STUDIES ON THE MUTAGENIC ACTION OF CHEMICAL AND PHYSICAL AGENCIES ON YEASTS

VI. Effect of Chrysene on the Rate of Growth and Fermentation of the Diploid Brewery Yeast

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CONTENTS

Summary

The immediate effect of chrysene on the growth rate and fermentation characteristics of the control diploid brewery yeast has been investigated. The chemical induces a stimulation in the rate of growth, discernible after an incubation period of about 36 hours. Under anaerobic conditions, however, the chemical affects adversely the normal fermentation rate of the strain.

The chrysene-induced tetraploids ferment sugar solutions much faster than the parent diploid, the improvement in the fermentative ability being identical with that obtained in the case of the acenaphthene-induced tetraploid.

Introduction

The influence of biologically active substances on the biochemical performance of micro-organisms has attracted the attention of many investigators (Goldstein, 1937; Hopper and Clapp, 1939; Dodge and Dodge, 1937; Dodge, Dodge and Johnson, 1941; Cook, Hart and Joly, 1939; Tolmatcheva, 1940; de Clerk, 1942). Yeasts have been the obvious choice for a number of such studies, since they lend themselves to easy manipulation with rigorous control of the experimental conditions, so necessary for any quantitative biological work. Particular importance has been attached to

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the action of carcinogenic chemicals in view of the possible significance of the results obtained in the wider context of the problem of carcinogenesis in higher animals. It is generally agreed that these substances accelerate the rate of growth of the organisms studied.

It was indicated in an earlier contribution from this laboratory (Mitra and Subramaniam, 1950) that the available literature does not throw any light on the question of the actual genetic consequences of such treatments. Bauch (1941 *a*, *b*; 1942 *a*, *b*; 1943) however has suggested that judged from the increased cell volumes, the yeast mutants obtained as a result of the action of biologically active chemicals may be polyploids. He has shown that these 'gigas' mutants could be perpetuated and that they, unlike the parent strains, produce a characteristic taste in beer. Unfortunately, the above suggestion is unsupported by any cytological evidence. The consideration that apart from the immediate metabolic effect, new cell types having entirely different physiological characteristics may be established in populations undergoing treatment with these chemicals (Haddow, 1944) emphasizes the need for a more elaborate study before attempting a correct evaluation of the phenomena.

Prema Bai and Subramaniam (1947) found that the curves representing the rates of growth of the diploid and the autotetraploid brewery yeast strains were related to each other in the same manner as the ones obtained by Richards (1938) on culturing his yeast strain with and without colchicine in the growth medium. This led them to suggest that Richards had in fact effected a doubling of the chromosome complement through the action of the chemical. Such an interpretation, however, appeared untenable when the extended observations on the effect of chrysene (Mitra and Subramaniam, 1950) confirmed the suspicion that the effect of polyploidogens need not be an all-or-none reaction. This necessitated a differentiation of the action of the chemical in various directions. Acceleration of the rate of mutation is only one property. Polyploidogens are known to affect cell division; they should, therefore, have some effect on the metabolism of the cells also. It is this specific problem of the immediate effect of chrysene on the growth rate and fermentation characteristics of the control diploid brewery yeast strain, BY 1, that is dealt with in this paper. Further, a study of the fermentative ability of the tetraploid strains, CHR 1 and CHR 2 isolated after prolonged treatment of the control strain with chrysene (Mitra and Subramaniam, 1949) was considered to be of interest.

EXPERIMENTAL

Effect of chrysene on the rate of growth of the control diploid yeast.— A synthetic glucose-peptone medium adjusted to a pH of 4·6–4·8 was

used in these experiments. Four ml. aliquots of this were pipetted out into 50 ml. erlenmeyer flasks and sterilised at 15 lb. steam pressure for 15 minutes. After cooling, six drops of absolute alcohol were added to the flasks designed to serve as controls, while a corresponding number received six drops of a saturated alcoholic solution of chrysene. The concentration of the chemical was thus approximately the same as the one used in the previous investigation (Mitra and Subramaniam, 1949). The final reaction mixture (5 ml.) contained 2% glucose, 1% peptone and 0·5 mg. of moist yeast as inoculum. The yeast for inoculation was obtained by harvesting a 24-hour old agar slant culture and suspending it in saline after repeated washings. The flasks were incubated at 28° C.

To get the growth data, two flasks from the experimental series and two from the control were withdrawn at intervals and growth was arrested by the addition of 0·25 ml. of 40% formalin. This was followed up for a period of 5 days at the end of which the yeast crop in each flask was estimated turbidimetrically using a 'Lumetron' photo-electric turbidometer. Uninoculated control was used as the blank to register zero per cent. absorption and the most turbid suspension was taken as the standard for 'cent. per cent. absorption'. Since even the uninoculated control of the experimental series showed an absorption of 1·5% due to the presence of the chemical in the colloidal state, this value was deducted from each reading of the series. A representative set of results is presented in Fig. 1.

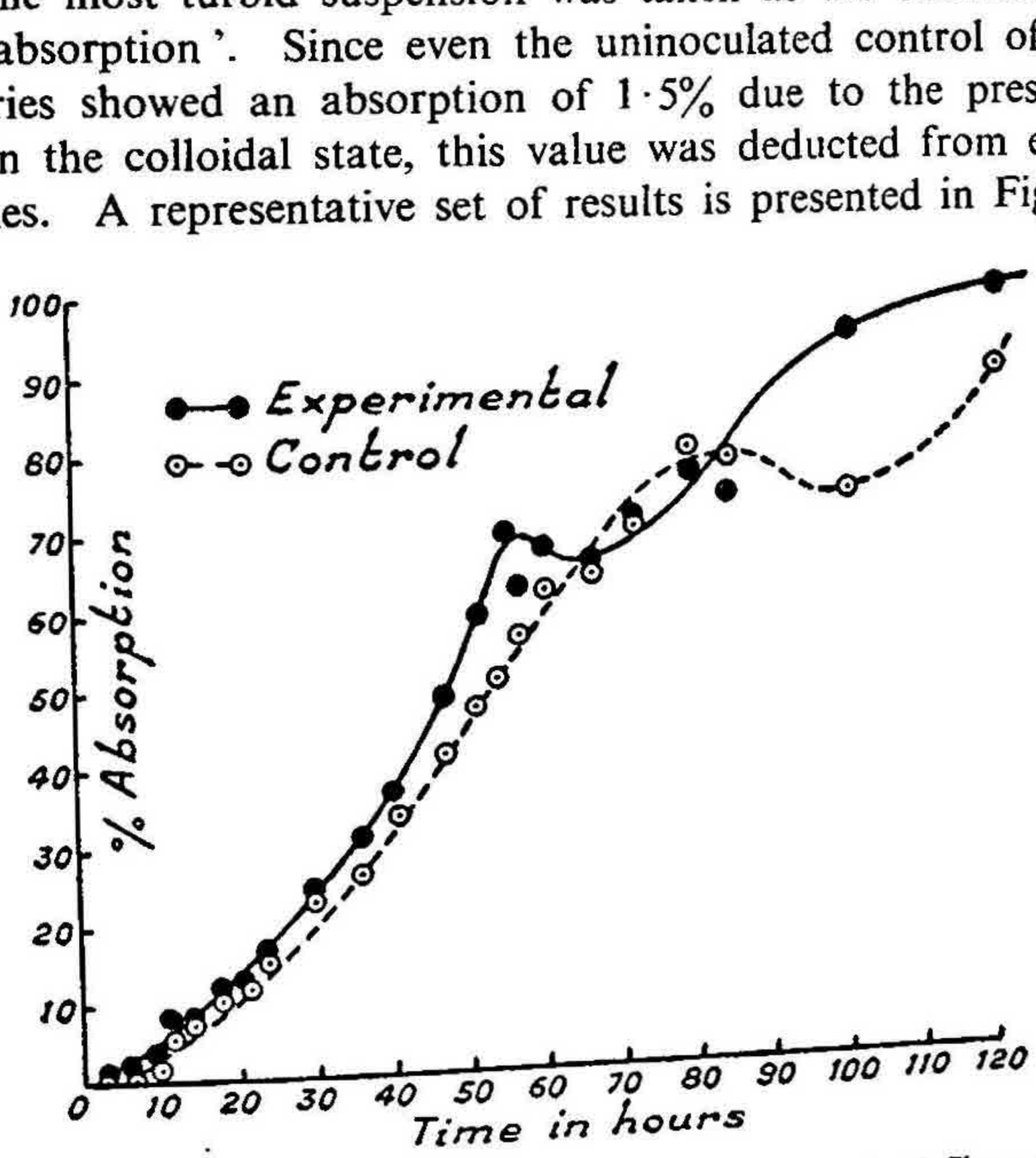


FIG. 1. Growth of the Diploid Strain in the Presence of Chrysene

2. *Fermentation characteristics in medium incorporated with chrysene.*—The rates of evolution of CO_2 by the strain under normal anaerobic conditions in wort and in the presence of chrysene were compared with the aid of a set of gasometers. The details of the apparatus and the experimental technique have been described elsewhere (Mitra, 1952). Two gasometers were connected to the tube containing the chrysene-incorporated medium and the other two to the 'control' tubes. Each of these tubes received 10 ml. of the medium. The concentration of the inoculum was kept sufficiently low so that the fermentation rates could be followed up for a considerable length of time. The proportion of the alcoholic solution of the chemical to the volume of the medium employed was maintained at the same level as in the foregoing studies. An equivalent amount of alcohol was added to the control cultures. The results are graphically represented in Fig. 2.

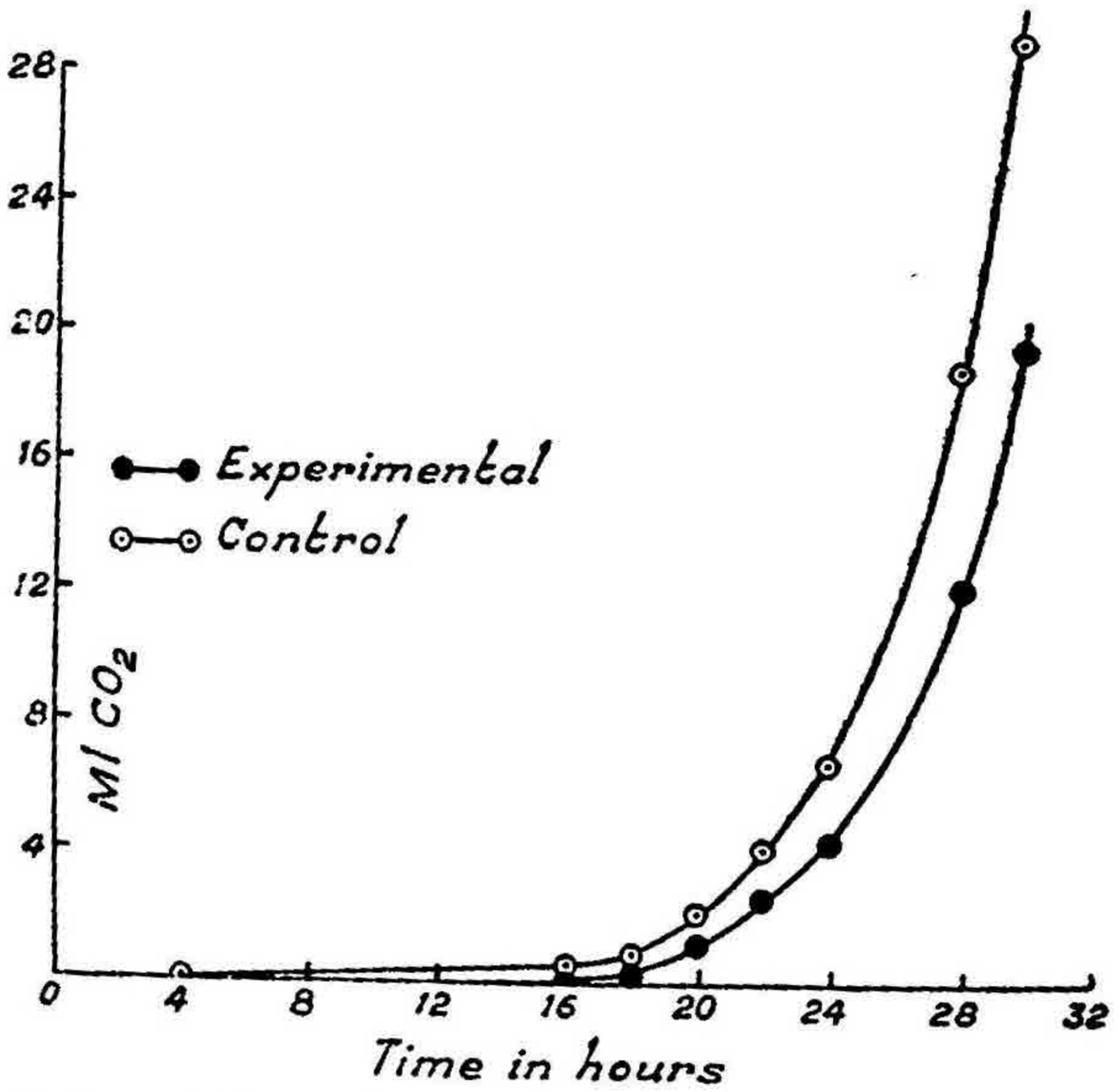
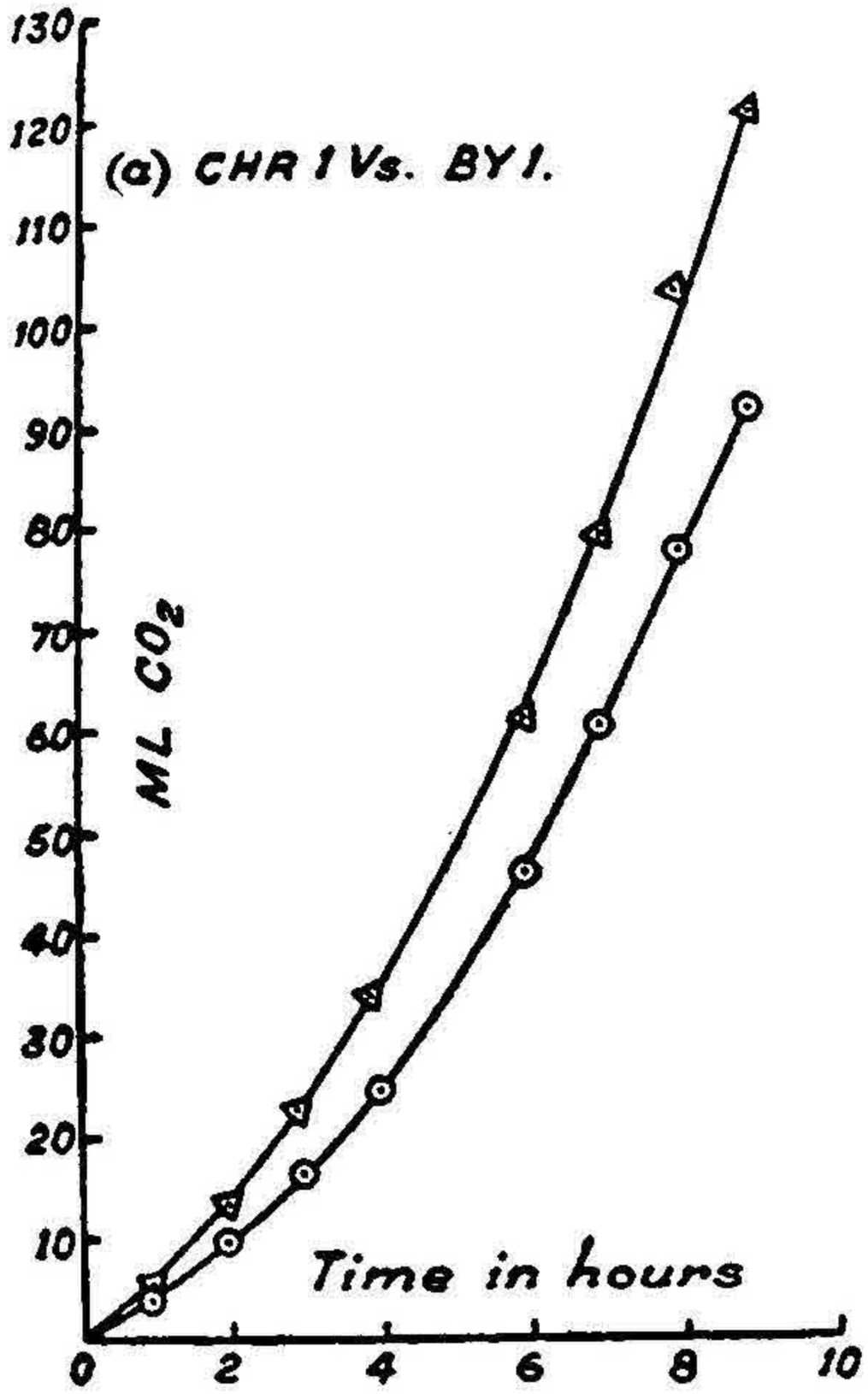
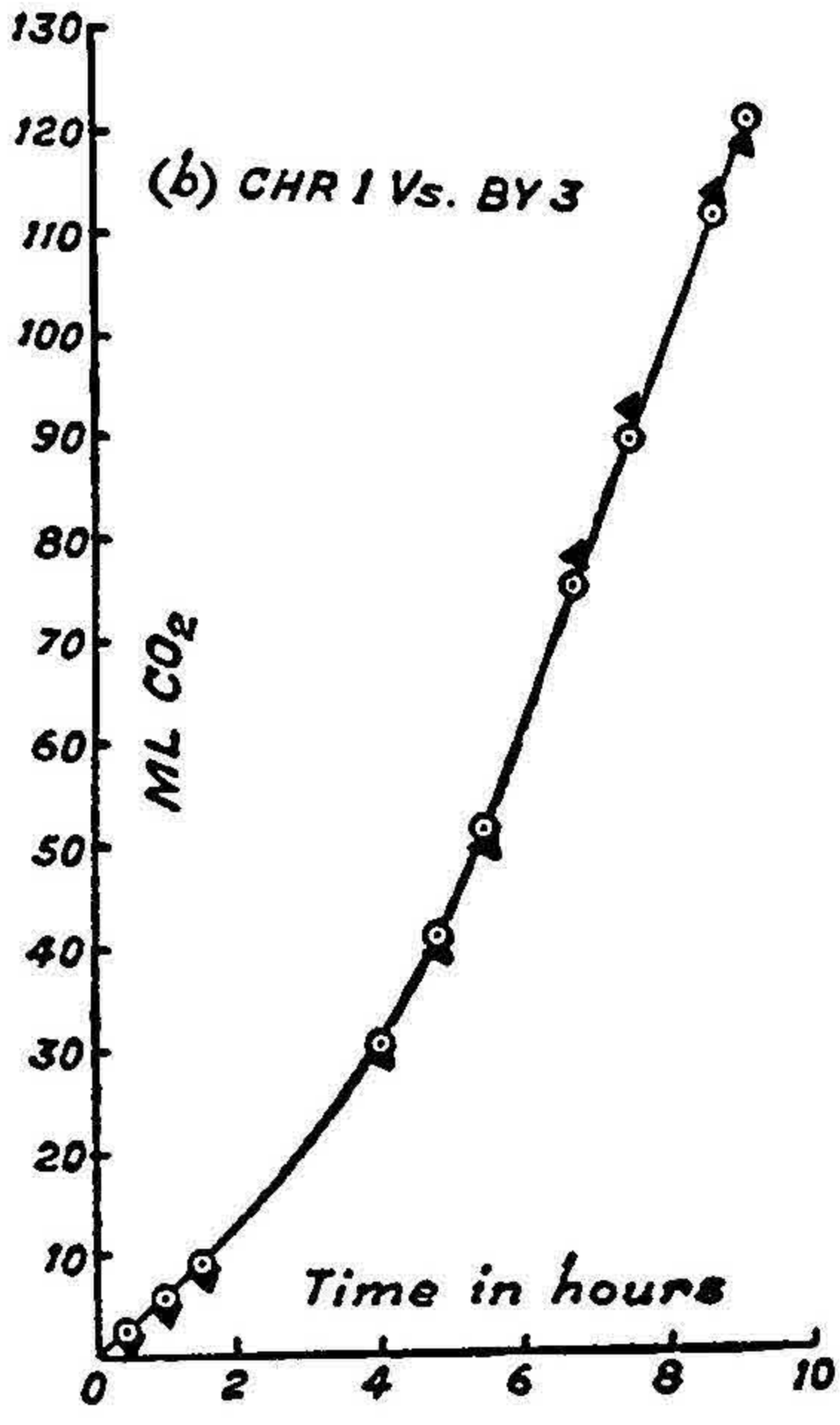


FIG. 2. Fermentation by the Diploid Strain in the Presence of Chrysene

3. *Fermentative ability of the chrysene-induced tetraploids.*—To facilitate the evaluation of the fermentative characteristics of the chrysene-induced tetraploids (Mitra and Subramaniam, 1949) CHR 1 and CHR 2, comparisons were made with the parent diploid strain BY 1 and the acenaphthene-induced tetraploid BY 3 (Mitra, 1952) using the gasometric technique. Since only two strains could be tested at a time in duplicate and





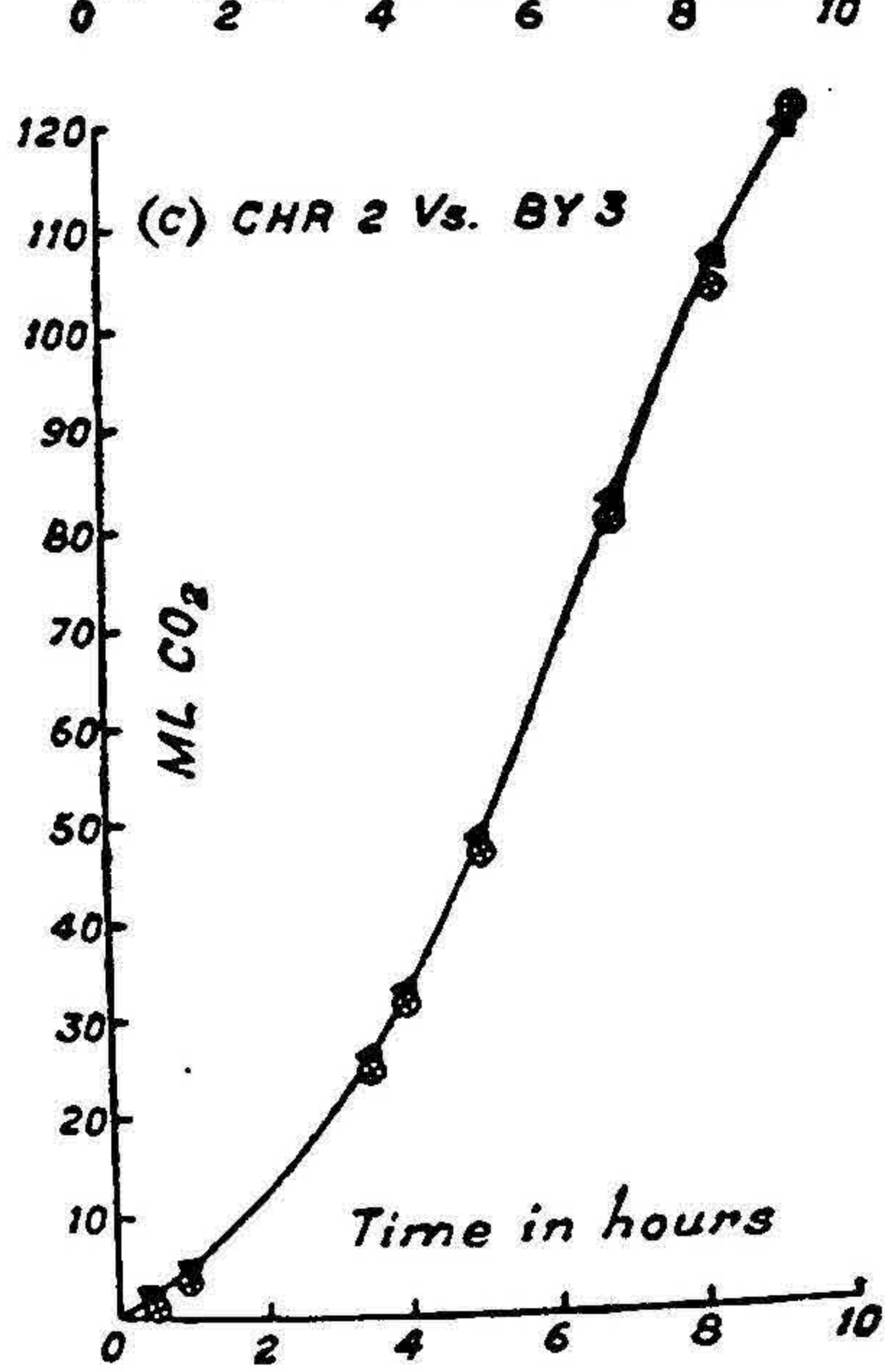
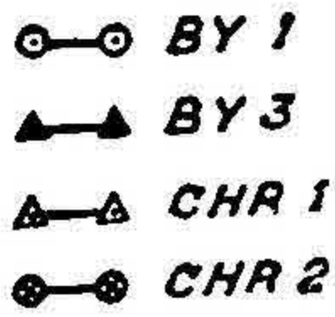


FIG. 3. Fermentative Ability of the Chrysene-induced Tetraploids



since the experiments were conducted at room temperature, the data obtained cannot be represented in a single graph. Comparisons of CHR 1 with BY 1, CHR 1 with BY 3 and CHR 2 with BY 3 have been shown graphically in Figs. 3 *a*, 3 *b* and 3 *c* respectively.

DISCUSSION

The behaviour of the diploid strain during the five days of continuous growth in the presence of chrysene shows a small but significant difference when compared with the control (Fig. 1). The chemical stimulates the rate of proliferation, an effect which is discernible after an incubation period of 36 hours. The characteristic single cycle of growth observed by Richards (1938) and by Prema Bai and Subramaniam (1947) is not evident in the present investigation. An earlier attainment of the end of the logarithmic phase of growth in the presence of chrysene appears to be significant. Since the genetical studies (Mitra and Subramaniam, 1950) indicated that gene mutants could get established even with five days of treatment with chrysene, it stands to reason that the growth characteristics of the population would also show some diversity when compared with the parent strain. The growth curve of the organism in the presence of chrysene when viewed in the light of the results obtained by Prema Bai and Subramaniam (1947) suggests that tetraploidy has not been induced within the period of observation. This negative finding does not however totally invalidate the interpretation these workers offered for the results obtained by Richards. The genetical investigations (Mitra and Subramaniam, 1950) while indicating that the effect of the chemical is not an all-or-none reaction, suggest also that tetraploids can appear after varying periods of treatment, even under identical experimental conditions. Even though the chemical accelerates the rate of mutation, the appearance of any particular mutant is sporadic. Chrysene which accelerates mutability at the locus governing the sculpturing of the giant colony (Mitra and Subramaniam, 1950), should have a similar effect on the locus for polyploidy also. Spontaneous tetraploidy being infrequent, the rate of mutation at the polyploidy locus should be low. A tetraploid can therefore arise after a very short exposure to chrysene or the culture may remain diploid even after prolonged treatment. It may be pointed out that tetraploids originate only when mutation in a particular direction takes place at a specific locus. The period necessary will also depend on the nature and the dosage of the chemical used. Whatever the correct interpretation may be, it thus appears untenable that the stimulation in growth or the disappearance of the second cycle in Richard's experiments could be due to the chemical " serving as a food and as a buffer in lessening

the adverse effect of the increasingly unfavourable medium ". The changes are much more deep-seated and have an intimate bearing on the genetical variations induced by the biologically active substance.

While effecting a stimulation in the rate of growth, chrysene influences adversely the fermentative behaviour of the yeast under anaerobic conditions. A sustained inferiority in the rate of fermentation is discernible throughout the period under observation (Fig. 2).

Investigation of the giant colony characteristics of the chrysene-induced variants suggested a similarity with the acenaphthene-induced tetraploid (Mitra and Subramaniam, 1949). The tetraploid character of the former was further confirmed by cytological examination. The results of the fermentation studies (Figs. 3 *a*, 3 *b* and 3 *c*) show that the degree of improvement obtained by chromosome doubling with chrysene is identical with that encountered in the case of the tetraploid produced by treatment with acenaphthene. It is remarkable that the two different polyploidogens, chrysene and acenaphthene, should induce identical biochemical and genetical effects.

The significance of a duplication of the chromosome complement for an improvement of yeast strains has already been discussed (Mitra, 1952). The genetical (Mitra and Subramaniam, 1949, 1950) as well as biochemical studies on the effect of chrysene indicate the steps involved in the production of polyploid strains through chemical agencies. Apart from the implications of the results from the industrial point of view, studies along these lines are expected to open up a new angle of approach to the elucidation of certain aspects of carcinogenesis. It is quite possible that unlike those with mice or tissue cultures, investigations on unicellular organisms like the yeast may offer a rational explanation for the changes produced in cells by carcinogens.

Acknowledgment

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