3. THE EFFECT OF SOME SATURATED FATTY ACIDS ON THE RESPIRATION OF BAKER'S YEAST

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IN the course of a study [Cook *et al.* 1938, 1] of the effects of certain fractions from yeast on the respiration of yeast and tissues it was observed [Cook & Kreke, 1938, 1] that a water-insoluble steam distillate obtained from an aqueous-alcoholic extract of yeast caused a marked increase in O_2 uptake when added to a yeast suspension but depressed the respiration of rat skin [Cook & Kreke, 1938, 2]. From its properties it soon became evident that the bulk of the distillate was composed of saturated fatty acids, and preliminary experiments showed similar respiratory activities to be possessed by decoic and undecoic acids [Cook & Kreke, 1938, 2; 1939].

It is well known that many animal tissues, such as liver, kidney and spleen, effect the oxidation of fatty acids *in vitro* [Quastel & Wheatley, 1933; 1934; Jowett & Quastel, 1935; Quagliarello, 1932, 1, 2; Quagliarello & Scoz, 1933]. Fatty acids inhibit the oxidation of pyruvate by brain, apparently by displacement of one of the members of the pyruvate-oxidase system [Peters & Wakelin, 1938], and also inhibit the respiration of rat skin [Cook & Kreke, 1938, 2; 1939]. Ciaranfi [1938] has shown that tumour tissue is unable to oxidize saturated and unsaturated fatty acids *in vitro* and that some acids inhibit tumour respiration, a fact which has been confirmed in our laboratory. Less has been written concerning the oxidation of fatty acids by yeast. It has been well established, particularly by the work of Smedley-Maclean [Smedley-Maclean & Hoffert, 1923; 1926; Macleod & Smedley-Maclean, 1938] and of Wieland & Wille [1935], that yeast, when incubated in oxygenated solutions, converts acetate into fat, at the same time burning part of it, but formate, propionate or butyrate do not seem to influence the production of fat [Smedley-Maclean & Hoffert, 1923]. Wieland & Sonderhoff [1932] have found that yeast oxidizes acetate to CO_2 and water and also in part to succinic and citric acids under the proper conditions. Winzler & Baumberger [1938] report that yeast oxidizes 58.7% of Na acetate to CO_2 and water and assimilates the remainder.

The purpose of this paper is to show the relative effects of a series of fatty acids on the O_2 uptake of baker's yeast and of certain of these acids on the R.Q. The facts observed are consonant with the oxidation of these acids by yeast, but proof of this must await a detailed chemical study of the fate of the acids.

EXPERIMENTAL

Fatty acids. The saturated fatty acids employed were Eastman Kodak products and were used without further purification. A specially purified sample of sodium ricinoleate was also tested and was supplied through the courtesy of

Dr R. S. Shelton of the Wm S. Merrell Co., Cincinnati, Ohio. Stock solutions of the fatty acids containing about 50 mg. per ml. were prepared and adjusted to pH 7.3 (glass electrode) with NaOH. This brought some of the lower acids into solution and partially dissolved the others with the formation of a uniform suspension. Warming to 37.5° increased the solubility. It is evident that at this pH mixtures of acid and Na salt existed [cf. Peters, 1931].

Respiration technique. The direct Warburg manometric technique was employed with slight changes from that described in previous papers [Cook *et al.* 1938, 1, 2; Cook & Kreke, 1939; Norris & Kreke, 1937]. Fleischmann's baker's yeast from the centre of a fresh 1 lb. cake was washed 3 times by centrifuging with the suspending medium and then was made up with fresh medium to a count of 250 as determined by a photoelectric densitometer (count of 1 = 250,000 cells per ml.). Ringer-phosphate-glucose solution (0.02 % glucose, pH 7.3) was used as the suspending medium in the earlier experiments.

1 ml. of the yeast suspension (containing an average of 3.9 mg. dry weight of yeast), the desired amount of the acid solution and sufficient Ringer-phosphate-glucose to bring the volume to 3.1 ml. were placed in the manometer flask, and 0.2 ml. *N* KOH was placed in the inner well. After an equilibrium period of 15 min. the manometers were shaken at 120 oscillations per minute for a 25 min. respiration period, in air, at 37.5°. The entire water bath was enclosed in a box maintained at approximately 27°.

Fig. 1. Effect of added fatty acids on O_2 uptake of baker's yeast in Ringer-phosphate-glucose (0.02 %) at pH 7.3 and 37.5° for 25 min. respiration period.

The effects of ten fatty acids on the respiration of yeast under these conditions are shown in Fig. 1. The fatty acids were not oxidized to a measurable extent in the absence of yeast. For the sake of readier comparison all of the results have been calculated to a control respiration of 50 μ l. The actual average control respiration (respiration of yeast without added acid) was 48 μ l. (range,

40–58 $\mu\text{l.}$) which corresponds to an average $Q_{O_2}^{\text{air}}$ of about 30 with a range of 25 to 36. It will be observed that the acids have optimum effective concentrations. Parallel staining experiments with methylene blue showed that inhibiting concentrations of the acids were toxic to the yeast cells. It has been shown that pyruvic acid, which is converted into fat by yeast, is also toxic to yeast in higher concentrations [Haehn & Glaubitz, 1927; Smythe, 1938]. An optimum concentration of fatty acids also exists for the respiration of liver slices, higher concentrations being inhibitory [Quastel & Wheatley, 1933; 1934; Jowett & Quastel, 1935].

It will be noticed that, in general, there is an increase in physiological activity of the acids with increase in length of the straight carbon chain over the range C_5 – C_{11} with the apparent exception of heptonic acid. This is evident both in the increased toxicity and the increased respiratory activity of lower concentrations. Stearic acid, on the other hand, shows a lowered activity. Ricinoleic acid, of interest because of its bacterial detoxifying properties, was found to be very toxic to yeast but very active in non-toxic concentrations. It is of interest that Beck [1935] has found the lower aliphatic acids (acetic to hexoic) to inhibit cytochrome reduction in yeast, and increase in inhibiting power accompanies an increase in length of carbon chain in acid concentrations of 0.01 M . Very little effect was observed in 0.001 M concentration. The addition of Na salts of the acids, however, in part overcomes the inhibitory effects of the free acids. These observations are suggestive in connexion with an ultimate consideration of the mechanism involved. It is to be remembered that the physical properties of the acids, e.g. the amount in solution, their surface activity and the ease of penetration into the cell, will vary with the size of the acid and must be taken into consideration in a final evaluation of the effects. It is, however, not within the province of the present paper to discuss cell penetration although it may be pointed out that the lower fatty acids and their salts rapidly penetrate yeast cells [Beck, 1935]. For discussion of cell penetration by these substances see, for example, Beck [1935], Harvey [1914], Crozier [1916], Smith & Clowes [1924], Smith [1925], Lillie [1926], and Howard [1931]. See also the suggestive film penetration studies of Schulman & Rideal [1937] and Schulman [1937].

Respiratory quotients. In order to investigate the above effects a little further, the influence of hexoic and decoic acids on the R.Q. was studied. It has been shown [Stier & Stannard, 1936; Warburg, 1927] that baker's yeast in a phosphate medium free of glucose oxidizes endogenous carbohydrates alone and that the CO_2 produced is purely respiratory uncomplicated by fermentative CO_2 . Under these conditions as well as in the presence of 0.02 % glucose we have found the addition of the fatty acids to lower the R.Q.

R.Q. were obtained by the direct two-vessel method [Dixon, 1934]. To obtain the conditions of Stier & Stannard and in order to avoid retention of CO_2 the suspending medium was changed from Ringer-phosphate to $M/15 \text{ KH}_2\text{PO}_4$ at pH 4.5, as suggested by Krebs [1928]. This made the addition of acid at the end of the experiment unnecessary, as shown by trial, and made it possible to construct time curves. In a number of experiments 0.02 % glucose was added to the medium and, although the total O_2 consumption was raised (Figs. 2 and 3), this amount of glucose did not alter the R.Q. within the limits of experimental error. The acids proved to be more toxic to yeast in the KH_2PO_4 than in Ringer-phosphate solutions and therefore less could be added. It should also be noted that free acids and not Na salts were present in suspension. Their essential behaviour, however, did not appear to be altered since cell penetration was evidently accomplished.

Fig. 2. Effect of 5.8×10^{-4} *M* decoic acid (0.1 mg. per ml.) on O_2 uptake of baker's yeast in *M*/15 KH_2PO_4 at pH 4.5 and 37.5°. Curve I, no acid; curve II, corresponding test with acid; curve III, no acid; curve IV, corresponding test with acid.

Fig. 3. Effect of 5.8×10^{-4} *M* decoic acid (0.1 mg. per ml.) on O_2 uptake of baker's yeast in *M*/15 KH_2PO_4 containing 0.02% glucose at pH 4.5 and 37.5°. Curve I, no acid; curve II, acid added.

A summary of the results is given in Table 1. It will be seen that the R.Q. of 0.94 in the absence of fatty acids was lowered to 0.80 and 0.73 by $5.8 \times 10^{-4} M$ decolic acid (0.1 mg. per ml.) and $4.3 \times 10^{-3} M$ hexoic acid (0.5 mg. per ml.),

Table 1. *R.Q. of baker's yeast in $M/15 KH_2PO_4$ with and without added fatty acids at pH 4.5 and 37.5°*

Acid	Glucose (0.02 %)	R. Q.		No. of determinations
		Average	Limits	
—	—	0.94 $\pm$ 0.03	0.90–1.01	10
—	+	0.93 $\pm$ 0.02	0.90–0.97	17
Decolic $5.8 \times 10^{-4} M$	—	0.80 $\pm$ 0.03	0.75–0.84	13
„	+	0.82 $\pm$ 0.01	0.80–0.84	8
Hexoic $4.3 \times 10^{-3} M$	—	0.73 $\pm$ 0.02	0.68–0.76	8

respectively. The R.Q. in the absence of acid approaches 1 by these measurements and indicates that carbohydrate is being burnt, as pointed out above. The respirations were measured over periods of 3–4 hr. (Figs. 2 and 3) and the R.Q. remained constant during these periods.

DISCUSSION

It is seen that non-toxic concentrations of the fatty acids increase the O_2 uptake of suspensions of baker's yeast. The acids studied depress the R.Q. in the absence of glucose or in the presence of a very low concentration of glucose. These facts suggest the oxidation of the acids by the yeast. Some further evidence is in accord with this suggestion. The flasks to which the acids were added usually gave slight positive tests for ketone bodies by the nitroprusside and *o*-nitrobenzaldehyde reactions. On the other hand, under the conditions of the experiments, insufficient β -keto-acids (acetoacetic acid) were present to be determined manometrically by the procedure of Edson [1935]. Since the amounts of ketone present were near the limits of sensitivity of the colour tests, some of the yeast suspensions were distilled. The first fractions gave definite positive reactions indicating the presence of volatile ketones. The addition of a saturated solution of 2:4-dinitrophenylhydrazine in 2*N* HCl to a few ml. of the distillate gave a very small amount of a yellow crystalline precipitate in the case of the yeast suspensions which had been treated with the fatty acids. Insufficient precipitate was obtained for M.P. determination but microscopically the crystals appeared to be identical with those of acetone-2:4-dinitrophenylhydrazone. These observations are suggestive but not conclusive. A thorough chemical study of the fate of the fatty acids must be carried out to explain the observed effects on yeast respiration. Since circumstances do not permit us to undertake this work at this time we feel it worth while to present our findings to date.

SUMMARY

The addition of non-toxic concentrations of saturated fatty acids to suspensions of baker's yeast causes an increase in O_2 uptake. The magnitude of the effect depends both upon the concentration of the acid and its mol. wt. Within limits (valeric to undecolic) the physiological effects, as determined by toxicity and respiratory activity, generally appear to increase with lengthening of the carbon chain but a lowering of activity is evident with stearic acid.

Measurements of the R.Q. and qualitative tests for the presence of ketone bodies suggest that the respiratory activity may be due at least in part to the oxidation of the fatty acids by the yeast.

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