

guinea pigs and hence may be assumed to exert a destructive effect on vitamin E. Such destruction was not evidenced under the conditions of this experiment, and administration of alpha tocopherol in cod liver oil appears to be an entirely practical procedure in experiments of this kind. Nondestearinated U.S.P. cod liver oil was used, and 100 mg of alpha tocopherol were dissolved and diluted in this oil at one time. Such an amount was sufficient to last for 7 to 10 days. It was kept in the refrigerator when not in use.

Summary. A large percentage of chicks fed a laboratory diet of dried skim milk, dextrinized corn starch, cod liver oil, and mineral salts develop a generalized edema and die. The most consistent post mortem finding is edema of the heart and pericardium. This disease can be prevented by administration in cod liver oil of synthetic d,l-alpha tocopherol. The disease has not been observed in any chicks fed practical rations.

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Effect of Amino Acids, of Vitamin B Complex and Other Compounds on Respiration of Bakers' Yeast.

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In our laboratories a considerable amount of work has been done on the effects of various fractions from yeast and other sources on the respiration and proliferation of yeast and tissues. In this connection experiments have been performed with pure substances some of which may be present in these preparations. The results of these experiments are the subject of the present paper.

Although we have used pure cultures of yeast in some of our respiration work (pure cultures are always used in proliferation studies), it has been desirable to have a readily available and reasonably constant source of yeast in quantity. We have found that Fleischmann's bakers' yeast answers these requirements fairly well. (Anheuser-Busch bakers' yeast has also been used with equal satisfaction in later work, but the experiments herein reported deal with

* Assistance in some experiments was given by Vincent Sacksteder.

Fleischmann's.) To insure sufficient uniformity we always use the yeast within 3 days after the "cutting" date.

All determinations were made by the direct Warburg method, using the technic previously described.^{1,2} Fleischmann's bakers' yeast from the center of a fresh 1-lb cake was washed 2 times by centrifugation with the suspending medium (Ringer-phosphate-glucose; 0.02% glucose; pH 7.3). The yeast was then made up with fresh medium to give a count of 250 as determined by a photoelectric densitometer. A count of 1 equals 250,000 cells per cc, or 0.064 mg (wet weight) of yeast per cc. One cc of the yeast suspension (containing an average of 3.9 mg dry weight of yeast), the desired amount of test solution and sufficient Ringer-phosphate-glucose to give a volume of 3.1 cc were placed in the outer well of the manometer flask and 0.2 cc of N KOH was placed in the inner well (trial showed the use of KOH papers to be unnecessary). After an equilibrium period of 15 minutes, the manometers were shaken for a 25-minute respiration period, in air, at 37.5°C. Under these conditions the control respirations usually ran between 40 and 60 mm³ corresponding to a $Q_{O_2}^{air}$ of about 25 to 37.

The substances tested were usually dissolved, or in some cases suspended, in Ringer-phosphate-glucose; a few of the less soluble materials were dissolved in distilled water. After adjustment of pH to 7.3 with sodium hydroxide or hydrochloric acid, the concentration was such that no more than 1 cc of the solution was added to the flasks. Certain of the substances, normally insoluble, were thus dissolved as the sodium salts.

Variations of $\pm 5-7\%$ are within experimental error. Only stimulations of 10% or greater are regarded as being significant. All data reported are the result of 2 to 6 determinations at each of not fewer than 4 concentration values within the limits given.

Of the amino acids examined in concentration ranges of 0.069 to 1.0 mg per cc, the following were inactive: d-alanine, arginine, cystine, glutamic acid, glycine, l-histidine, dl- β -phenylalanine, l-proline, and l-tryptophane. The following, in different experiments, varied from inactive to slightly active (10-15% stimulation): dl-alanine, l-alanine, β -alanine, l-aspartic acid, l-leucine and tyrosine.

In another series of experiments several members of the vitamin B complex were examined.† Of these, nicotinic acid (0.077-0.77

¹ Cook, E. S., Kreke, C. W., and Nutini, L. G., *Studies Inst. Divi Thomae*, 1938, **2**, 23; Cook, E. S., Hart, M. J., and Joly, R. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 169.

² Cook, E. S., and Morgan, M. N., *Biochem. J.*, 1940, **34**, 15.

TABLE I.
Effect of Thiamin Hydrochloride on Respiration of Bakers' Yeast.

Conc., mg/cc	% stimulation
.008	8
.017	6
.042	12
.083	26
.166	26
.420	34
.830	0

mg/cc) and riboflavin (0.0097-0.97 mg/cc) were inactive. Vitamin B₆ (0.01-0.98 mg/cc) was generally inactive although occasional stimulation of the order of 10% was found with some batches of yeast. Thiamin hydrochloride showed an activity dependent upon the concentration, there being an optimum concentration range as shown by the results of a typical experiment in Table I. Of the 2 components of the thiamin molecule, 4-methyl-5- β -hydroxyethyl thiazole showed no significant activity in concentrations of 0.012 to 1.15 mg per cc. 2-Methyl-4-amino-5-ethoxymethyl pyrimidine was inactive between 1.05 and 0.11 mg per cc; slight activity (10-13%) was found in concentrations down to 0.01 mg per cc. However, an equimolecular mixture of the 2 showed the same activity as thiamin in total concentration ranges of 0.05 to 0.50 mg per cc (*e.g.*, at 0.1 mg/cc, thiamin gave 29% and the mixture 30% stimulation).

The following substances were also inactive in stimulating respiration: adenosine phosphate (Hoffmann-LaRoche) (0.076-0.76 mg/cc), yeast nucleic acid (0.08-0.8 mg/cc), thymus nucleic acid (0.08-0.8 mg/cc), inositol (0.097-0.97 mg/cc) and hippuric acid (0.094-0.94 mg/cc).

Insulin (Iletin, Lilly) was inactive in a concentration of 0.016 mg (or 0.13 unit) per cc but caused 18% stimulation in 10 times this concentration. Concentrations of 0.8 mg (6.5 units) and 1.6 mg (13 units) per cc gave, respectively, 240% and 330% stimulation.

From the results it is seen that most of the amino acids, under the conditions used, were inactive or of a very low order of activity. It will be recalled that l-leucine³ and β -alanine^{3,4} (by itself or com-

† We wish to thank Drs. R. T. Major and J. M. Carlisle of Merck and Co. for gifts of synthetic thiamin, 2-methyl-4-amino-5-ethoxymethyl pyrimidine, 4-methyl-5- β -hydroxyethyl thiazole, riboflavin and vitamin B₆.

³ Miller, W. L., *Trans. Roy. Soc. Can.*, III, 1936, **30**, 99.

⁴ Williams, R. J., and Rohrmann, E., *J. Am. Chem. Soc.*, 1936, **58**, 695.

bined in pantothenic acid^{5,6}) have been identified as bios components for various yeasts. Pratt and Williams⁷ found that, while both pantothenic acid and β -alanine increased the respiration of deficient Gebrüder Mayer yeast, pantothenic acid was ineffective on Fleischmann's cake yeast, presumably owing to an adequate supply of pantothenic acid in the latter yeast. This checks our observations on the usual inactivity of β -alanine. The relative ineffectiveness of the amino acids contrasts markedly with the stimulating effects of non-toxic concentrations of saturated fatty acids on bakers' yeast; these effects appear to be due to the ability of the yeast to oxidize the fatty acids.²

Among the other substances examined, thiamin, vitamin B₆, and inositol may also act as bios components for certain strains of yeast. Of these, only thiamin was definitely effective in stimulating the respiration of bakers' yeast. Williams⁷ found similar results with both Gebrüder Mayer and Fleischmann's yeasts. It is of interest that, while the 2 components of the thiamin molecule are essentially inactive by themselves (the pyrimidine portion showing only very slight activity), an equimolecular mixture is as effective as the intact molecule. This suggests an ability of the yeast to combine the thiazole and pyrimidine moieties into the whole molecule which would seem to be essential for an increase in respiration. In contrast, Schultz, Atkin and Frey⁸ found the pyrimidine portion to be equally as effective as thiamin in stimulating fermentation by bakers' yeast, the thiazole portion being ineffective.

The inactivity of yeast nucleic acid checks the observation of Pourbaix⁹ who found that sodium nucleinate has no effect on the normal respiration of yeast; it will, however, restore to normal respiration which has been depressed by styryl 430.

The very marked effect of insulin in increasing the oxygen uptake of yeast is particularly interesting in view of the reports that it does not accelerate the fermentation of glucose by yeast.¹⁰ These experiments are being extended in our laboratories.

⁵ Williams, R. J., and Saunders, D. H., *Biochem. J.*, 1934, **28**, 1886.

⁶ Rainbow, C., *J. Institute Brewing*, 1939, **45**, 533; Rainbow, C., and Bishop, L. R., *ibid.*, 1939, **45**, 593.

⁷ Pratt, E. F., and Williams, R. J., *J. Gen. Physiol.*, 1939, **22**, 637.

⁸ Schultz, A. S., Atkin, L., and Frey, C. N., *J. Am. Chem. Soc.*, 1937, **50**, 2457.

⁹ Pourbaix, Y., *Compt. rend. soc. biol.*, 1939, **131**, 1306.

¹⁰ Fürth, O., *Biochem. Z.*, 1923, **150**, 265; Laufberger, V., *Z. ges. exp. Med.*, 1924, **42**, 570; Travell, J. G., and Behre, J. A., *Proc. Soc. Exp. Biol. and Med.*, 1923-4, **21**, 478; Euler, H. von, and Myrback, K., *Z. physiol. chem.*, 1925, **150**, 1.

Obviously, as with growth factors, the effectiveness of various substances on the respiration of yeast will depend upon the strain of yeast, the medium, and the general technique. The present results are of value in checking against the activity of various fractions from yeast and animal tissues which we are assaying by means of the technique used in this paper. Under different conditions certain of the apparently inactive materials may assume importance when they become limiting substances. Suggestions of this are seen in the case of several amino acids and vitamin B₆.

Summary. A number of amino acids, members of the vitamin B complex, and miscellaneous substances have been examined for their effects on the respiration of Fleischmann's bakers' yeast. Most of the substances are inactive or only slightly active, but thiamin, or a mixture of its pyrimidine and thiazole components, and insulin have marked activity.

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Quantitative Studies of Cell Types in Rat Hypophysis Following Administration of Antigonadotropic Serum.*

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Several reports in the literature have shown that treatment of animals with antigonadotropic serum causes a condition in the pituitary gland analogous to that found in castrated animals. Severinghaus and Thompson^{1, 2} have described cytological changes in the hypophyses of dogs injected with antihormones. In these animals there was an increase in the basophile cells and a corresponding decrease in the chromophobes which was associated with an atrophy of the gonads, thyroids, and adrenals. Physiological effects have been demonstrated in rats by Meyer and Kupperman³ who

* Aided in part by a grant from the Wisconsin Alumni Research Foundation and by assistance furnished by the personnel of W.P.A. Official Project No. 65-1-53-2349.

¹ Severinghaus, A. E., and Thompson, K. W., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 627.

² Severinghaus, A. E., and Thompson, K. W., *Am. J. Path.*, 1939, **15**, 391.

³ Meyer, R. K., and Kupperman, H. S., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 285.