution of calcium lactate which had been acted on by yeast. Whereas shaking the solution of acetate in which yeast is incubated produces comparatively little effect on the amounts of carbohydrate and fat stored by the yeast, provided that a good current of oxygen is maintained, shaking a corresponding solution of lactate during the period of incubation leads to a much increased storage of both fat and carbohydrate, and the lactate then appears to be as effective as the acetate. When the effects of the yeast on equal quantities of lactate and acetate are compared, more of the acetate disappears than of the lactate and it seems possible that the lactate is converted into acetate before it is finally burnt to carbon dioxide. We were able to make out a satisfactory carbon balance sheet in which the carbon of all the lactate which had disappeared could be satisfactorily accounted for as CO_2, as stored fat and hydrolysable carbohydrate and as small quantities of volatile acids.

Lieben [1923] also examined the action of yeast on an oxygenated solution of sodium pyruvate and found that solutions of lactate and pyruvate behaved similarly. We found that, if a moderate current of oxygen is passed through the solution and similar amounts of yeast are incubated in solutions of acetate and pyruvate respectively, the acetate is much more efficient in producing storage compounds in the yeast. Pyruvate is also less efficient in this respect than a corresponding solution of lactate. In experiments, however, in which the solution was vigorously shaken during the course of the experiment, the effect on the fat formation was very similar to that of the acetate. Probably the acetaldehyde which Neuberg and Hildesheimer [1911] have shown to be formed from pyruvic acid by the action of carboxylase acts injuriously on the yeast cells if it is allowed to accumulate. With glycerol and acetone negative results were obtained and there was no evidence that either of these could be utilised to any appreciable extent by the yeast.

Compounds containing Four Carbon Atoms.

The four-carbon atom compounds examined include aldol and the sodium salts of hydroxybutyric, acetoacetic and butyric acids. With all these, negative results were obtained (Tables IV and V) and there was no evidence of any

Table II. *Effects of acetate, lactate and pyruvate compared.*

Original yeast first incubated in oxygenated water			Change in 12·5 g. yeast after being incubated in 1500 cc. of a solution of					
			(*a*) Na acetate (0·20 % acetic)			(*b*) Na lactate (0·20 % lactic)		
Carbohydrate g.	Fat g.	Protein g.	Carbohydrate g.	Fat g.	Protein g.	Carbohydrate g.	Fat g.	Protein g.
0·53	0·16	1·42	−0·03	+0·06	−0·16	−0·07	+0·07	−0·09
0·48	0·15	1·31	+0·04	+0·12	−0·20	−0·01	+0·05	−0·09
0·70	0·12	1·56	−0·02	+0·27	−0·03	−0·04	+0·09	−0·07
0·43	0·15	1·58	−0·07	+0·13	−0·14	−0·07	+0·04	−0·11
0·27	0·15	1·50	+0·31	+0·21	−0·10	+0·18	+0·03	−0·06
x	0·08	1·43	+0·32 − *x*	+0·22	±0·00	+0·46 − *x*	+0·13	±0·00
			2·11 g. acetate in 250 cc. water, shaken for 46 hours			3·85 g. lactate in 250 cc. water, shaken for 46 hours		
*0·70	0·08	1·30	−0·48	+0·15	−0·03	−0·50	+0·17	−0·03
*0·78	0·07	1·29	−0·45	+0·16	−0·01	−0·52	+0·18	−0·01
			(*a*) Na acetate			(*c*) Na pyruvate		
*0·98	0·07	—	−0·71	+0·15	—	−0·76	+0·10	—
0·53	0·17	1·43	+0·18	+0·18	−0·01	−0·11	+0·01	−0·11
			(*b*) Na lactate					
0·49	0·13	1·50	+0·02	+0·05	−0·04	−0·13	+0·01	−0·07
			2·3 g. Na acetate in 250 cc. solution, shaken for 46 hours			2·3 g. Na pyruvate in 250 cc. solution, shaken for 46 hours		
*1·02	0·07	1·24	−0·82	+0·21	−0·06	−0·23	+0·21	—

* Yeast not previously incubated in oxygenated water.

Table III. *Carbon balance of yeast after incubation in solutions of sodium acetate, lactate and pyruvate respectively.*

	Original yeast	Yeast after incubation
(*a*) Sodium lactate.		
Carbohydrate	0·78 g. = 0·312 g. C	0·22 g. = 0·088 g. C
Fat	0·07 „ = 0·052 „	0·25 „ = 0·188 „
Protein	1·29 „ = 0·645 „	1·13 „ = 0·650 „
CO_2 (free)	0·02 „ = 0·006 „	1·03 „ = 0·281 „
„ (bound)	— —	0·32 „ = 0·087 „
Volatile acids (acetic and pyruvic)	— —	0·06 „ = 0·024 „
Total lactic acid in original medium	2·71 „ = 1·084 „	— —
Residual lactic acid	— —	1·78 „ = 0·712 „
	2·099 „	2·030 „
(*b*) Sodium acetate.		
Carbohydrate	0·53 „ = 0·21 „	0·70 „ = 0·28 „
Fat	0·17 „ = 0·13 „	0·35 „ = 0·26 „
Protein	1·43 „ = 0·71 „	1·42 „ = 0·71 „
CO_2 (free)	0·27 „ = 0·07 „	0·70 }
„ (bound)	0·00 „ —	1·83 } „ = 0·69 „
Acetic acid: original	2·93 „ = 1·17 „	— —
„ residual	— —	0·76 „ = 0·30 „
	2·29 „	2·24 „
(*c*) Sodium pyruvate.		
Carbohydrate	0·53 „ = 0·21 „	0·42 „ = 0·17 „
Fat	0·17 „ = 0·13 „	0·18 „ = 0·11 „
Protein	1·43 „ = 0·71 „	1·32 „ = 0·66 „
CO_2 (free)	0·27 „ = 0·07 „	0·61 }
„ (bound)	0·00 —	0·79 } „ = 0·38 „
Pyruvic acid: original	3·30 „ = 1·35 „	— —
„ residual	— —	2·63 „ = 1·08 „
	2·47 „	2·40 „

increased storage of either fat or carbohydrate by the yeast which could be regarded as significant.

The results in the literature as to the effect of yeast on a solution of acetoacetic acid are conflicting; our results support the view that acetoacetic acid is not utilised by yeast to form storage products. Lundin [1923] found no increase in the amount of free acetone when a solution of potassium acetoacetate was acted upon by yeast and concluded that either acetone is not formed or if formed is either assimilated or burnt by the yeast cells. He found that in an oxygenated solution of acetoacetic acid (0·95 %) about 17 %, in the non-oxygenated solution about 11 %, of the acetoacetic acid had disappeared: in another experiment where the solution contained 2·93 % of acetoacetic acid the respective quantities which disappeared from the oxygenated and non-oxygenated solutions were 7·8 and 6·2 %. Lundin regarded the acetoacetic acid as being utilised by the yeast to form carbohydrate; he found an increase of dry weight had taken place in the yeast after incubation in the oxygenated acetoacetate solution and considered that this increase was due to the formation of carbohydrate, an assumption which was not confirmed by direct experiment. In any case only a small proportion of the acetoacetic acid present is acted on by the yeast.

In our experiments, after incubating the yeast for 22 hours in a solution of acetoacetate, a loss of only about 6 % of the acid originally present was observed and there was no indication that the yeast incubated in the acetoacetate solution had increased its store of carbohydrate or fat when compared with the control: our results indicate that acetoacetic acid is only utilised by yeast to a very small extent; we could not find any evidence that incubation in the sodium acetoacetate solution led to the formation of storage products in the yeast cells.

Table IV. *Comparison of original acetoacetate solution with solution after the yeast has been incubated in it.*

	(*a*) Original	(*b*) After incubation
Free CO_2	0·00 g.	0·80
Bound CO_2	0·70	0·56 g.
Total CO_2	0·70 g.	1·36 g.
Free acetone in medium and bubbler	0·09 g.	0·60
Acetone from acetoacetic acid	1·67	1·05
Total acetone	1·76 g.	1·65 g.

Amount of acetoacetic acid which has disappeared = 0·19 g.
" " " originally present = 2·94 "

		After incubation in	
	Original	(*a*) Acetoacetate	(*b*) Control alcohol
	g.	g.	g.
Dry weight yeast	2·07	2·40	2·68
Protein	1·35	1·31	1·32
Carbohydrate	0·36	0·38	0·40
Fat	0·10	0·23	0·27

No evidence of storage of carbohydrate or fat detected.

Table V. *Incubation of yeast in solutions of butyrate, β-hydroxybutyrate and aldol.*

	Original yeast			Yeast after incubation		
	Protein g.	Carbohydrate g.	Fat g.	Protein g.	Carbohydrate g.	Fat g.
Aldol	1·65	0·32	0·09	1·46	0·19	0·09
Acetate	1·49	0·49	0·13	1·37	0·45	0·27
Butyrate	—	—	—	1·18	0·36	0·16
Hydroxybutyrate	—	—	—	1·21	0·31	0·15
Butyrate	1·50	0·23	0·11	1·33	0·19	0·12
Acetate	1·66	0·37	0·11	1·42	0·43	0·28
Hydroxybutyrate	—	—	—	1·52	0·18	0·14

Incubation of yeast in a solution of sodium acetoacetate.

The solution of acetoacetate was obtained by adding slightly more than the calculated quantity of $N/2$ sodium hydroxide to 15·285 g. of ethyl acetoacetate in 750 cc. water and allowing it to stand overnight at the laboratory temperature: the solution was then neutralised and evaporated under reduced pressure (40 mm.) for $2\frac{1}{2}$ hours. The alcohol was determined in the solution and a corresponding amount of alcohol dissolved in water and used as a control, both solutions being made up to a litre, of which 262 cc. were diluted to 1500 cc. and used for the experiment (cp. Table IV).

Effect of Sulphite on Storage of Fat and Carbohydrate by Yeast.

As the result of these experiments we came to the conclusion that two-carbon atom compounds are more readily utilised by yeast than the three- and four-carbon atom compounds we had examined. Of the two-carbon atom compounds, acetic acid and ethyl alcohol furnish equally efficient sources of building material for the formation of the fat and carbohydrate the yeast stores within its cell. It seemed to us possible that both these acted as potential sources of acetaldehyde, supplying it in such a way that it was never allowed to accumulate in the cell but was immediately condensed to some less injurious product.

Neuberg has shown in his work on fermentation that acetaldehyde is formed as a decomposition product of sugar and is then reduced to alcohol; for when sodium sulphite is added to a fermenting sugar solution, the acetaldehyde reacts with the sulphite and the aldehyde-bisulphite compound can be isolated from the medium. The yield of alcohol is therefore diminished and may fall to about one-third of that which would be produced in the absence of the sulphite. There is always considerable dissociation of the bisulphite compound so that some alcohol is always formed. The excess of hydrogen which has not been used to reduce the acetaldehyde withdrawn by the sulphite is effective in producing a much increased supply of glycerol in the medium and the method has been used for the large scale preparation of glycerol. There seemed a possibility therefore that the addition of sulphite to the medium

might be used as a means of testing whether either the alcohol or acetate went through the stage of aldehyde before being converted to storage carbohydrate or fat by the yeast. If alcohol or acetic acid were first converted into acetaldehyde a considerable proportion of this would be held by the sulphite and we might expect to find a diminished store of fat or carbohydrate in the yeast; this proved to be the case.

The amounts of stored fat and carbohydrate were much less in the yeast which had been incubated in the solutions to which sulphite had been added than in the yeast incubated in the pure solutions of alcohol or acetate.

Table VI. *Effect of sulphite on storage of fat and carbohydrate by yeast.*

Original yeast		After incubation in			
		(*a*) Alcohol		Alcohol and sulphite	
Carbohydrate	Fat	Carbohydrate	Fat	Carbohydrate	Fat
g.	g.	g.	g.	g.	g.
0·435	0·11	0·545	0·38	0·29	0·26
0·46	0·09	0·68	0·43	0·28	0·21
—	—	—	—	0·28	0·22
0·71	0·08	0·54	0·40	0·33	0·29
		(*b*) Acetate		Acetate and sulphite	
0·44	0·08	0·44	0·31	0·25	0·19
		(*c*) Alcohol and sulphite		Acetate and sulphite	
0·43	0·11	0·31	0·22	0·36	0·25
0·76	0·10	0·48	0·23	0·42	0·21

The nature of the fat or rather of the ether-soluble substance was examined and proved to be practically the same in the yeast incubated in the media with and without the addition of sulphite. It consisted of from 53 to 60 % of fatty acids, having an iodine value of from 76 to 78 and of from 32 to 37 % unsaponifiable matter, which from its very high iodine value (about 200) must have consisted chiefly of the ergosterol characteristic of yeast [Smedley MacLean and Thomas, 1920].

In all these experiments however the processes of carbohydrate and fat storage always occurred together. From the results so far obtained it was possible that acetaldehyde was first formed and then built up directly either to form carbohydrate molecules or to form fatty acid chains; but there was also the possibility that the acetaldehyde was first condensed to a hexose molecule and that the latter was either further condensed to form storage carbohydrate or that the hexose molecules were directly condensed with each other to form long carbon chains which by subsequent reduction were converted to fatty acids as originally postulated by Emil Fischer.

These yeast experiments seemed for the first time to offer an opportunity of testing whether a fatty aldehyde occurred as an intermediate substance on the path from sugar to fatty acid. If this were the case the addition of sulphite to a solution of sugar in which yeast was incubated should produce a diminution of the fat and carbohydrate in yeast when compared with the same

sample of yeast incubated in a sugar solution to which sulphite had not been added. The results should be similar to those obtained when yeast is incubated in solutions of alcohol and acetate with and without the addition of sulphite which have already been described above.

It was found, however, that the addition of sulphite to a solution of fructose or glucose produces a result quite different from that obtained when the sulphite is added to the solution of alcohol or acetate. In the case of the yeast incubated in the sugar solution to which sulphite has been added there is no constant diminution in the amounts of stored fat and carbohydrate when compared with the yeast incubated in the sugar solution itself; the amount of carbohydrate stored is always greater in the yeast taken from the sulphite-containing medium, whilst the amount of fat is sometimes greater and sometimes less than the amount stored in the yeast incubated in the sugar solution. When, instead of considering the total amount of ether-soluble substance which we have termed the fat, the amount of fatty acids actually present is considered, we find the total amount of fatty acid present after the addition of the sulphite to the medium is in very much closer agreement with the corresponding amount produced in the hexose solution without the addition of sulphite. Further the effect of increasing the quantity of sulphite in the medium was to increase both the amounts of fat and of carbohydrate stored in the yeast. The fat, or rather the ether-soluble substance, isolated from the yeast after its incubation in the hexose solution differs in composition from that isolated from yeast incubated in the hexose-sulphite medium. In the latter case the percentage of fatty acids in the fat is considerably higher than in the former case and the percentage of unsaponifiable matter or sterol is considerably lower.

When yeast was incubated in a solution of fructose also containing about 4 % of a mixture of alkali phosphates, the addition of sulphite diminished the total amount of ether-soluble substance considerably, but here again the most marked diminution was in the weight of sterol, which was very much reduced. The effect on the carbohydrate was variable, and in the two experiments carried out there was a drop of about 20 % in the amount of fatty acid formed.

From these experiments it seems justifiable to infer that since the quantity of fatty acids formed from hexose by yeast is very little if at all diminished by the addition of sulphite to the medium, a fatty aldehyde does not occur as an intermediate stage between the hexose molecule and the fatty acid. The view of Fischer that the hexose chains are directly condensed to form chains containing fatty acid chains built up in multiples of six carbon atoms cannot then be disregarded. It would appear that in the action of yeast on ethyl alcohol or on acetic acid the first stage is the conversion to acetaldehyde; the next, the condensation of aldehyde to form a hexose molecule. This change does not take place at all unless there is a free supply of oxygen and oxidation must indeed form an essential part of the process for the change is represented by the following equation: $3CH_3.CHO + 3O = C_6H_{12}O_6$.

In a non-oxygenated solution of either alcohol or acetate there is no evidence of increased carbohydrate or fat storage; these materials are only formed in the presence of a plentiful oxygen supply. With an inadequate oxygen supply less carbohydrate is stored in the yeast incubated in the solution of alcohol than in the acetate solution, a result explained by the fact that oxidation of the alcohol to aldehyde has also to be brought about. We have previously shown that when yeast is incubated in a solution of a fermentable sugar the amounts of fat and carbohydrate stored are increased by the oxygenation of the medium but an appreciable amount of storage takes place in the absence of a free supply of oxygen. It seems therefore that hexose can only be formed from acetaldehyde in the presence of a free supply of oxygen, but that fat and carbohydrate can be formed from hexose in the absence of a supply of oxygen though the process is very much aided by such a supply. A plentiful supply of oxygen is an essential condition in order that good results may be obtained and in carrying out these experiments care must be taken that the oxygen is being passed through the control and the experiment at approximately the same rate.

The fact thus established that the yeast uses acetaldehyde as a starting material to build up carbohydrate is of particular interest in connection with the recent work of Neuberg and Gottschalk [1924, 1925], who have brought forward convincing evidence that acetaldehyde is a normal step in the oxidation of carbohydrate by muscle.

After these experiments were completed we noticed a paper by Haehn and Kinttof [1925] on the formation of fat by *Endomyces vernalis*. These authors find that this organism can utilise acetaldehyde, alcohol, aldol and pyruvic acid and store up fat from such solutions, carbon dioxide being simultaneously formed. They conclude that sugar is first decomposed with the production of acetaldehyde and that the latter is then condensed to produce fatty acids. Glycerol is also used by this fungus and built into fat, but less readily than alcohol. On the whole their conclusion agrees with the one that we have drawn from our experiments with yeast, namely that the simpler carbon compounds pass through the stage of acetaldehyde on their way to conversion to fat. Haehn and Kinttof also tested the effect of sulphite on the growth of *Endomyces vernalis* in a cane sugar solution; they found, however, that sulphite is very unfavourable to the growth of this organism and cannot be used in concentrations of more than 1 %. The fact that yeast is much less adversely affected by the presence of sulphite in the medium makes it easier to carry out such experiments and we think that Haehn and Kinttof's experiments with the *Endomyces* cannot be taken as furnishing conclusive evidence that the acetaldehyde passes directly to fat without being first converted to hexose. It is of course possible though unlikely that the path of metabolism in these two organisms is different. The evidence we have obtained from the study of the effect of the addition of sulphite to solutions of alcohol and sugar respectively in which yeast is incubated seems to us to point quite definitely to the con-

clusion that the hexose is transformed directly to fat without being first broken down to acetaldehyde. We feel that it is more difficult to interpret the experiments on *Endomyces* in which the sulphite is added to the sugar solution, because the conditions of growth are so adversely affected that it is necessary to prolong the experiments for very long periods in order to show the increase of fat.

We have previously brought forward evidence that phosphates [Smedley MacLean and Hoffert, 1923] play an important part in the change from hexose to fat, and that the amount of phosphate taken up by the yeast incubated in a solution containing both hexose and phosphate depends on the concentration of the *hexose*. It seems probable therefore that the hexose is converted into a hexosephosphate and that this substance undergoes further changes which result in the direct formation of the fatty acids; there is no evidence available to support the view that this compound breaks down into a simple substance from which the fatty acids are synthesised.

Table VII. *Effect of sulphite and phosphates on storage of carbohydrate, fat and sterol by yeast.*

12·5 g. pressed yeast after incubation in 1500 cc. of a solution of	Weight sulphite added ($Na_2SO_3.7H_2O$) g.	Amount carbohydrate g.	Amount ether-soluble substance g.	Saponification value	Amount fatty acids g.	% fatty acids	I.V. fatty acids	Amount sterol g.	% sterol	I.V. sterol
1. Glucose, 0·5 %	—	0·71	0·39	201·6	0·26	66·2	79·48	0·056	17·4	113·0
1 *a*. „ „	20	0·85	0·43	204·7	0·35	81·7	72·70	0·055	12·8	114·0
1 *b*. „ „	40	0·89	0·35	—	—	—	—	—	—	—
2. „ „	—	0·60	0·22*	—	—	—	—	—	—	—
2 *a*. „ „	20	0·88	0·33	220·0	0·27	81·4	72·7	0·41	12·4	—
3. Fructose, 0·5 %	—	0·82	>0·22	206·6	>0·17	79·7	66·4	>0·18	16·9	—
3 *a*. „ „	20	1·07	0·38	—	0·25	65·4	77·35	0·37	17·6	175·1
4. „ „	—	0·73	0·202	218·7	0·15	73·0	—	0·031	15·5	—
4 *a*. „ „	10	0·66	0·299	—						
4 *b*. „ „	20	0·75	0·239	—	0·68	76·0	—	1·32	14·9	—
4 *c*. „ „	30	1·04	0·354	196·0						
5. „ „	—	0·88	0·35	—	—	62·0	—	—	27·5	—
5 *a*. „ 0·25 %	30	1·06	0·28	201·9	0·20	—	76·47	0·06	20·8	155·8
5 *b*. „ 0·5 %	30	1·00	0·28	—	—	—	—	—	19·8	—
5 *c*. „ 0·75 %	30	1·29	0·35	—	—	—	—	—	—	—
6. „ 1 %	—	1·17	0·73	153·0	0·44	60·1	—	0·25	35·4	232·1
6 *a*. „ „	30	1·40	0·42	170·8	0·32	74·4	79·0	0·10	25·1	158·0
7. „ 2·4 %	—	0·97	0·55	153·2	0·35	63·7	72·01	0·18	33·1	158·6
7 *a*. „ „	60	1·65	0·67	181·8	0·48	72·6	76·47	0·14	21·7	159·1
9. Fructose, 2 % + phosphates†	—	1·15	1·15	136·4	0·60	57·9	73·24	0·37	32·1	176·3
9 *a*. Fructose, 2 % + phosphates	30	1·29	0·58	169·8	0·45	72·5	78·52	0·10	17·0	147·7
10. Fructose, 3 % + phosphates	—	1·77	0·80	149·9	0·51	65·0	76·17	0·25	31·6	205·1
10 *a*. Fructose, 3 % + phosphates	30	1·18	0·50	172·5	0·41	81·5	77·76	0·07	14·4	163·6

* Some of fat in control was lost.

† 0·39 % Na_2HPO_4 + 0·029 % KH_2PO_4.

With regard to the synthesis of sterol or unsaponifiable matter by the yeast, in every case the amount present was very much diminished in the yeast by the addition of sulphite to the hexose medium in which the yeast was incubated, a result, which suggests that the sulphite is exerting an effect on the internal metabolism of the yeast cell. There seem good grounds for believing that in the synthesis of sterol from hexose, an aldehyde forms an intermediate stage. In some cases the amount of sterol is only one-third or one-quarter that present in the yeast which has been incubated in the corresponding medium without the addition of sulphite.

Discussion of Results.

These experiments undoubtedly seem to furnish evidence that a path of chemical transformation exists from hexose to fatty acid without passing through any intermediate stage of aldehyde, and the question arises: Are we really justified in regarding the lower fatty acids containing even numbers of carbon atoms which occur in such fats as butter and coconut oil as stages in the synthesis of the fatty acids? Actually the only experimental evidence which is available is directly against this view and suggests that they are rather to be regarded as degradation products; this is furnished by some experiments of Meigs, Blatherwick and Carey [1919] on the difference of the composition of the blood before and after its passage through the active mammary glands of cows. These observers estimated the amount of inorganic phosphate and of lipin phosphate in the blood of the jugular and mammary veins of cows, both of those which were actively lactating and of those which were not giving milk at the time. They found that the inorganic phosphate in the actively lactating animal was increased and the lipin phosphorus diminished in the blood leaving the mammary gland of the actively lactating animal; they argued from this that fat is split off from the lipins of the plasma by the gland cells and they calculated that the change in lipin phosphorus would account for the setting free of the whole of the fat contained in the milk. If this conclusion be justified and if the whole of the fat be formed in this manner, the lower fatty acids present in milk must be formed by a degradation process from the higher fatty acids, for no fatty acids with less than sixteen carbon atoms have been shown to occur in lipins. Chiefly owing to the work of Levene we know that arachidonic, oleic, stearic and sometimes palmitic acids occur in the lipins of the blood, so that, if the acids containing from four to fourteen carbon atoms which are present in milk fat are derived from lipin, they must be formed by a process of degradation. One of the few facts which has been definitely established about the fate of the fatty acids is that they undergo β-oxidation in the body, and that the resulting products would therefore be the even-numbered members of the fatty acid series. Having regard to the experiments of Meigs, Blatherwick and Carey, it cannot be considered as definitely established that the lower fatty acids are intermediate

stages on their way to being built up into the higher fatty acids. The point is of fundamental importance in its bearing on the question of the method of formation of the fatty acids, but since the only two pieces of experimental evidence at present available seem to be that the higher fatty acids undergo β-oxidation in the body, presumably with formation of the lower even-numbered fatty acids as intermediate stages, and that fat is split off from the lipins of the blood by the active mammary gland, we are not justified in regarding these lower fatty acids as necessarily formed by a synthetic process.

The evidence obtainable from a study of the yeast organism is distinctly against the view that either acetaldehyde or pyruvic acid is an intermediate stage between the hexose molecule and the fatty acid. This is in agreement with the view of Shaffer [1922], who has opposed the hypothesis that fatty acids are built up from acetaldehyde on the ground that the first step would be the formation of the aldehyde of β-hydroxybutyric acid, a ketogenic substance, and this fact would render it necessary for us to regard the carbohydrates as ketogenic substances. It seems probable that the two-carbon atom compound is first converted to acetaldehyde, then synthesised to a hexose and that the hexose serves as the unit for further condensation with the ultimate production of the fatty acid. The phosphate group has previously been shown by us [1924] to play an important part in the process; it probably enters into combination with the hexose molecule or with some product derived from it, and there is no evidence that any breaking down of the six-carbon chain takes place before it is built up into the higher fatty acids.

There is certainly a good deal to be said in favour of the view that the hexose molecules are able to join directly together, in a way which we cannot imitate in the laboratory, to form long chains of carbon atoms linked together in multiples of six.

Summary.

1. The action of yeast on the following substances has been investigated: ethyl alcohol, acetaldehyde, sodium acetate, glycol, glycollic aldehyde, sodium glycollate, sodium glyoxylate, sodium oxalate, sodium lactate, sodium pyruvate, acetone, glycerol, aldol, sodium butyrate, sodium β-hydroxybutyrate, sodium acetoacetate.

2. Of these ethyl alcohol and sodium acetate were found to be best utilised by the yeast to form storage fat and carbohydrate, part being at the same time burnt and carbon dioxide formed.

3. Sodium lactate is also used by the yeast to build up fat and carbohydrate and to form carbon dioxide; the action is increased by vigorously shaking the solution and then resembles that of the acetate. Sodium pyruvate can also be utilised; the action is increased by vigorously shaking the solution but it is not as favourable as the lactate. Probably the acetaldehyde known to be formed acts unfavourably on the yeast, as we find that even at low con-

centrations the presence of acetaldehyde in the solution is unfavourable to the formation of metabolic products by yeast.

4. In all cases there must be a plentiful supply of oxygen to the medium, without which no storage takes place in the yeast.

5. The addition of sodium sulphite to the alcohol or acetate medium diminishes the storage of both fat and carbohydrate and it is inferred that the first stage is the transformation of the alcohol and acetate to acetaldehyde, which however is never allowed to reach a concentration which becomes injurious to the action of the cell. The ether-soluble substance formed in this way contains the same proportion of unsaponifiable matter (sterol) and fatty acids as that formed from the alcohol or acetate in the absence of sulphite.

6. The addition of sulphite to a solution of hexose in which yeast is incubated results in an *increase* of stored carbohydrate; the ether-soluble substance may be increased or diminished; the proportion of fatty acids it contains is considerably raised and the proportion of sterol much diminished. On the whole the amount of fatty acids formed is little influenced by the addition of sulphite to a medium containing hexose.

7. The deduction is made that the acetaldehyde is first condensed to hexose and that the latter is either converted to storage carbohydrate or else directly condensed to form the higher fatty acids without passing through a fatty aldehyde stage, probably by a direct linking of the hexose molecules.

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