part as such in the meal. Such a meal is more toxic than a properly cooked meal. In these cases gossypol may be extracted with ether and the amount estimated by the aniline method. It is interesting to note that gossypol in crude oil behaves much the same as free fatty acid. This was shown by dissolving some gossypol in neutral cotton oil, after which alkali was required to render the oil again neutral to phenolphthalein.

Thus the presence of a considerable amount of gossypol in crude oil would increase the refining loss. The writer has been informed that commercial "cold pressed" oil tends to show a smaller refining loss than hot pressed oil. Provided the same seed were used in each process the author believes that the reverse would be true owing to the presence of considerable amounts of gossypol in the crude cold-pressed oil.

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STUDIES ON THE BACTERIAL METABOLISM OF SULFUR.

II. FORMATION OF HYDROGEN SULFIDE FROM CERTAIN SULFUR COMPOUNDS BY YEAST-LIKE FUNGI.

BY FRED W. TANNER.

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In a series of studies which are being made on the relation of sulfur to bacterial metabolism, it seemed important to investigate this question with regard to the yeasts. Even though these microörganisms are not bacteria, the report of this investigation is included in the series. In a former paper, the author[1] has shown that bacteria are important in forcing sulfur through its cycle. Little data are available in the literature with regard to the relation of sulfur compounds to the metabolism of yeasts. A great part of that which does exist must be taken from reports of investigations which are directly concerned with other things. Lafar[2] who has given a fairly complete résumé of the work on sulfur has stated that the sulfur metabolism of yeasts is very much in the dark.

Small amounts of sulfur are probably necessary in the nutrition of the budding fungi. Ash analyses of yeasts made by different investigators are reported by Lafar[2] which show a variation in sulfur trioxide content of from 0 to 6.38 per cent. Since sulfur has been found in most yeasts, it seems reasonable to assume that it may be closely connected with their metabolism.

De Rey-Pailhade[3] reported the formation of hydrogen sulfide by yeasts and believed that its formation was due to the reducing enzyme which he called *philothion*. These data were later verified by Sostegni and San-

[1] Tanner, F. W., *Jour. Bact.*, **2**, 585–593 (1917).

[2] Lafar, F., *Handbuch der Technischen Mykologie*, **4**, 83–84 (1904).

[3] De Rey-Pailhade, M. J., *Bull. soc. chim.*, [3] **23**, 666–668 (1900).

nino.[1] To prove this, a pure culture of yeast was inoculated into a sugar solution containing sulfur. The evolution of hydrogen sulfide was soon observed. Pozzi-Escot[2] stated that *philothion* could also reduce selenium and phosphorous compounds and possibly arsenic and tellurium salts. Euler[3] does not agree with de Rey-Pailhade nor Pozzi-Escot that *philothion* is a reducing enzyme. It is now known under the name of *hydrogenase* (Grüsse;[4] de Rey-Pailhade[5]). That hydrogen sulfide is given off from certain industrial fermentations has been known for a long time.

Experimental.

The strains of yeasts which were used in this investigation came from different sources. The most of them, however, were received through the kindness of Doctor H. W. Anderson[6] who has recently published an extended study of the yeast-like fungi. No sharp line may be drawn between the different groups of fungi and in this paper some are included which might not be regarded as yeasts.

The technique of the investigation was quite similar to that described in a former paper. Strips of bibulous paper which had been treated with a saturated solution of lead acetate to which a small amount of glycerol had been added, were suspended in test tubes over the sulfur-containing substrate. The medium which was used had the following composition:

Triple distilled water	1000 cc.
Asparagin	2 g.
Dibasic sodium phosphate	1 g.
Dextrose	50 g.
Ammonium chloride	Trace
Ferric chloride	Trace
Magnesium chloride	Trace

This medium amply supplied the requirements of the yeasts, and all of them grew vigorously after the sulfur-containing compound had been added. Several control tubes were incubated which checked the chemicals used in the special medium and showed that the hydrogen sulfide which was observed, came from the special sulfur compound upon which the action of the yeast was being studied. Inoculations were made from dextrose agar slants, great care being taken to prevent taking any of this medium over to the culture tubes. Since the yeasts grew abundantly with a distinctly raised growth, this was accomplished with no difficulty. The culture tubes were incubated for 30 days at room temperature which

[1] Sostegni, L., and Sannino, A., *Chem. Centr.*, 61, 2, 112 (1890).

[2] Pozzi-Escot, M. E., *Bull. soc. chim.*, [3] **27**, 280 (1902).

[3] Euler, H., "General Chemistry of the Enzymes," Trans. Pope, John Wiley & Sons, New York, 1912.

[4] Grüsse, J., *Woch. Brau.*, **27** (1903). Original not seen. Quoted by Guilliermond in Les Levures, Paris, 1912.

[5] *Loc. cit.*

[6] Anderson, H. W., *J. Infect. Dis.*, **21**, 341–386 (1917).

varied from 24° to 30°. A summary of the results which were secured with the various compounds is given in Table I wherein the extent of hydrogen sulfide formation is roughly expressed.

Peptone.—A three per cent. solution of Witte's peptone in the special medium was used. Vigorous growth was observed in all cases but not all of the yeasts liberated hydrogen sulfide from peptone. Eleven of the 30 pure cultures of yeasts formed it, with *Mycoderma lactis* forming the largest amounts. With the bacteria as reported in a former paper it was found that a few reduced the sulfur in peptone but not that in cystine to hydrogen sulfide. This was not observed with the fungi which form the basis of this investigation. Fearing that the dextrose in the medium might be inhibiting the decomposition of the peptone a portion of the special medium was prepared without the addition of the dextrose. This was inoculated with those strains which formed no hydrogen sulfide from the original medium. Under these conditions *Saccharomyces ellipsoideus* Hansen and *Torula monosa* formed hydrogen sulfide.

Cystine.—This was prepared directly from wool according to Folin's[1] method. In order to conserve the supply of this compound, a 2% solution was distributed into small test tubes. Not over 4 cc. were used in any of the culture tubes. All of the strains of fungi used in this investigation reduced the sulfur in cystine to hydrogen sulfide. Some of these formed no hydrogen sulfide from peptone according to the method used in this investigation. The detailed results for each strain may be obtained from Table I.

Sodium Taurocholate.—A 2% solution of Merck's sodium taurocholate was used. This yielded a medium in which there was vigorous growth. *Oidium albicans* and *Mycoderma lactis* were the only strains which gave unmistakable evidence of hydrogen sulfide formation from this compound.

Sodium Phenolsulfonate.—A 3% solution of this compound in the special medium was used. None of the strains used in this investigation attacked the sulfur linkage in sodium phenolsulfonate. All of them grew well, many of them showing the characteristic growth of top yeasts.

Sodium Sulfate.—A 3% solution of this compound in the special medium was used. Beijerinck,[2] has stated that yeasts are unable to reduce sulfates to hydrogen sulfide. Kossowicz and Loew[3] reported an investigation which confirmed the statements of Beijerinck. Among the strains of yeast which they used and which were used in this investigation were *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus*.

In this investigation, 9 of the 30 pure cultures of yeast-like fungi formed

[1] Folin, O., *J. Biol. Chem.*, **8**, 9–10 (1910).

[2] Beijerinck, M. W., *Centr. Bakt. Parasitenk., II Abt.*, **6**, 194–206 (1900).

[3] Kossowicz, A. L., and Loew, W., *Z. Gärungsphysiol.*, **2**, 87–103 (1912).

TABLE I.—HYDROGEN SULFIDE FORMATION BY YEASTS.

Name of fungus	Peptone.	Cystin.	Sodium thio-sulfate.	Sodium sulfate.	Sodium sulfite.	Potassium sulfo-cyanate.	Thio-urea.	Sodium phenol sulfon.	Sodium tauro-cholate.	Sulfur.
Saccharomyces marxianus	—	+	—	—	+	—	+	—	—	+
Saccharomyces ellipsoideus Hansen	—	1+	4+	—	+	—	—	—	—	+
Saccharomyces hominis Busse	—	+	—	—	—	—	—	—	—	—
Saccharomyces anomolous Hansen	1+	2+	+	+	+	+	—	—	—	2+
Saccharomyces anomolous Hansen	1+	2+	4+	+	+	+	—	—	—	+
Saccharomyces albus	—	+	2+	—	+	—	2+	—	—	—
Saccharomyces glutinis (Fres) Cohn	—	+	1+	—	+	—	—	—	—	—
Zygosaccharomyces bisporus Anderson	—	+	2+	—	+	—	—	—	—	1+
Saccharomyces of Cutris	—	+	1+	+	+	—	—	—	—	+
Parasaccharomyces Thomasii Anderson	—	+	4+	—	+	—	—	—	—	4+
Parasaccharomyces Ashfordii Anderson	—	+	3+	—	+	+	1+	—	—	—
Willia belgica Lindner	+	+	—	—	+	—	—	—	—	+
Mycoderma monosa Anderson	+	3+	4+	1+	5+	3+	—	—	—	4+
Mycoderma vini	+	3+	4+	1+	5+	3+	2+	—	—	—
Pseudosaccharomyces Stevensii Anderson	+	+	1+	—	—	—	—	—	—	1+
Monilia candida Bon	+	1+	4+	+	+	2+	3+	—	—	1+
Schizosaccharomyces Pombe Lindner	—	+	2+	—	+	—	—	—	—	1+
Cryptococcus glabratus Anderson	+	+	—	—	+	—	—	—	—	1+
Cryptococcus verrucosus Anderson	—	+	—	—	+	—	—	—	—	+
Oidium albicans Ch. Robin	—	+	3+	—	+	—	2+	—	+	1+
Mycoderma lactis	2+	1+	4+	1+	3+	3+	+	+	+	+
Torula humicola Daczewska	—	—	—	—	—	—	—	—	—	—
Saccharomyces of Binot	—	+	3+	—	+	—	+	—	—	+
Champagne yeast	—	+	—	+	+	—	+	—	—	+
Burgundy wine yeast	—	1+	4+	1+	+	—	+	—	—	+
Brewers' yeast	1+	4+	—	—	+	3+	—	—	—	1+
Saccharomyces cerevisiae Hansen	—	+	1+	—	—	—	+	—	—	—
Cryptococcus agregatus Anderson	—	+	1+	—	—	+	+	—	—	+
Torula monosa	—	+	2+	—	—	—	—	—	—	—
Torula datilla	+	1+	—	+	—	—	+	—	—	+

\+ = edge of the lead acetate paper darkened.
1+ = about 1 cm. of the paper darkened.
2+ = about 2 cm. of the paper darkened.
3+ = etc.
4+ = etc.

hydrogen sulfide from sodium sulfate. None of these strains were among those used by Kossowicz and Loew. This may possibly explain their statement that yeasts do not reduce sulfates to hydrogen sulfide. Had they used more than four strains, it is possible that they would have observed hydrogen sulfide formation from some of them.

Sodium Sulfite.—A 3% solution of sodium sulfite was used. Twenty-three of the strains reduced the sulfur in sodium sulfite to hydrogen sulfide. In this compound, the sulfur is partly reduced, which may explain why a greater number of the yeasts produced hydrogen sulfide from sodium sulfite than from sodium sulfate. The bacteria are not able to reduce sulfates under aerobic conditions but could reduce the less highly oxidized forms of sulfur. Also, the yeasts seem to be less sensitive to sulfites than the bacteria. Nagaeli[1] has stated that sulfates, hyposulfites and sulfites may serve as sources of sulfur for yeasts.

Sodium Thiosulfate.—Beijerinck[2] found that yeasts could form hydrogen sulfide from thiosulfates. Hahn (1903) showed that the juice pressed from yeasts cells exhibited a reducing action on sulfur. Kossowicz and Loew[2] extended our knowledge on this subject by asserting from data which they present, that yeasts are able to use thiosulfate sulfur in their metabolism. No free sulfur nor sulfates could be detected by these investigators in their culture flasks. To study this question more extensively the following work was carried out:

A 3% solution of Merck's sodium thiosulfate in the special medium was used. In this the control culture tubes showed the formation of hydrogen sulfide. This probably took place according to the following equations:

$$Na_2S_2O_3 + NaH_2PO_4 = H_2S_2O_3 + Na_3PO_4$$

$$H_2S_2O_3 + H_2O = H_2S + H_2SO_4$$

The use of the normal sodium phosphate reduced the amount of hydrogen sulfide formed spontaneously without the aid of the microörganisms but it was not used in any of the culture media. In no case did the blank tubes show as much darkening of the lead acetate paper as the culture tubes. In order to secure accurate data with regard to this compound, the contents of the culture tubes were precipitated with cadmium carbonate and filtered through a dry filter paper. Five cc. of the filtrate were then titrated with 0.1 *N* iodine solution. The results are shown in Table II and are calculated to the amount of thiosulfate which disappeared from 100 cc. of medium. Corrections were made by means of two blanks so that the loss there indicated was directly due to the action of the microorganisms.

[1] Nagaeli, see "Technical Mycology," by Franz, Lafar, trans. Salter. Vol. 2, Part I, p. 48. C. Griffen and Company, London, 1912.

[2] *Loc. cit.*

TABLE II.—DECOMPOSITION OF SODIUM THIOSULFATE.

Culture No.	Name of fungus.	Mg. $Na_2S_2O_3$ decomposed in 100 cc. of medium.
1	*Saccharomyces marxianus*	0
2	*Saccharomyces ellipsoideus*	30.02
3	*Saccharomyces hominis*	0
4	*Saccharomyces anomolous*	23.70
5	*Saccharomyces anomolous*	26.22
6	*Saccharomyces albus*	14.85
7	*Saccharomyces glutinis*	11.06
8	*Zygosaccharomyces bisporus*	12.64
9	*Saccharomyces* of Curtis	12.64
10	*Parasaccharomyces Thomasii*	12.64
11	*Parasaccharomyces Ashfordii*	20.54
12	*Willia belgica*	0
13	*Mycoderma monosa*	31.60
14	*Mycoderma vini*	28.44
15	*Pseudosaccharomyces Stevensii*	11.48
16	*Monilia candida*	23.70
17	*Schizosaccharomyces Pombe*	12.64
18	*Cryptococcus glabratus*	0
19	*Cryptococcus verrucosus*	0
20	*Oidium albicans*	23.70
21	*Mycoderma lactis*	34.76
22	*Torula numicola*	0
23	*Saccharomyces* of Binot	23.70
24	Champagne yeast	0
25	Burgundy wine yeast	22.12
26	Brewers' yeast	0
27	*Saccharomyces cerevisiae*	11.48
28	*Cryptococcus agregatus*	12.08
29	*Torula monosa*	13.07
30	*Torula datilla*	0

Twenty-one of the pure strains of yeasts used in this investigation formed hydrogen sulfide from sodium thiosulfate. No sulfates nor free sulfur could be detected in the culture tubes. Sulfites were demonstrable. This is in accord with the work of Neuberg and Welde[1] who report that yeasts break up thiosulfate according to the following equation:

$$Na_2S_2O_3 + H_2 = H_2S + Na_2SO_3$$

and not according to the following

$$Na_2S_2O_3 + H_2O = NaSO_4 + H_2S$$

Potassium Thiocyanate.—Thirty g. of the pure salt were added to a liter of the special medium, in which all of the strains grew well. Gröller[2] studied the thiocyanates as sources of carbon, nitrogen and sulfur for bacteria yeasts and molds. Thiocyanates were utilized as sources of sulfur

[1] Neuberg, C., and Welde, E., *Biochem. Z.*, **67**, 111–118 (1915).

[2] Kossowicz, A., and Gröller, L., 1912–1913; *Z. Gärungsphysiol.*, **2**, 159–165 (1912).

and nitrogen but not for carbon. Hydrogen sulfide was liberated in some cases but no free sulfur was produced.

Ten of the pure strains of yeasts attacked potassium thiocyanate with the liberation of hydrogen sulfide. *Mycoderma vini, Mycoderma monosa, Mycoderma lactis* and Brewers' yeast formed especially large amounts of hydrogen sulfide from this compound. Nagaeli has reported that ammonium thiocyanate was unsuitable as a source of sulfur for yeasts.

Thiourea.—A 3% solution was used. Fourteen of the yeasts formed hydrogen sulfide from it. Nagaeli has reported that this urea was unsuitable as a source of sulfur for yeasts.

Free Sulfur.—About 0.2 g. of Merck's resublimed sulfur was placed in the bottom of each culture tube, to which were added about 10 cc. of the special culture medium. After sterilization, this sulfur collected in the bottom of the culture tubes in the shape of a button but available for the yeasts. Eight of the strains formed no hydrogen sulfide from free sulfur. In each of these 8 tubes there was good evidence of vigorous growth.

Summary.

The budding fungi used in this investigation are able to reduce the sulfur in cystine to hydrogen sulfide. With regard to peptone this characteristic is less extensive. Most of the strains were able to attack the sulfur linkage in thiosulfate to produce hydrogen sulfide. Thiosulfates are probably reduced to sulfite and hydrogen sulfide. Contrary to statements in the literature that yeasts are unable to reduce sulfates, 10 of the strains used in this investigation reduced sodium sulfate to hydrogen sulfide. A few of the yeasts reduced sodium sulfite. Sodium taurocholate was reduced to hydrogen sulfide by 2 strains, while sodium phenolsulfonate was not, although there was good growth in this latter substrate. Potassium thiocyanate and thiourea were also reduced. Hydrogen sulfide was also formed by many of the yeasts from free sulfur. Yeasts seem to be able to split some of the more stable linkages of sulfur, a characteristic which is probably not so wide spread among the bacteria.

ANALOGUES OF ATROPINE AND HOMATROPINE.

By Louis F. Werner.

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The alkamine tropine, one of the hydrolysis products of atropine, is changed upon oxidation to the ketone tropinone. This ketone is closely related to another ketone known as pseudopelletierine, which occurs naturally in the bark of the pomegranate tree. The relationship between the two is shown by the following structural formulas: