GROWTH RESPONSE OF A MARINE YEAST, *CRYPTOCOCCUS LAURENTII*, TO THIAMINE

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Received April 15, 1955

Summary

Repeated experimentations during the course of a month on the growth response of a marine yeast, *Cryptococcus laurentii*, to graded dosages of thiamine showed that the organism could respond linearly to micro-quantities of this vitamin. Continuation of these experiments after a break of five months revealed the loss in ability of the yeast to respond to the minute dosages earlier employed though the strain still exhibited a linear response to higher amounts of thiamine. Subsequent studies revealed that the organism had totally lost its dependency on the vitamin.

Introduction

Because of the fixity with which they respond to certain of their nutrilites, the lactobacilli have come to be adopted as suitable organisms for microbiological assays. However, time and again, other micro-organisms including yeasts have been reported to be of value in this respect. Burkholder and co-workers, for instance, have reported (Burkholder and Moyer, 1943; Burkholder *et al.*, 1944) the existence of certain yeasts which respond to the addition of vitamins in their growth media, the vitamins concerned being effective as growth promoters rather than as essential growth factors. Claims have been made by several workers here as well as elsewhere (DeSouza and Sreenivasaya, 1946; Raghavendra Rao, 1950; Snell, 1950; Venkatesh, 1951; Bheemeswar, 1954) that certain select strains of yeasts such as *Saccharomyces cerevisiæ*, *Saccharomyces carlsbergensis*, *Torula creamoris* and *Klœckera brevis* can yield consistently linear responses to the addition of increasing amounts of certain vitamins of the B complex into the basal medium. But it has been the experience of the present investigators that yeasts, although may be employed in the microbiological assays, fail to give as consistent and as reliable data as the lactic bacteria. The object of the present communication is to emphasise the above point by demonstrating that even a marine yeast which for a long time was observed to be dependent on the external source of thiamine had done away with it on storage in the laboratory for a period of about four years. This report should also serve to bring home the fact that the nutritional response of a yeast varies from time to time despite adherence to careful methods of assay and uniformity in all experimental procedures.

Materials and Methods

Preparation of the media.—The basal medium employed for the nutritional experiments was a modification of Burkholder's medium (1944) into which was added 3 per cent. extra pure sodium chloride in order to simulate the marine environs for the test organism. The source for nitrogen at all times was a mixture of ammonium sulphate and asparagine. The constituent salts used in the basal solution were all of 'Analar' grade. Vitamin-free glucose (Roche) constituted the source of carbon and its solution was always sterilized separately and added on into the sterile base in such a way as to attain a final concentration of 2 per cent. The pH of the medium was throughout maintained at 5·0 (Burkholder, 1944). All the stock solutions of the vitamins excepting those of riboflavine and biotin were prepared to a concentration of 1 mg./ml. The stock solution of riboflavine was prepared to a concentration of 25 μg./ml., whilst that of biotin was restricted to 1 mμg./ml. The final composition of the basal medium was as follows:—

Glucose, 2 g; KH_2PO_4, 4·4 g.; KCl, 3·4 g.; $MgSO_4.7H_2O$, 1 g.; $CaCl_2.2H_2O$, 1 g.; $MnSO_4.4\,H_2O$, 20 mg.; $FeCl_3.6H_2O$, 20 mg.; H_3BO_3, 20 mg.; $ZnSO_4$, 8 mg.; $CuSO_4$, 0·8 mg.; KI, 0·8 mg.; $(NH_4)_2SO_4$, 1 g.; Asparagine 1·0 g.; NaCl, 3 g.; distilled water, 1000 ml.

In the first series of experiments different vitamin mixtures were compounded by additing individual vitamin solutions but eliminating one vitamin at a time. For preparing the final medium, to 4·4 ml. of the basal solution placed in a 50 ml. Erlenmeyer flask, 0·1 ml. of the vitamin mixture was added. The various flasks were then sterilized at 15 lb. pressure for 20 minutes, cooled and 0·5 ml. of the sterile glucose solution (20%) aseptically added.

In the second series of experiments thiamine solution alone was added in graded doses with a view to see the specific response of the selected marine yeast to this singled out vitamin. The experiments were so designed as to be concluded within the course of a month by which period the variations in response by the yeast to minute dosages of thiamine could be studied repeatedly. Thiamine levels incorporated into the medium during this study were as under:—

0·1 mμg., 0·25 mμg., 0·5 mμg., 0·75 mμg., and 1·0 mμg./5 ml. of the medium.

In both the series control flasks containing the basal medium fortified with 0·1 ml. of a 5 per cent. solution of Difco yeast extract were inoculated with the experimental culture suspension and maintained for comparative observation.

Maintenance of the stock culture employed.—The yeast strain was isolated from sea water off the coast of Bombay well over four years ago (Kachwalla, 1953) and identified as *Cryptococcus laurentii* by Bhat and Kachwalla (1955). The dependence of the organism for thiamine was shown in a subsequent study by Bhat *et al.* (1955). The stock culture of this organism at all times was maintained on the enrichment agar medium (Lobo *et al.*, 1953, but with only 3 per cent. sucrose

instead of 10 per cent. as described) containing 3 per cent. salt. Maintenance and growth was at the room temperature (25–29° C.) and subculturing was attended to every month.

Inoculum and the measurement of growth response.—For the preparation of inocula, the growth (a loopful) was carefully removed from a 24–48 hour old enrichment agar slant taking care to see that the loop does not cut into the medium. This growth was used for inoculating 5 ml. of the basal medium (given above) kept in a 50 ml. Erlenmeyer flask. After 24 hour growth, two loopfuls of the culture were transferred into another flask containing the same medium. This passage through two aliquots of basal medium (without added vitamins) resulted in a harvest of cells with no free vitamins to carry over into the media for assay and yielded a thin growth which could be centrifuged with ease, washed with saline, recentrifuged and rewashed with saline before final centrifugation. The deposit

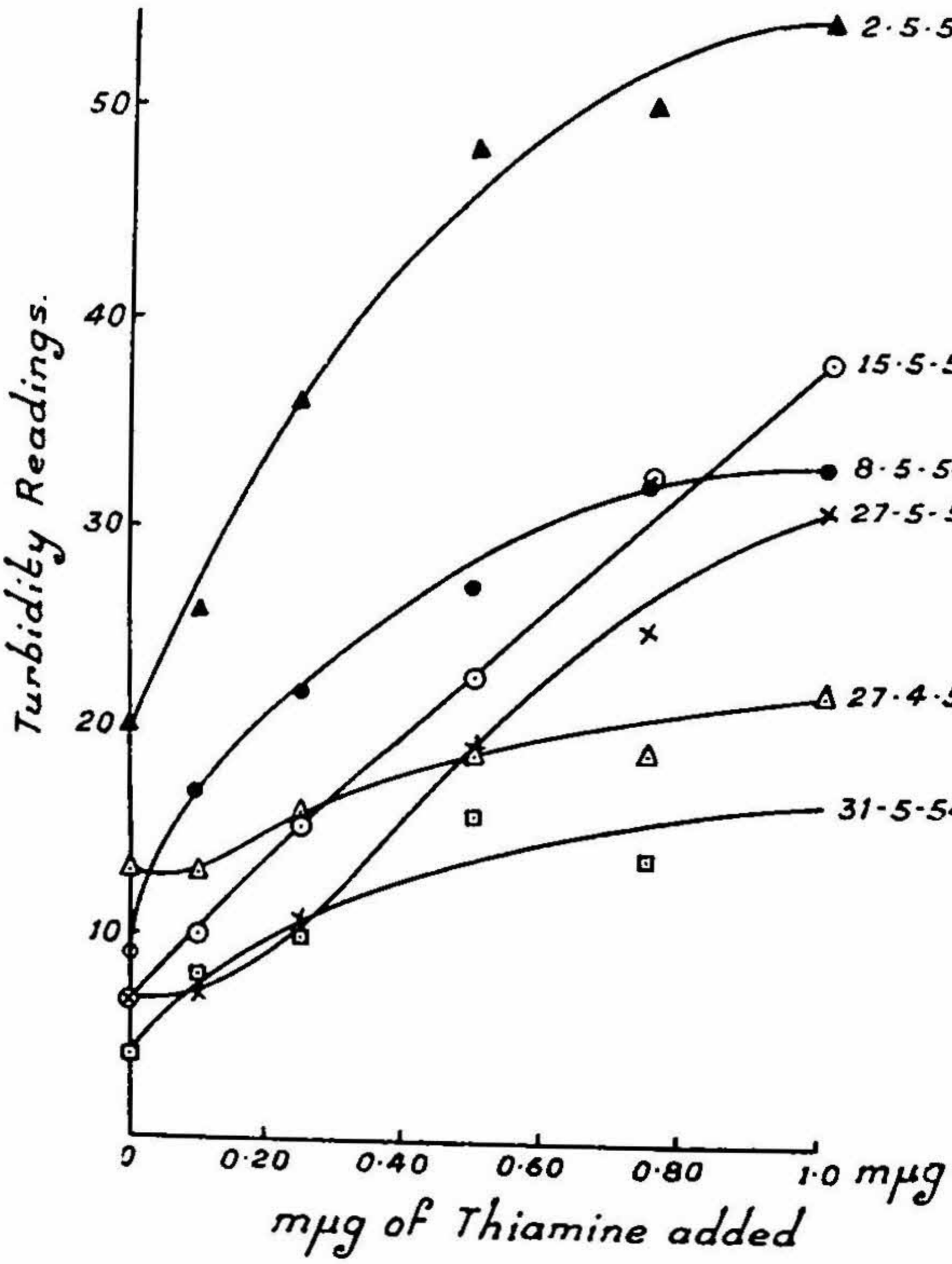

FIG. 1

was suspended in a known amount of saline to yield a suitable suspension for inoculation into the assay media. The inoculum so prepared was every time examined under the microscope for checking the uniformity of cells and for their freedom from contaminants.

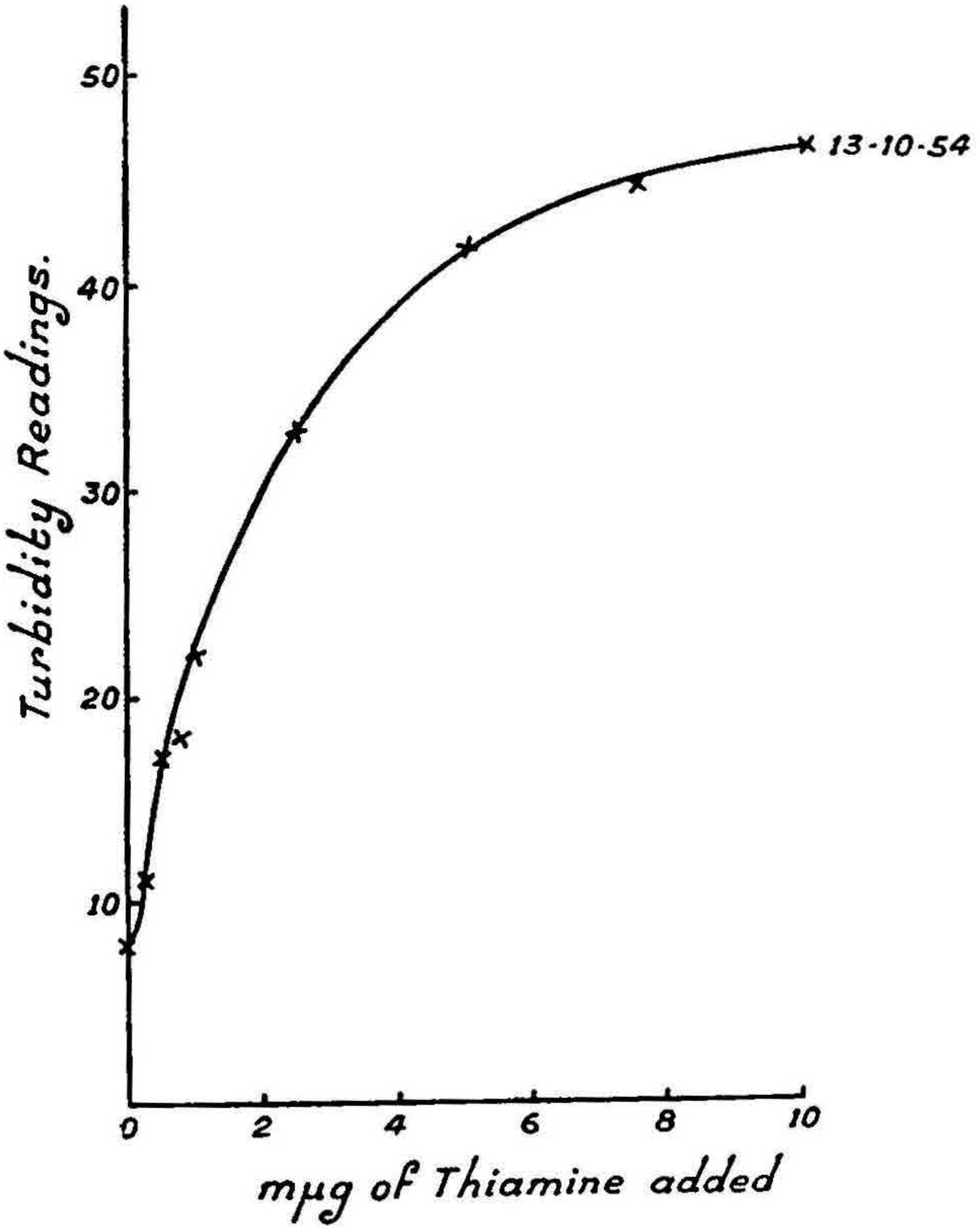

FIG. 2

Aliquots of 0·1 ml. of the suspensions prepared as above served as inocula for the assay tubes. Growth responses were measured in Klett Summerson photo-electric colorimeter using 42 filter.

All the above series of experiments together with the controls were repeated after a comparatively long lapse of five months when it came to be noted that the observations made earlier were difficult of attainment inasmuch as the yeast strain which earlier had responded to minute doses of thiamine had already lost its capacity to so respond; however, it still could respond linearly to higher dosages of thiamine as 1·0 mμg., 2·5 mμg., 5·0 mμg., 7·5 mμg., and 10 mμg./5 ml. of the basal medium.

RESULTS AND DISCUSSION

It became abundantly clear from the first series of experiments that the addition into the basal medium of the vitamin mixture could boost the growth of the yeasts by two to three times the growth recorded for the controls, *viz.*, the basal medium without any added vitamin. The response exhibited by the yeast in the medium with vitamins other than thiamine was also clearly indicative of the dependency of the organism for this individual vitamin. However, the growth secured with yeast extract in the basal medium was by far the very best recorded indicating thereby that the yeast extract contained some other stimulatory factors besides the vitamins put to the test. It was also revealed that no single vitamin other than thiamine could support as much growth of the yeast as did the mixture of the vitamins.

In the second series of experiments carried out a month later for the possible exploitation of the yeast for the assay of micro amounts of thiamine, it became abundantly clear from the results (see Fig. 1) that the incorporation of graded doses of thiamine in the basal medium was accompanied by a steadily increasing growth of the yeast and that the response recorded repeatedly was such as to warrant the exploitation of the strain for the microbiological assay of micro quantities of thiamine. However, when these experiments were again repeated after a break of five months, the test organism had already lost its ability to respond linearly (as it did earlier) to the micro amounts of thiamine even though at that stage it still did possess the ability to respond so to higher dosages of thiamine as may be judged from Fig. 2. Subsequent experiments showed (see Table I) that the organism had

TABLE I

Subsequent responses of C. laurentii *to thiamine*

Experiment with dates	Relative growth* in the presence of mμg./5 ml. thiamine					
	0	1·0	2·5	5·0	7·5	10·0
I 19–11–1954	40	40	39	40	39	40
II 25–11–1954	27	35	32	30	34	35

* Klett Summerson colorimetric readings.

altogether failed to respond quantitatively to the graded dosages of the vitamin although its presence in the basal medium still continued to stimulate its growth. All these experiments seem to suggest that the yeast strain though originally was dependent on thiamine for its nutritional requirements had subsequently developed its mechanism for thiamine synthesis to an extent as to do away with its extraneous

supply indicating thereby that the vitamin served to promote growth instead of as an accessory growth factor. The situation encountered here is perhaps similar to that described by Lindegren and Raut (1947) who had explained the inability expressed by a particular yeast to grow in a vitamin deficient medium on the lack of ability of that organism to grow under certain conditions rather than as due to its intrinsic disability to synthesise the vitamins in question under all conditions. The experiments described here should help explain the inconsistency in results obtained on the use of yeasts in some microbiological assays.

Acknowledgements

The authors wish to thank the Director, Indian Institute of Science, for his kind interest in this work.

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