distilled at 200–210°/0.05 mm. as a pale yellow oil which solidified on cooling with ice, and melted at about 20°; $[\alpha]_D + 11.1^\circ$ (in ether; $c=11.1$); C-methyl, 12.8%. The amide was obtained as a low-melting solid which was very soluble in organic solvents; the tribromoanilide separated from aqueous methanol as an oil.

SUMMARY

1. A scheme of separating mixtures of the component fatty acids from the lipids of human tubercle bacilli is described. The scheme, which may be found of value in other connexions, depends upon the fractional crystallization of derivatives, obtainable by converting the acids into acetol esters, $R.CO_2CH_2.CO.CH_3$, and treating the latter with an appropriate reagent for ketones, such as semicarbazide or 2:4-dinitrophenylsemicarbazide.

2. The acids isolated by this process include a saturated C_{19} -acid, resembling tuberculostearic acid in respect of the melting points of derivatives; how-

ever, the melting points of the free acids are found to differ by about 12°, and the available evidence seems to indicate the presence of an additional acid of similar composition, having a lower melting-point.

3. From a mixture of the acids of higher molecular weight three dextrorotatory products are obtained by the above procedure; of these, two appear to contain four terminal methyl groups, and the third, three such groups.

4. The presence of an unsaturated branched-chain acid of high molecular weight is indicated by the isolation of its oxidation product; for the oxidation hydrogen peroxide in formic acid has been employed.

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The Determination of Glutathione and other Substances Contained in Yeast

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In the course of an investigation of the action of glutathione on the growth of tissue cells *in vitro* (Astrup & Fischer, 1946) we made some determinations of the amount of glutathione present in Danish yeasts. During these determinations some difficulties were encountered which are of importance also for the determination of other substances contained in yeast and which, as far as we could find, have not previously been described.

METHODS

Glutathione was measured colorimetrically with nitroprusside (Fujita & Numata, 1939; Numata, 1940), using, however, $2N-NH_3$ instead of a N -solution for developing

the colour, and a standard curve made under identical conditions for comparison. Reduction of GSSG to GSH was carried out electrolytically after Dohan & Woodward (1939). Phosphorus was estimated according to Fiske & Subbarow (1925), using a Pulfrich photometer (Tørell, 1931). Glutathione was prepared by the method of Pirie (1930).

RESULTS

When metaphosphoric acid was used for extracting GSH from yeast no definite results could be reached. Thus, Fig. 1 shows a curve *A* the values of which were obtained by grinding different 1 g. lots of baker's yeast with fine sand in a mortar until a paste was formed, then 4 ml. of 5% (w/v) freshly prepared metaphosphoric acid solution were added and

the mixture allowed to stand for varying lengths of time, 5 ml. of water added and the GSH measured in the centrifuged extract. The results are given as mg. GSH/g. of fresh yeast (Fig. 1).

Fig. 1. The extraction of glutathione from yeast by treatment with metaphosphoric and sulphosalicylic acids for various periods. Curve A, mg. GSH/g. of fresh yeast obtained by extraction with metaphosphoric acid. Curve B, mg. GSH/g. of fresh yeast (another sample) after extraction with sulphosalicylic acid. ----- Total GSH obtained after treatment with ethanol followed by sulphosalicylic acid.

A treatment with sulphosalicylic acid in the same manner yielded a curve B of similar appearance, although higher values were obtained. It was observed that a sulphosalicylic acid extract of dried yeast (prepared with ethanol) gave still higher values. The paste from 1 g. of yeast was therefore first treated with 2 ml. ethanol, and then 4 ml. 5% (w/v) sulphosalicylic acid were added followed by 3 ml. water. The amount of GSH found was now further increased and did not change with time. In this manner the amount marked 'total GSH' in Fig. 1 was obtained. Similar results were obtained when metaphosphoric acid was added after ethanol treatment.

The influence of different concentrations of sulphosalicylic acid on the extraction was investigated (Table 1). The same amount of sulphosalicylic acid/g. of yeast was used, and the concentrations of both yeast and sulphosalicylic acid during the extraction were varied simultaneously. After the treatment water was added until the total volume was 10 ml., the final concentration of sulphosalicylic acid being 2%. Even when sulphosalicylic acid was present in the highest concentrations during the denaturation period not all GSH was removed, the total amount found after treatment with ethanol and sulphosalicylic acid being 1.86 mg. GSH/g. yeast.

With trichloroacetic acid, however, a treatment with 2 ml. of a 10% (w/v) solution for 5 min. resulted in total extraction of GSH (1.93 mg. GSH/g.

yeast). Addition of ethanol (2 ml.) did not significantly alter this figure (1.85 mg.), while a lower concentration of trichloroacetic acid (4 ml. 5% solution) during the denaturation was quite insufficient, yielding a value (0.50 mg.) almost as low as that (0.20 mg.) obtained by treatment with 2 ml. 10% sulphosalicylic acid alone.

Table 1. Determination of GSH after treatment of 1 g. yeast with different concentrations of sulphosalicylic acid

Sulphosalicylic acid solution added		Water added after extraction (ml.)	GSH (mg./g. yeast)
Initial concentration (%)	Volume used (ml.)		
2.5	8	1	0.25
5.0	4	5	0.47
10	2	7	1.18
20	1	8	1.52
40	0.5	8.5	1.55
100	0.2 g.	8.8	1.65

From these results it is seen that both the nature and the concentration of the denaturing agents are of importance for the extraction. The quantitative determination of GSH in yeast may be performed, according to these investigations, either by treatment of 1 g. of yeast with sand and 2 ml. 10% trichloroacetic acid for 5 min., or with sand and 2 ml. ethanol followed by 4 ml. of either sulphosalicylic or metaphosphoric acid in 5% solution. For comparison three 1 g. samples from the same batch of Danish baker's yeast were treated with trichloroacetic acid and with ethanol and sulphosalicylic acid. Trichloroacetic acid treatment gave 2.15, 2.38 and 2.27 mg. of GSH/g. of yeast; while ethanol and sulphosalicylic acid gave 2.25, 2.35 and 2.38 mg. Three types of brewer's yeast were obtained from the Carlsberg brewery: F, U (bottom yeasts) and O (a top yeast). They were washed several times with water and filtered through a sintered-glass filter (G 4) until the dry substance amounted to 25–26%, corresponding to the dry substance in the baker's yeast used. Yeasts F and U contained 1.40 mg. GSH/g., but yeast O only 0.86 mg. The GSH content was thus considerably lower than in the baker's yeast investigated, in which the amount of GSH in the different samples investigated by us usually varied between 1.80 and 2.30 mg. GSH/g.

In order to see whether the denaturing agents also acted differently on cells other than yeast cells we measured the GSH content of rat's liver. The liver, removed immediately after killing the rat, was kept frozen in a freezing mixture. Samples (1 g.) were

treated as described for yeast. The following results are typical:

Treatment	mg. GSH/g. liver
Trichloroacetic acid (10%)	1.76, 1.74
Metaphosphoric acid (5%)	1.75, 1.85
Sulphosalicylic acid (5%)	1.74, 1.78
Ethanol and sulphosalicylic acid (5%)	1.80, 1.74

They are concordant and independent of the method, and the differences observed with yeast cells must therefore be due to some peculiarity of these cells. It was of interest to find out whether other constituents of the yeast cell would behave in the same manner as GSH. We compared, therefore, the extraction of GSH with that of total phosphorus. The results are shown in Fig. 2. The mode of release of

Fig. 2. Comparison of the extraction of GSH and total P from fresh yeast by treatment with 5% sulphosalicylic acid. ----- Total GSH obtained after treatment with ethanol followed by sulphosalicylic acid.

GSH and of phosphorus from the yeast cells is evidently of a similar nature, and the difficulty of obtaining total extraction is most probably due to the cell membrane. When the yeast was treated directly with 5% sulphosalicylic acid without sand, similar curves were obtained, but the values for the first minutes were considerably lower than with previous treatment with sand. The sand may act solely by destroying the cell membrane, so that the cell contents are more easily released. With water alone only very small amounts of GSH, <0.2 mg./g. yeast, were found; even after 3 hr. extraction without sand no phosphorus was found. Treatment with 2 ml. 10% trichloroacetic acid followed by 7 ml. of water yielded after 10 min. without treatment with sand the total amount of GSH and phosphorus. All these results are best explained by the retaining action of the undestroyed cell membrane.

We therefore tried to destroy the cell membrane with some plasmolyzing agents. 5 g. of yeast were treated for 1 hr. with (a) 0.25 ml. chloroform, (b) 0.25 ml. of Pirie's (1930) mixture (20 ml. conc.

sulphuric acid, 100 ml. ethanol and 80 ml. ether), (c) 0.25 ml. of benzene, (d) 5 g. NaCl, (e) 25 ml. of saturated NaCl solution, (f) 25 ml. of a 33% (w/v) solution of urea. With benzene and sodium chloride almost no GSH was extracted (about 0.2 mg./g. yeast). Chloroform and urea were considerably better, yielding about 50% of the total GSH. Pirie's mixture, which released almost all GSH, was best.

Repeated freezing of a sample of yeast acted in the same manner. Eight (1 g.) samples of yeast were placed in centrifuge tubes, frozen in a mixture of ether and solid CO₂, thawed and frozen again, the process being repeated six times in all. The yeast became fluid after the first freezing. Then different denaturing agents were added followed by water until the volume totalled 10 ml. The following amounts of GSH were found:

Treatment	mg. GSH/g. yeast
Sulphosalicylic acid (4 ml. 5%)	2.41, 2.36
Trichloroacetic acid (2 ml. 10%)	2.53, 2.36
Ethanol (2 ml.) and sulphosalicylic acid (4 ml. 5%)	2.33, 2.39
Metaphosphoric acid (4 ml. 5%)	(2.91, 2.95)

In this case all the denaturing agents yielded similar results. Metaphosphoric acid was unsuitable as a slight turbidity appeared on addition of the ammonia solution, resulting in too high values for the GSH content.

DISCUSSION

These results suggest that the difficulties met with in determining substances contained in yeast cells may be caused by the resistance of the cell membrane to the action of the various denaturing agents. However, the large differences observed between the action of agents commonly used for the precipitation and denaturation of proteins were unexpected. While trichloroacetic acid yielded the total amount of GSH, sulphosalicylic and metaphosphoric acids did so only after treatment with ethanol. Our use of ethanol originated from the observation that yeast dried with it yields considerably higher amounts of GSH than fresh yeast. Similar observations had already been made with ethanol (Régnier, 1933), and acetone (Schroeder, Collier & Woodward, 1939), in the isolation of GSH from yeast. Treatment of yeast with ethanol in the analytical determination of GSH has been used previously by Weller (1936) and Bolomey (1939), but in connexion with removal of proteins by means of 10% trichloroacetic acid, where according to our experience it is of no use.

The total GSH determined in yeast after electrolytic reduction was often identical with the amount of reduced GSH present, and in no case did the amount rise by more than 10% after reduction. Our

results thus confirm those of Bolomey (1939), and it is our opinion that the large amounts of oxidized GSH found by some authors in yeast may be the result of inaccurate analytical procedures (cf. Fink & Just, 1940).

SUMMARY

1. Different denaturing agents (trichloroacetic acid, sulphosalicylic acid, metaphosphoric acid)

release different amounts of GSH and phosphorus from yeast cells.

2. This must be taken into consideration in the estimation of substances contained in yeast cells. Most probably the cell membrane is the cause of the difficulties.

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Preliminary Experiments in the Study of the Respiratory Activity of Micro-organisms Suspended in Thin Films of Fluid Adhering to Solid Surfaces

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It is obvious that the metabolic events taking place in soil occur, under normal field conditions, in the films of water adhering to the soil surfaces. These films vary greatly in extent and thickness, according to soil conditions, e.g. soil structure and moisture content. Evidence secured recently by Lees & Quastel (1946) in their investigations of nitrification in soil show that, even in a soil which is water-saturated but not waterlogged as in their soil perfusion apparatus (Lees & Quastel, 1944), the process of conversion of ammonia into nitrate is brought about by bacteria proliferating at the soil surfaces. It is therefore evident that, for quantitative studies of the biochemical changes taking place in soil, techniques are required which will allow investigations to be made of the metabolism of micro-organisms when these are suspended in films of fluid adhering to solid surfaces, such as those presented by soil.

The well-known Warburg or Barcroft manometric apparatus may be very conveniently adapted for the measurement of the respiration of micro-organisms, when a suspension of these is spread over suitable solid surfaces.

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Usually, for estimations of the respiration of micro-organisms, a suspension of the cells in 3 or 4 ml. fluid is placed in the manometer vessel of the Warburg or Barcroft apparatus. The oxygen consumption of the cells, when the apparatus is not shaken, proceeds slowly, the rate being determined by the surface of the fluid in the vessel and the rate of diffusion of oxygen into the fluid. When the apparatus is vigorously shaken new fluid surfaces are constantly exposed to the air in the manometer vessel with the result that the fluid becomes saturated with oxygen. There results an immediate rise in the rate of oxygen consumption by the micro-organisms. The effect of shaking the apparatus is the same as that of spreading the volume of fluid in the vessel over an area so large that the thickness of the film of fluid no longer makes diffusion of oxygen into the fluid a limiting factor. When, therefore, a suspension of the micro-organisms is spread over the surfaces of suitable substances, placed in a vessel attached to a manometer, so that the area of spread is sufficiently great to ensure that diffusion of oxygen into the fluid is not a limiting factor, the resulting rate of oxygen uptake is precisely the same as