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COMPARTMENTATION IN HISTIDINE BIOSYNTHESIS

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COMPARTMENTATION IN HISTIDINE BIOSYNTHESIS

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SUMMARY

1. Biochemical compartmentation, in the sense of intermediates in a pathway not being freely miscible with other cellular pools of the same compounds, was investigated in histidine biosynthesis in Salmonella typhimurium and Saccharomyces cerevisiae.

2. The experimental approach involved measurement of the degree of dilution by unlabelled histidinol, added to the medium, of ^{14}C incorporated endogenously from labelled glucose into the histidine of the cell proteins.

3. Compartmentation, on this basis, was absent in Salm. typhimurium but present in Sacc. cerevisiae.

4. In Sacc. cerevisiae, the absence of competition between internally-formed and externally-added histidinol was not due to a failure of this substance to penetrate the cell membrane.

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INTRODUCTION

Metabolic compartmentation is dependent on the structural and functional organization of intracellular components. In eucaryotes, some metabolic activities are associated with membrane-delineated subcellular organelles, while others occur free in the "soluble" cytoplasm. Procaryotes are devoid of subcellular organelles. One might ask whether the so-called "soluble" enzymes are randomly distributed in the cytoplasm, with substrates and products diffusing freely, or whether a more tightly organized system is present, with defined spatial relationships between enzymes cooperating in metabolic sequences, and in which intermediates are passed directly from one enzymatically active site to the next. There are numerous indications that the latter may be the case. Thus, in several systems a specific metabolite participating in one sequence was found not to mix with the same compound participating in a different sequence[1]. A number of multi-enzyme complexes have been physically isolated. Moses[2] has recently reviewed some aspects of metabolic compartmentation.

In the present work we have investigated, with these considerations in mind, the organization of histidine biosynthesis in the procaryote Salm. typhimurium, and the eucaryote Sacc. cerevisiae. The histidine pathway in both organisms is well understood and possesses features which make it particularly interesting from our point of view.

In Salm. typhimurium, ten enzymes mediate the conversion of phosphoribosyl 1-pyrophosphate + ATP to histidine[3,4]. The final step is the oxidation, catalyzed by L-histidinol dehydrogenase, of

L-histidinol to L-histidine, using NAD as the electron acceptor. The pathway is not branched. Of special interest to us was the fact that all ten enzymes of the biosynthetic sequences are specified by contiguous sequences of DNA. The order of the genes on the chromosome, however, is not the same as the order of the enzymes in the biosynthetic sequence. Expression of these genes is controlled as a single operon, and the entire operon is transcribed into a single mRNA molecule. It thus appeared not unlikely that the histidine biosynthetic enzymes in Salm. typhimurium might be organized into a macrostructure, possibly leading to a situation of non-equilibration of intermediates.

The histidine biosynthetic pathway in Sacc. cerevisiae contains the same reaction sequence as that in bacteria[5], although the histidine genes are largely scattered throughout the genome[5,6]. The only evidence of genetic clustering is for the his-4 region, which codes for three enzymatic activities, occupying (in the order of their polarity) positions 3, 2 and 10 of the biosynthetic pathway[7]. SHAFFER, MYTHA AND FINK[8] have shown that the three activities specified by the his-4 region remain physically associated during gel filtration and ultracentrifugation. A chain-terminating mutation in that part of the his-4 region which codes for histidinol dehydrogenase lowers the apparent molecular weight associated with the other two enzyme activities. The his-4 region might thus code for a multiprotein complex, a single protein with three distinct enzymatic activities, or a single protein subsequently cleaved to form three individual protein molecules which nevertheless remain physically associated with one another.

We have studied compartmentation in the histidine biosynthetic pathway by measuring the mixing of (^{14}C)histidinol, formed endogenously from (^{14}C)glucose, with unlabelled histidinol added externally. Compartmentation appeared to exist in the histidine pathway of Sacc. cerevisiae, but not in that of Salm. typhimurium.

MATERIALS AND METHODS

L-Histidine. HCl, hydrate was obtained from Calbiochem, Los Angeles, California, and L-histidinol. 2HCl from Cyclo Chemical Corp., Los Angeles, California.

D-($^{14}C_6$)Glucose (180 mC/mmole) was from ICN Corp., Irvine, California; L-($^{14}C_6$)histidine (250 mC/mmole) was supplied by New England Nuclear Corp., Boston, Mass. L-($^{14}C_6$)Histidinol was prepared from labelled histidine by reduction with $LiAlH_4$ [9].

Membrane filters, type B-6 (Schleicher and Schuell, Keene, New Hampshire) were used for the filtration of cells, and of material precipitated with trichloroacetic acid.

Organisms

Strains of Salm. typhimurium and Sacc. cerevisiae were obtained through the kindness of Professors B.N. Ames and R. Mortimer, respectively. The strains used were: (a) wild type: Salm. typhimurium LT-2 and Sacc. cerevisiae X 2180-1B (a); (b) mutants of phosphoribosyl 1-pyrophosphate-ATP pyrophosphorylase, the first enzyme of the histidine biosynthetic pathway, which are capable of growth with added histidinol or histidine: Salm. typhimurium his G-46, and Sacc. cerevisiae JB 13 (a ade 2, his 1); (c) mutants of the last enzyme, histidinol dehydrogenase,

which require histidine for growth: Salm. typhimurium his D-1 and Sacc. cerevisiae JB 60 (a ade 2, his 4 AEC). The yeast strain JB 13 required a training period for efficient growth on histidinol. While it is possible that an additional mutation was selected in this way, we confirmed that a revertant to wild type had not been selected, since a requirement for histidinol or histidine remained through several subcultures.

Growth

Salm. typhimurium was grown aerobically at 37°, with shaking, in minimal medium[10] supplemented as required. For routine growth, 0.5% glucose was used as the carbon source. However, during experiments involving the uptake of (^{14}C)glucose, the glucose concentration was reduced to 0.1% in order to achieve a high specific radioactivity without the use of excessive quantities of labelled material. Growth was monitored by A_{650}, using a Beckman DK-2 double-beam spectrophotometer. The growth rates were identical at both glucose concentrations, and care was taken when using 0.1% glucose that the cells remained in exponential growth throughout the experimental period.

Sacc. cerevisiae cultures were shaken aerobically at 30° in "yeast nitrogen base without amino acids" (Difco Laboratories, Detroit, Mich.) containing 2% glucose for routine growth, and 0.1% glucose in labelled substrate uptake experiments. The growth rates were not affected by glucose concentration. Growth was followed by A_{540} after thorough shaking of the culture to disperse clumps of cells.

Determination of ^{14}C incorporated into protein

Cells were grown exponentially in the presence of (^{14}C)glucose. At intervals, 1 ml portions of the culture were mixed with 0.5-1 ml of 30% (w/v) trichloroacetic acid, containing unlabelled cells to aid subsequent manipulation; the final concentration of trichloroacetic acid was 10% (w/v). The precipitates were heated to 90° for 15 min. to solubilise nucleic acids, recovered by centrifugation, and washed successively with 1 ml portions of 50% (v/v) ethanol (twice), absolute ethanol, and ether. The washed residues, after drying at room temperature, were hydrolysed by heating for 24 hr. at 110° in sealed tubes with a mixture of equal parts of 12N HCl and glacial acetic acid. After cooling, the hydrolysis mixtures were evaporated to dryness in a stream of N_2.

The residues were dissolved in small volumes of water, and applied to 48 cm. x 60 cm. sheets of Ederol No. 202 filter paper (J.C. Binzer G.m.b.H., Batzfeld/Eder, Germany). The amino acids were separated by two-dimensional chromatography, using n-butan-1-ol - pyridine - water (1:1:1, by vol.) followed by phenol - water (4:1, w/w) containing ca. 0.1% NH_4OH[11]. The running time in each dimension was 20-24 hr. at about 23°. Labelled spots were found by radioautography, they were excised from the chromatograms, placed in vials charged with toluene containing 0.382% 2:5-diphenyloxazole and 0.008% p-Bis 2-(5-phenyloxazolyl)-benzene. The ^{14}C content was measured with a Tri-Carb Liquid Scintillation Spectrometer Model 3375 (Packard Instrument Co., Inc., Downers Grove, Ill.). The geometry of the chromatogram spots within the vials did not significantly affect the counting efficiency so long as the entire paper was immersed in the scintillator solution.

Determination of histidine and histidinol

These were assayed colorimetrically by a modification of the method of NEUMAN AND TURNBULL[12]: the pH was adjusted with 0.1 vol. of 0.1M Na_3PO_4 instead of with NaOH. This gave the correct pH of 11-12, and there was no need to check individually the pH of each tube. The pyridine must be redistilled, and the KI_3 reagent freshly prepared. Standard curves must be made to determine the amount of KI_3 reagent to be used for any particular concentration range of histidine or histidinol; the method is not satisfactory if a large excess of KI_3 is present[12].

RESULTS

Design of the basic experiment

We wished to determine whether or not the histidine biosynthetic pathway exhibited compartmentation in the sense that L-histidinol, an intermediate of that pathway, might be retained in a separate compartment, and not be in equilibrium with one or more other pools of the metabolite not involved in the biosynthetic process. To do this, organisms were grown on (^{14}C)glucose as sole carbon source; thus, all cellular components containing carbon, including endogenously-formed L-histidinol, were labelled. In the absence of competition from an unlabelled exogenous pool, the label in histidinol was converted to histidine and ultimately incorporated into protein. The relative specific radioactivity of histidine in protein, compared with that of other amino acids, thus afforded a measure of the competition between endogenously-formed and externally-added histidinol.

Concentration of L-histidinol to be used for competition

It was reasoned that in order to observe possible competition, the internal concentration of the externally-added histidinol should be as high as that of histidinol generated in the biosynthetic sequence. Since this parameter would be difficult to measure directly, growth criteria were used: the required amount of added histidinol in the medium would be that concentration which would support growth in an appropriate mutant at the same rate as would an excess of histidine. This concentration of histidinol was then used with the wild-type strain in the competition experiment, making the assumption that histidinol would enter the wild-type strain as it had the mutant. If competition were to be observed, the assumption would need no further substantiation, since clearly histidinol would have penetrated the cells. In the absence of competition, however, it would become necessary to explore further the ability of histidinol to enter the cells from the medium.

Using Salm. typhimurium his C-46, which requires either histidinol or histidine for growth, it was found that 0.1 mM histidine supported growth at the maximum rate (cf. MARTIN et al.[13]); 3 mM histidinol was necessary to achieve an equally rapid growth rate (Table I).

(INSERT TABLE I NEAR HERE)

In a parallel experiment with Sacc. cerevisiae, using strain JB 13 adapted for growth on histidinol as described in MATERIALS AND METHODS, it was found that the presence of 1.4 mM histidinol resulted in a growth rate only slightly less than that produced by 0.1 mM histidine (Table II).

(INSERT TABLE II NEAR HERE)

Effect of histidinol on histidine incorporation

It has been suggested[14] that histidinol is a competitive inhibitor of the histidine activating enzyme. It was therefore necessary to demonstrate that the concentration of added histidinol to be used in the competition experiments did not inhibit the incorporation of histidine into protein.

Using Salm. typhimurium his D-1 (Fig. 1) and Sacc. cerevisiae JR 80 (Fig. 2), both of which require histidine for growth, and cannot use histidinol, it was found that the concentrations of histidinol chosen for the competition experiments did not affect histidine incorporation into protein.

(INSERT FIGS. 1 AND 2 NEAR HERE)

Competition experiments

The wild type strains, Salm. typhimurium LT-2 and Sacc. cerevisiae X 2180 (a), were grown exponentially in minimal media containing 0.1% glucose (see MATERIALS AND METHODS). Three parallel identical cultures were set up for each experiment. Each initially received a charge of (^{14}C)glucose sufficient to bring the specific radioactivity of glucose in the medium to 3.53 mC/mmole. In each experiment, one culture received no histidinol, and served as a control. The second culture received histidinol (3mM for Salm. typhimurium; 1.4 mM for Sacc. cerevisiae) simultaneously with the labelled glucose. The third culture received a similar quantity of histidinol 1-1.5 generations after the introduction of (^{14}C)glucose. At various times, aliquots of the suspensions were removed into trichloroacetic acid for measurement of ^{14}C in the protein amino acids (see MATERIALS AND METHODS).

The following amino acids, which were stable to hydrolysis and well separated chromatographically, were studied kinetically: aspartic acid (including asparagine), glutamic acid (including glutamine), glycine, alanine, arginine, lysine, valine, proline, histidine, and a combined spot of leucine, isoleucine and phenylalanine. All showed a linear relationship between ^{14}C incorporated and growth as measured optically. Figs. 3A and 4A report data for alanine for Salm. typhimurium and Sacc. cerevisiae, respectively; all the other amino acids, except histidine, gave comparable results. With the exception of histidine, the incorporation of ^{14}C into these amino acids was in no way affected by the presence in the medium of L-histidinol.

(INSERT FIGS. 3 AND 4 NEAR HERE)

The corresponding data for ^{14}C incorporation into histidine is shown in Figs. 3B and 4B. Replicate experiments yielded similar results. There was always more scatter in plots of labelled histidine than was found for other amino acids, partly because of the relatively low abundance of labelled histidine, and partly as a result of the somewhat diffuse character of histidine spots on paper. It was nevertheless clear that external histidinol totally replaced the endogenously formed compound as a precursor for histidine in Salm. typhimurium, but not in Sacc. cerevisiae. We conclude that in Salm. typhimurium external histidinol mixes freely with the internal pool, and compartmentation does not occur. In yeast, the suggestion of possible compartmentation made it necessary to ascertain that histidinol did, in fact, penetrate to the interior of the cell.

Chemical measurement of the entry of L-histidinol into wild-type yeast cells

Known amounts of L-histidinol were added to dense suspensions of yeast cells. After appropriate periods, the cells were sedimented by centrifugation, and the concentration of histidinol measured in the supernatant medium.

If the whole cell volume were inaccessible to histidinol, the latter would be confined entirely to the external medium. Knowing the total amount of histidinol in the whole suspension and the volume of the cell mass, measurement of the extracellular histidinol concentration permits determination of the exclusion volume. For a totally excluding cell, the extracellular histidinol concentration would rise rapidly with increasing cell concentration (Fig. 5). However, even for a fully accessible cell there would be a small increase in the extracellular histidinol concentration as the cell density is increased, since all cells contain a proportion of solid material. From published data on the cellular composition of packed yeast cells, and their intracellular and interstitial water contents[15], a curve for external histidinol concentration versus cell density may be calculated assuming maximum penetrability (Fig. 5). A concentration of histidinol in the medium lower than the known average concentration for the suspension as a whole would indicate either failure of the assay procedure, or the removal of histidinol from the medium by the cells.

(INSERT FIG. 5 NEAR HERE)

It was observed experimentally (Fig. 6) that the external histidinol concentration indeed decreased with increasing cell density. Table III shows that this was not due to interference with the

chemical assay resulting from the presence of yeast cells.

(INSERT TABLE III NEAR HERE)

(INSERT FIG. 6 NEAR HERE)

Making allowance for the non-available space within the cells (v. sup.), it can be calculated that the intracellular concentration (2.6 mM) was 1.85 times the theoretical uniform concentration (1.4 mM).

The accumulation of histidinol by the cells represented genuine penetration, and was not the result of simple sticking to the membranes. Table IV reports the results of an experiment in which standard quantities of histidinol were added to standard volumes of medium containing known

(INSERT TABLE IV NEAR HERE)

amounts of yeast cell ghosts. The presence of cell ghosts did not decrease the concentration of histidinol in the supernatant medium. There was thus no evidence for histidinol binding to the membranes; we conclude that the fall of supernatant histidinol concentration in the presence of live cells (Fig. 6) was due to uptake of this material and its concentration within the cells.

These measurements, however, were performed with very dense cell suspensions in phosphate buffer containing glucose, conditions quite unphysiological and different from those used in the competition experiments. A more direct approach to the problem of histidinol penetration was therefore adopted by observing the incorporation of labelled histidinol into growing cells.

Radiochemical measurement of the entry of histidinol into wild-type yeast cells

Direct measurement of (^{14}C)histidinol uptake under conditions of cell growth presents certain technical difficulties. At the cell

density at which the competition experiments were performed (A_{540} about 1.0), the proportion of the total volume of the suspension (i.e. cells plus medium) occupied by the cells themselves was very small. By centrifugation to constant packed cell volume in haematocrit tubes, we have found the packed cell volume to be 5.5-6.0 x 10^{-4} ml/ml of cell suspension at A_{540} = 1.0. From this value, one must subtract 32% for non-available space, plus 23% for interstitial water[15], to obtain the total available intracellular volume; this is about 2.6 x 10^{-4} ml/ml of cell suspension. Thus, if a labelled substance is taken up from the medium into the cells, without concentration or accumulation, only about 0.025% of the total radioactivity in the system will be associated with the cellular fraction. This results in low count rates compared with relatively high background values, and the consequent need for considerable replication in order to acquire statistically significant data.

The experiments were performed with wild-type Saco. cerevisiae X 2180 - 1B(a), growing exponentially as described earlier. In some experiments 0.1% glucose was used as the carbon source in order to maintain conditions as close as possible to those in the competition experiments. In other cases the glucose concentration was raised to 1% to permit more dense cultures to be used, with a consequently greater incorporation of radioactivity. To each experimental culture was added 1.4 mM (^{14}C)histidinol, containing about 3.2 x 10^{5} counts/min/nmole. Aliquots (1 ml) were withdrawn at intervals. In one series, the cell suspension was filtered directly on a membrane filter, the cells rapidly washed with 4 x 2 ml of medium containing unlabelled histidinol (1.4 mM),

and the filters immersed in scintillation fluid for measurement of total cellular radioactivity; all these operations were performed at 23°. In the second series, the cells were killed with 10% trichloroacetic acid, the precipitated protein collected on membrane filters, washed, and the radioactivity in protein measured. From the difference between ^{14}C in the whole cells and ^{14}C in the trichloroacetic acid-precipitated material, and knowing the specific radioactivity of the (^{14}C)histidinol, and the proportion of the whole cell suspension represented by the available intracellular space, the intracellular histidinol concentration was calculated. From 16 pairs of measurements in 4 experiments, using 3 different (^{14}C)histidinol preparations, the mean intracellular histidinol concentration ± S.E. was 1.54 ± 0.42 mM, the external concentration being 1.4 mM in each case.

A similar experiment in which cells were supplied with 1.4 mM (^{14}C)histidine showed that the intracellular concentration of histidine was 0.18 M (in agreement with the findings of CRABEEL AND GRENSON[16]), and established a rate for the incorporation of histidine into protein. The rate of ^{14}C incorporation from (^{14}C)histidinol into trichloroacetic acid-insoluble material was equal to 3-4% of the rate of histidine incorporation into protein. A decrease of 3-4% in the rate of ^{14}C incorporation into histidine in the presence of histidinol in the competition experiment would have fallen within the limits of experimental error, and would not have been detected (Fig. 4).

DISCUSSION

Competition experiments with histidinol suggest a compartmented metabolic organisation in *Sacc. cerevisiae* but not in *Salm. typhimurium*,

even though there is genetic clustering of the histidine biosynthetic genes on the bacterial genome. It seems probable that in Salm. typhimurium, externally added histidinol gains ready access to histidinol dehydrogenase, and is rapidly oxidized to histidine. The latter then acts as a retro-inhibitor of the endogenous pathway[2,3], resulting in a total cessation of histidine formation through the complete pathway, the whole histidine requirement being met by the oxidation of external histidinol. Thus, the incorporation of ^{14}C from glucose into histidine is entirely prevented by added unlabelled histidinol.

Competition was not observed in yeast. Chemical and radiochemical determination of histidinol uptake into yeast, as well as the ability of certain mutants to satisfy their histidine requirement from an external source of histidinol, indicated the ability of histidinol to penetrate into the yeast cell. Radiochemical measurements suggested a concentration of histidinol inside the cells equal at least to that in the medium.

In the competition experiments the concentration of histidinol in the medium was 1.4 mM, and the concentration inside the cells no lower. The precision of our experimental data with yeast would readily have permitted the observation of a fall of 20% in the rate of ^{14}C incorporation from glucose into histidine (Fig. 4). Thus, if the lack of observed competition were due to a high relative concentration of endogenously-produced labelled histidinol compared with externally-added unlabelled histidinol, the concentration of endogenous histidinol must have been at least 5.6 mM. Such a high concentration is unlikely for the following reason: in the competition

experiments (Figs. 3 and 4) there was always a lag in the appearance of ^{14}C in some protein amino acids, including histidine. This showed that ^{14}C, supplied as (^{14}C)glucose, had to pass through, and equilibrate with, the carbon in a series of metabolic pools between glucose and the protein amino acids. The sizes of these pools may be estimated approximately from the length of the lag and the known specific radioactivity of the (^{14}C)glucose supplied to the cells. In two experiments, the sizes of the combined pools of all intermediates (presumably including histidinol) between glucose and histidine (calculated on the basis of 6 carbon atoms in each intermediate) were 3.8 mM and 5.3 mM, respectively. Thus, the size of the histidinol pool alone cannot have been 5.6 mM. It may also be noted that the lag in yeast (about 0.3 generation) was not very different from that in Salm. typhimurium (about 0.2 generation), in which free competition was observed.

The evidence for metabolic compartmentation in histidine biosynthesis in yeast is based upon the lack of equilibration for histidinol. It should be noted nevertheless, that although externally added histidinol does not have access to histidinol dehydrogenase when the cell is producing histidinol endogenously, it can be converted to histidine in a mutant (JR 13) unable to synthesize histidinol endogenously owing to a lesion in phosphoribosyl 1-pyrophosphate-ATP pyrophosphorylase. Presumably, when both are present, endogenously-generated histidinol competes successfully for access to the catalytic site, but in its absence the enzyme will catalyze the oxidation of histidinol derived from the medium. It is possible that histidinol is unique among the intermediates of the sequence. However, we note

again the observation[8] that at least three of the yeast histidine biosynthetic enzymes form a physical aggregate, one of the constituents of which is histidinol dehydrogenase; the enzyme responsible for histidinol formation, histidinol phosphate phosphatase, does not form part of the aggregate. Thus, by itself, this aggregate could not account for the lack of equilibration shown by histidinol. We suggest that in the living yeast cell the aggregate isolated by MARTIN et al.[8] might form part of a larger macrostructure in which others of the histidine biosynthetic enzymes are also present. It is possible that other intermediates, in addition to histidinol, are compartmented, and that the whole biosynthetic mechanism forms both a structural and a metabolic unit. Such a unit might be envisaged as a multi-enzyme structure, in which intermediates are channelled from one catalytic site to the next, with a high probability of direct capture. Alternatively, the unit might itself be an "organelle", in which the biosynthetic mechanism is contained within a limiting membrane. In such a situation, compartmentation would result from the impermeability of the limiting membrane to intermediates of the sequence. In either case it might be possible to isolate the whole unit by physical means.

Compartmented systems in yeast have been described for nucleic acid bases[17] and amino acids[19] in Candida utilis; COHEN AND WOOLLEY[18] suggested that an internal pool from which amino acids are selected for incorporation into protein, the amino acids are complexed with macromolecular components of the cells, possibly with the proteins. Compartmentation in bacteria is more doubtful. In accord with our own findings on histidine biosynthesis in bacteria

and yeast, BERLYN AND GILES[19], and AHMED AND GILES[20], found that a number of enzymes of the aromatic amino acid biosynthetic pathway are physically aggregated in some fungi, but not in bacteria. Genetically, however, this system differs from histidine biosynthesis; an aromatic amino acid gene cluster probably occurs in the fungi, but not in the bacteria.

Metabolite channeling has previously been explored in the biosynthetic pathway of arginine from glutamic acid via ornithine. In bacteria, including Escherichia coli and Proteus mirabilis, all intermediates between glutamic acid and ornithine are N-acetylated. N-Acetyl-γ-glutamyl phosphate, unlike free γ-glutamyl phosphate, is a stable intermediate in aqueous solution, and does not cyclize and hydrolyze. The next intermediate, N-acetylglutamic γ-semialdehyde, cannot cyclize in the manner in which free glutamic γ-semialdehyde spontaneously forms Δ^1-pyrroline 5-carboxylate in the proline pathway. Thus, N-acetylglutamic γ-semialdehyde in the arginine pathway cannot serve as an intermediate in the proline pathway[10, 21-24], and there is no evidence for channeling in these bacterial systems.

In Neurospora crassa and Torulopsis utilis the intermediates are not acetylated[25,26]. Evidence exists for channeling in these organisms, since exogenous ornithine gains access to the proline pathway (via transamination to glutamic γ-semialdehyde), while endogenous ornithine in the arginine pathway does not[27]. Channeling prevents the cyclization and hydrolysis of the intermediary γ-glutamyl phosphate, and the cyclization of glutamic γ-semialdehyde to Δ^1-pyrroline 5-carboxylate, which would reduce the efficiency of the transamination to ornithine.

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TABLE I

GROWTH RATES OF *Salmonella typhimurium his* G-46

WITH VARIOUS SUPPLEMENTS

This *his*⁻ strain was grown in minimal medium supplemented with various concentrations of L-histidine or L-histidinol. The cultures were grown aerobically, with shaking, at 37°, and growth was followed by measurement of A_{650}.

Supplement	Concentration (mM)	Growth rate (doublings/hr)
L- Histidine	0.1	0.85
L- Histidine	0.5	0.86
L- Histidinol	0.5	0.58
L- Histidinol	1.0	0.61
L- Histidinol	3.0	0.90
L- Histidinol	5.0	1.09

TABLE II

GROWTH RATES OF *Saccharomyces cerevisiae* JB 13

WITH VARIOUS SUPPLEMENTS

This *his*$^-$ strain was grown in minimal medium supplemented with various concentrations of L-histidine or L-histidinol. The cultures were grown aerobically, with shaking, at 30°, and growth was followed by measurement of A_{540}.

Supplement	Concentration (mM)	Growth rate (doublings/hr)
L-Histidine	0.1	0.41
L-Histidinol	0.94	0.32
L-Histidinol	1.4	0.37
L-Histidinol	2.3	0.37

TABLE III

EFFECT OF PREINCUBATION OF MEDIUM WITH YEAST CELLS ON THE COLORIMETRIC DETERMINATION OF HISTIDINOL

Cells were preincubated with buffer, and then removed by centrifugation. To the supernatants were added standard quantities of histidinol. Assays were then performed for histidinol, and these were compared with similar assays in which histidinol was added to medium which had not been used to suspend yeast cells, (see caption to Fig. 6). During the preincubation in buffer, the cells had a relative wet packed volume of 63%; at this cell concentration, there was more than a 50% decrease in histidinol concentration when the assay was performed in the presence of cells (Fig. 6).

Sample	Absorbance units in standard histidinol assay
1. Control	0.320
plus supernatant from a 30 min preincubation	0.304
2. Control	0.342
plus supernatant from a 16 hr preincubation	0.396

TABLE IV

EFFECT OF THE PRESENCE OF YEAST CELL GHOSTS ON THE COLORIMETRIC DETERMINATION OF HISTIDINOL

The cells were broken in a French pressure cell at 15 - 20,000 psi. The experiment depicted in Fig. 6 was repeated in the presence of washed cell ghosts alone, instead of whole cells. Further experimental details as for Fig. 6.

Sample	Absorption units
Experiment no. 1	
Control	0.407
*Plus membranes corresponding to about 53% cell volume	0.465
*Plus membranes corresponding to about 26% cell volume	0.485
Experiment no. 2	
Control	0.373
*Plus membranes corresponding to about 57% cell volume	0.341
*Plus membranes corresponding to about 28% cell volume	0.373

*i.e. the membranes from that concentration of cells, which, if composed of unruptured cells, would constitute x% of the total volume of the reaction mixture.

Fig. 1 Effect of histidinol on the incorporation of (^{14}C) histidine into Salm. typhimurium his D-1. The cells were grown to A_{650} of 0.5 in glucose-minimal medium, supplemented with 0.1 mM histidine. The culture was divided into four portions; these received a standard quantity of (^{14}C) histidine, together with varying amounts of unlabeled histidinol. Aliquots were withdrawn at intervals, the protein precipitated in 10% (w/v) trichloroacetic acid, filtered, washed, dried and the precipitated ^{14}C measured in a liquid scintillation counter. O—O, control; ⊙—⊙, plus 1 mM L-histidinol; X—X, plus 3 mM L-histidinol; △—△, plus 5 mM L-histidinol.

Fig. 2 Effect of histidinol on the incorporation of (^{14}C) histidine into Sacc. cerevisiae JR 30. The cells were grown to A_{540} of 0.35 in glucose-minimal medium, supplemented with L-histidine and adenine (20 µg/ml of each). (^{14}C)Histidine was then added, and the culture divided into three portions, two of which received unlabeled histidinol, 1.4 mM and 2.3 mM, respectively. Aliquots were withdrawn at intervals, the protein precipitated in 10% (w/v) trichloroacetic acid, filtered, washed, dried, and the precipitated ^{14}C measured in a liquid scintillation counter. O—O, control; X—X, plus 1.4 mM L-histidinol; Δ—Δ, plus 2.3 mM L-histidinol.

Fig. 3 Effect of L-histidinol on the incorporation of ^{14}C from (^{14}C) glucose into the protein-amino acids of Salm. typhimurium. Salm. typhimurium LT-2 was grown exponentially in minimal medium containing 0.1% glucose. At the first arrow, (^{14}C)glucose (180 mC/mmole) was added to a final concentration of 20 μC/ml, and the culture was divided into three portions: O—O, control; ⊕—⊕, plus 3 mM L-histidinol added at the same time as the labeled glucose; X—X, L-histidinol (3mM) added at the second arrow. Samples were withdrawn at various times, and treated as described in the text. The straight lines were calculated using a least squares averaging procedure. Fig. 3a: ^{14}C incorporation into alanine; Fig. 3b: ^{14}C incorporation into histidine.

Fig. 4 Effect of L-histidinol on the incorporation of ^{14}C from (^{14}C)glucose into the protein-amino acids of Sacc. cerevisiae. Sacc. cerevisiae X 2180-1B was grown exponentially in minimal medium containing 0.1% glucose. At the first arrow, (^{14}C)glucose (180 mC/mmole) was added to a final concentration of 20 µC/ml, and the culture was divided into three portions: O — O, control; ● — ●, plus 1.4 mM L-histidinol added at the same time as the labeled glucose; X — X, L-histidinol (1.4 mM) added at the second arrow. Samples were withdrawn at various times, and treated as described in the text. The straight lines were calculated using a least squares averaging procedure. Fig. 4a: ^{14}C incorporation into alanine; Fig. 4b: ^{14}C incorporation into histidine.

Fig. 5 Hypothetical relative extracellular concentration of a fixed amount of compound in a fixed total (cells plus supernatant) volume, as a function of the proportion of cells present. X — X, assuming whole cell is not accessible; ⊙ — ⊙, assuming 22% of cells (actual solid material in yeast[15]) is not accessible.

Fig. 6 Measured relative concentrations of a fixed amount of histidinol in the supernatant of reaction mixtures containing various yeast cell:liquid ratios. Sacc. cerevisiae X 2180-1B was grown in the usual way and harvested in log phase at A_{540} about 1.6. The cells were washed twice with 0.1 M phosphate buffer containing 0.2% glucose, and then suspended in a minimum amount of the same buffer to make the suspension fluid enough for accurate dilution. The relative cell volume measured in hematocrit tubes, after centrifugation for 20 minutes at 1450 x g (max.). A number of cell dilutions were made, and a standard amount of histidinol was added to each tube. The tubes were incubated for 1 hr at 30° with agitation, then spun for 20 minutes in the bench-top centrifuge and aliquots of the supernatant withdrawn and used for the colorimetric assay of histidinol (see Methods). The amount of histidinol added would have yielded a concentration of 1.4 mM if it were dispersed uniformly throughout the suspensions. This was the concentration used in competition experiments with Sacc. cerevisiae.

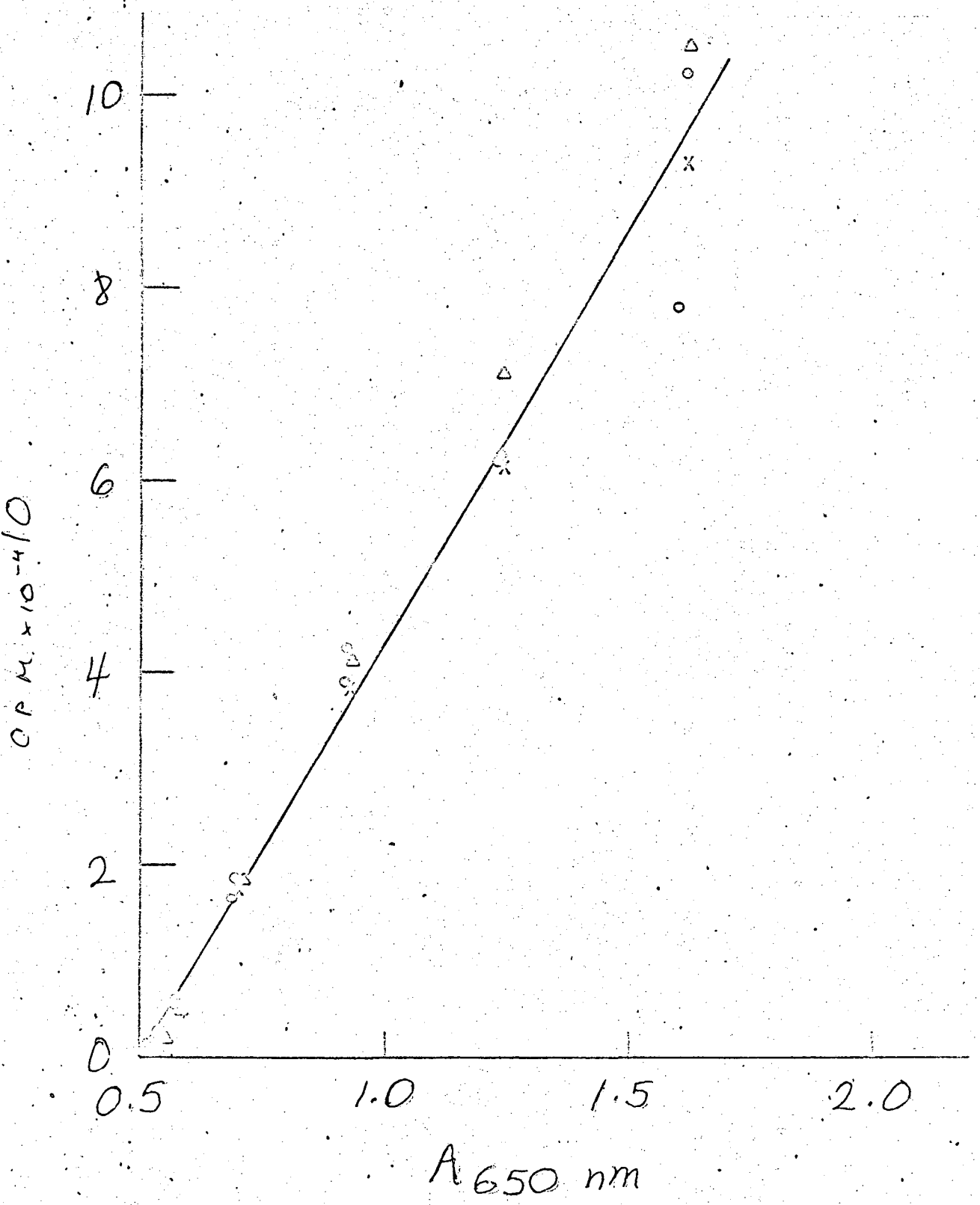


Fig. 1

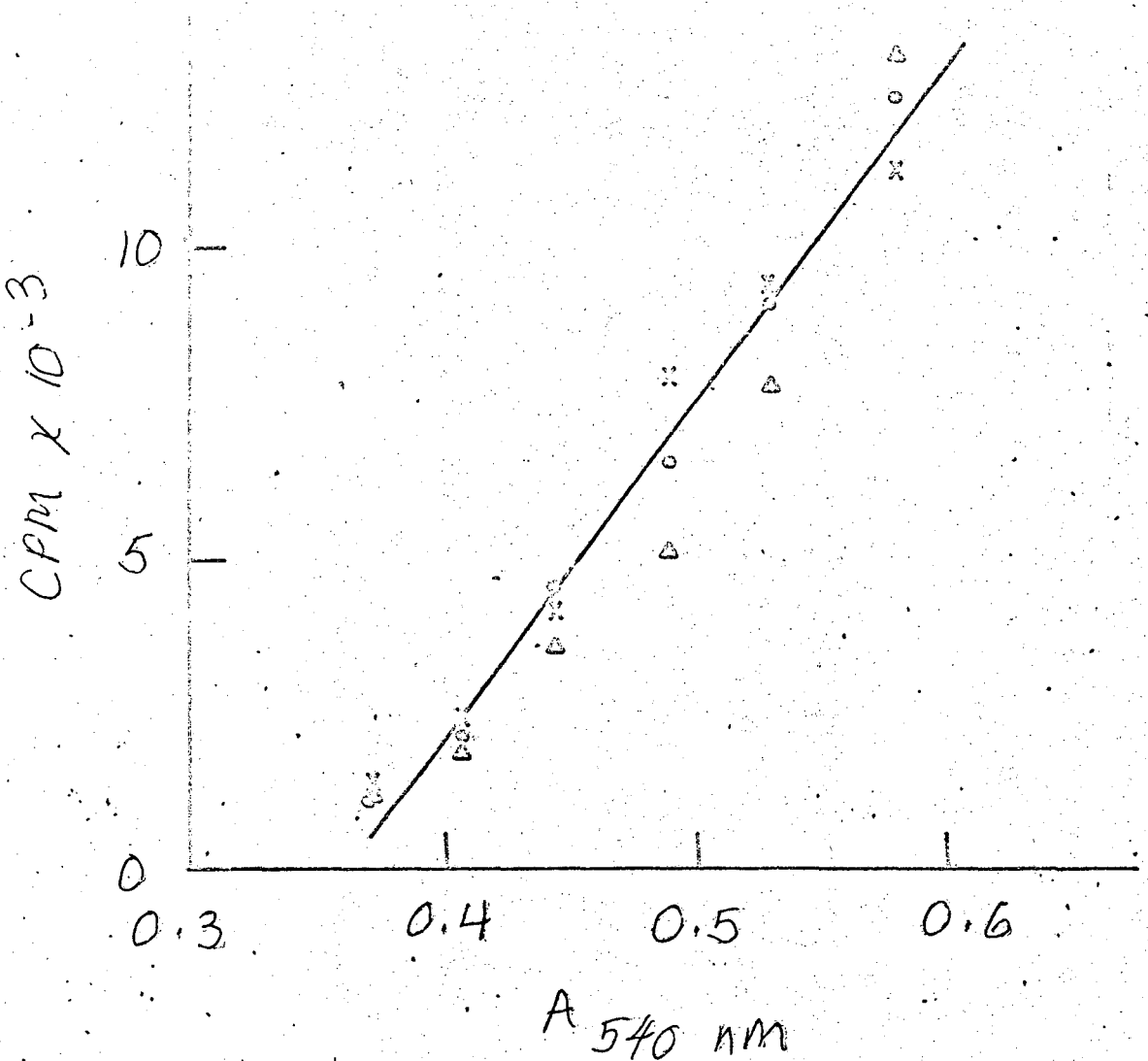


Fig. 2

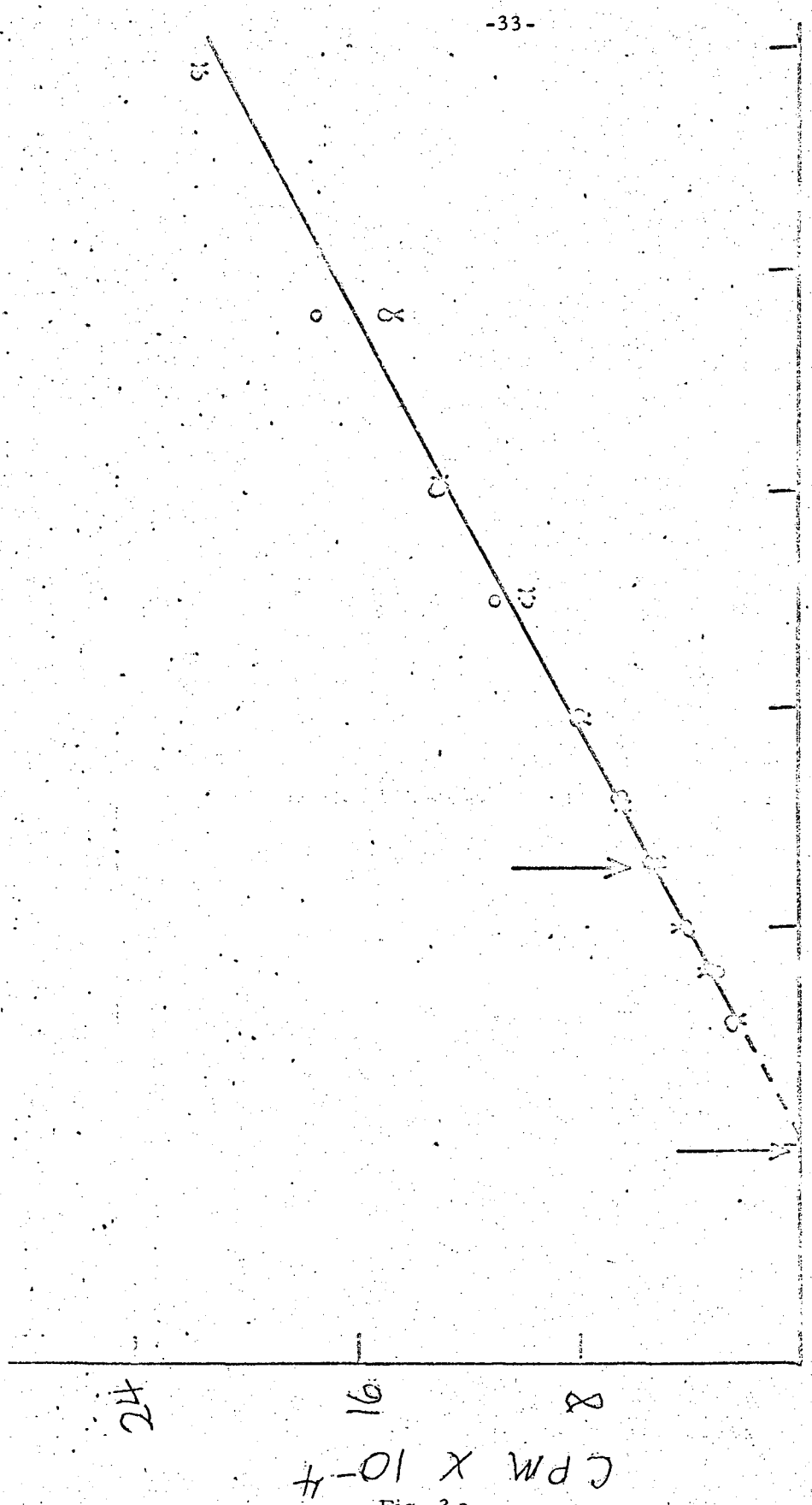


Fig. 3 a

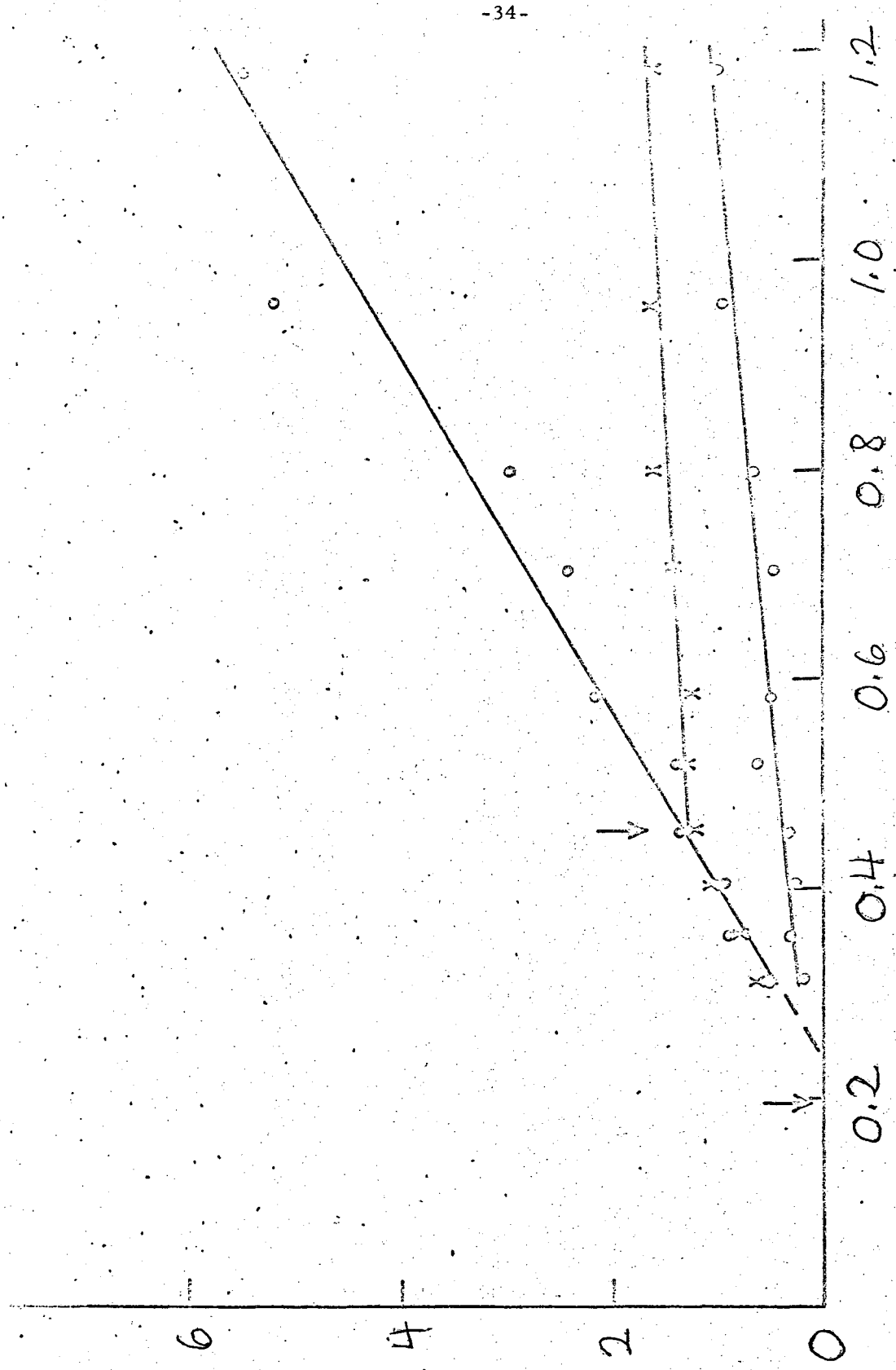
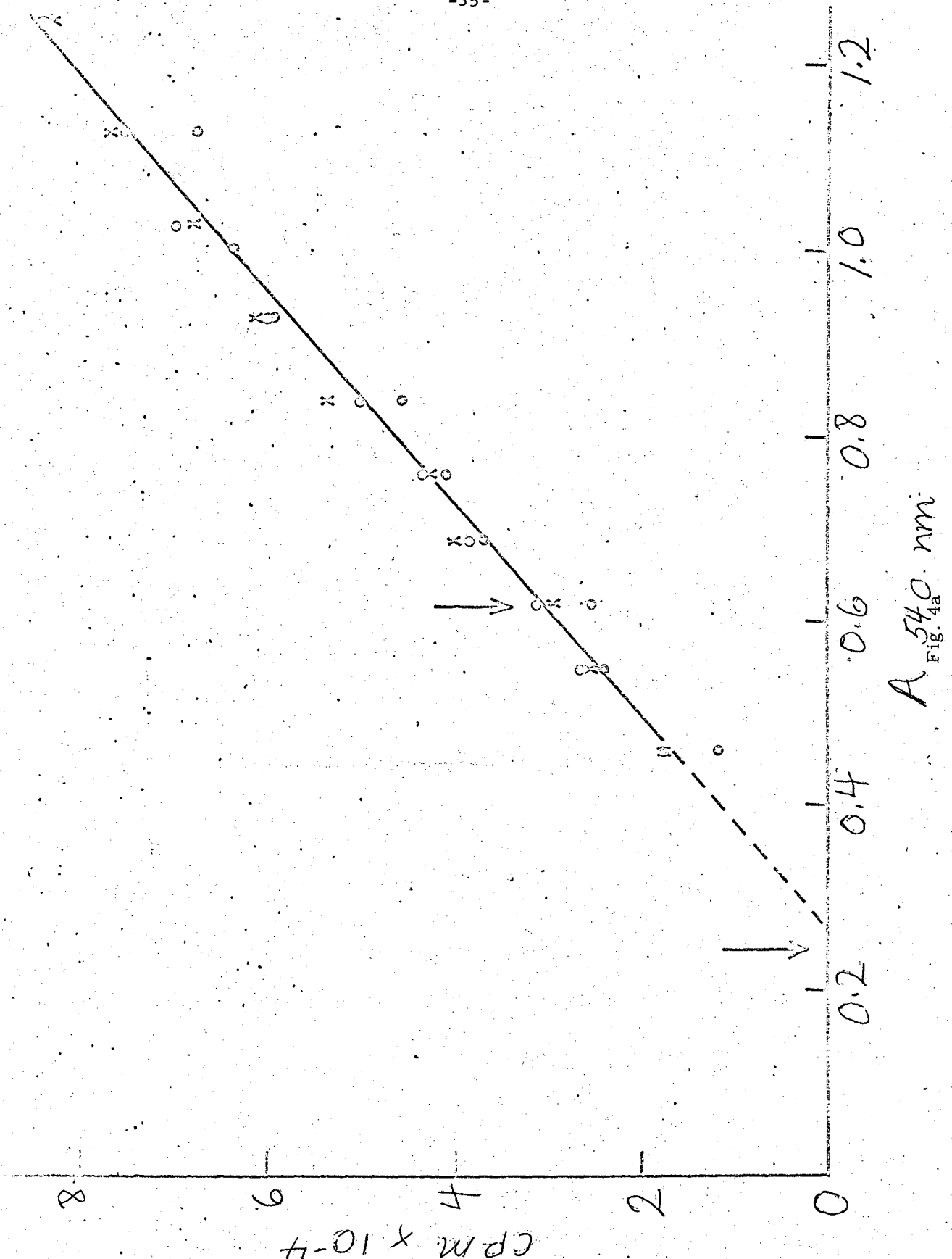


Fig.3b


CPM x 10-4
0
2
4
6
8
0.2
0.4
0.6
0.8
1.0
1.2
A 540 nm
Fig. 4a

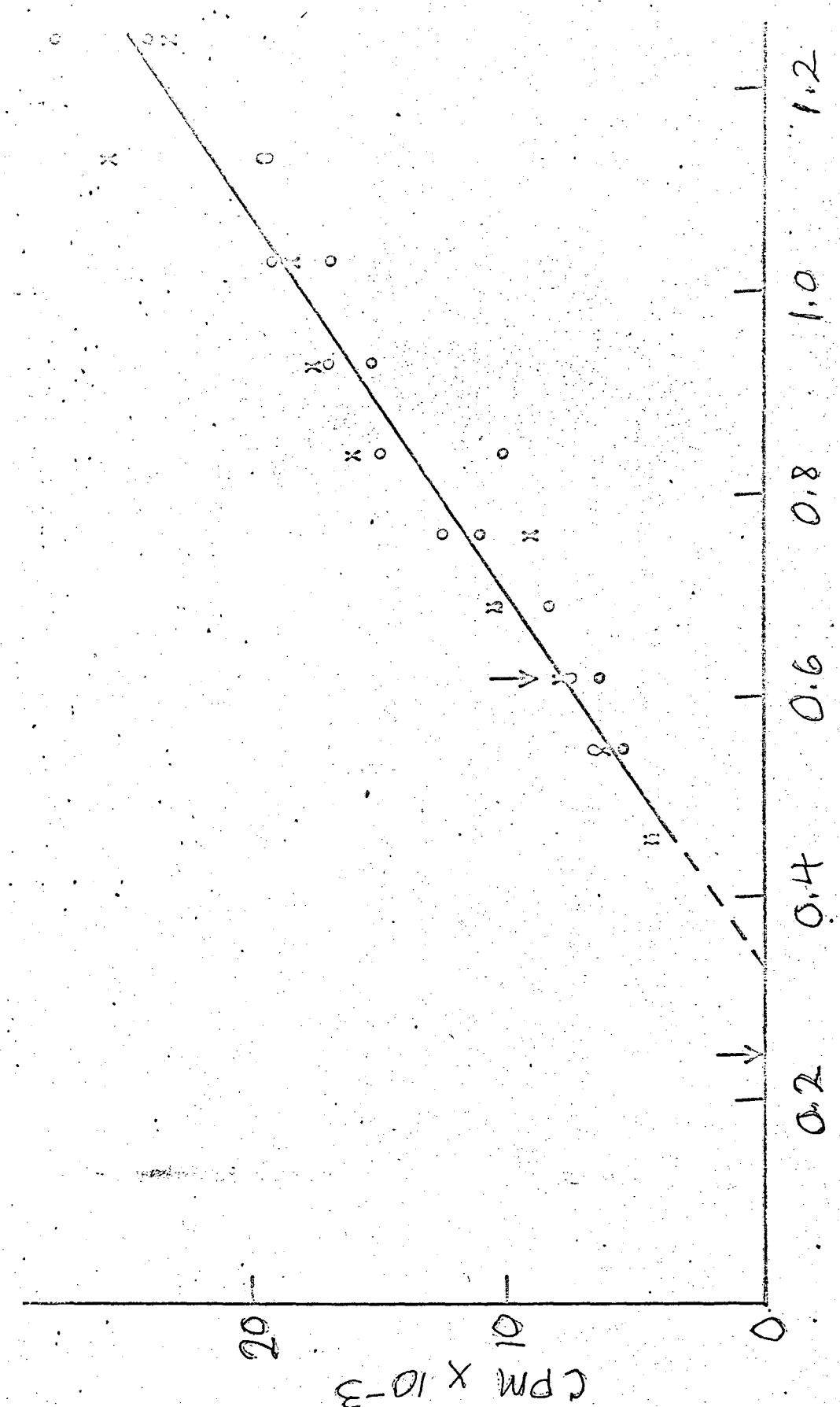


Fig. 4b

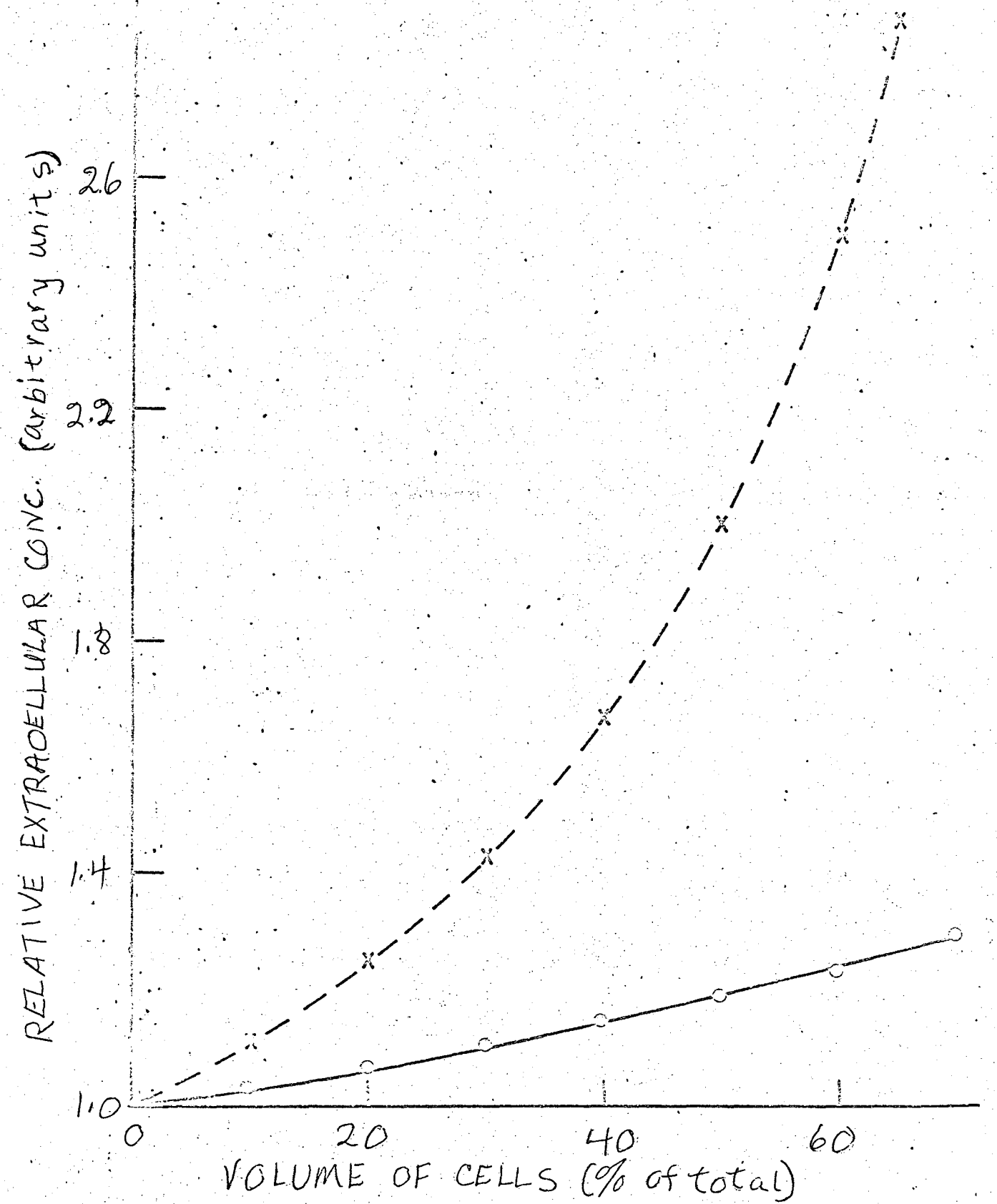

RELATIVE EXTRACELLULAR CONC. (arbitrary units)
2.6
2.2
1.8
1.4
1.0
0
20
40
60
VOLUME OF CELLS (% of total)

Fig. 5

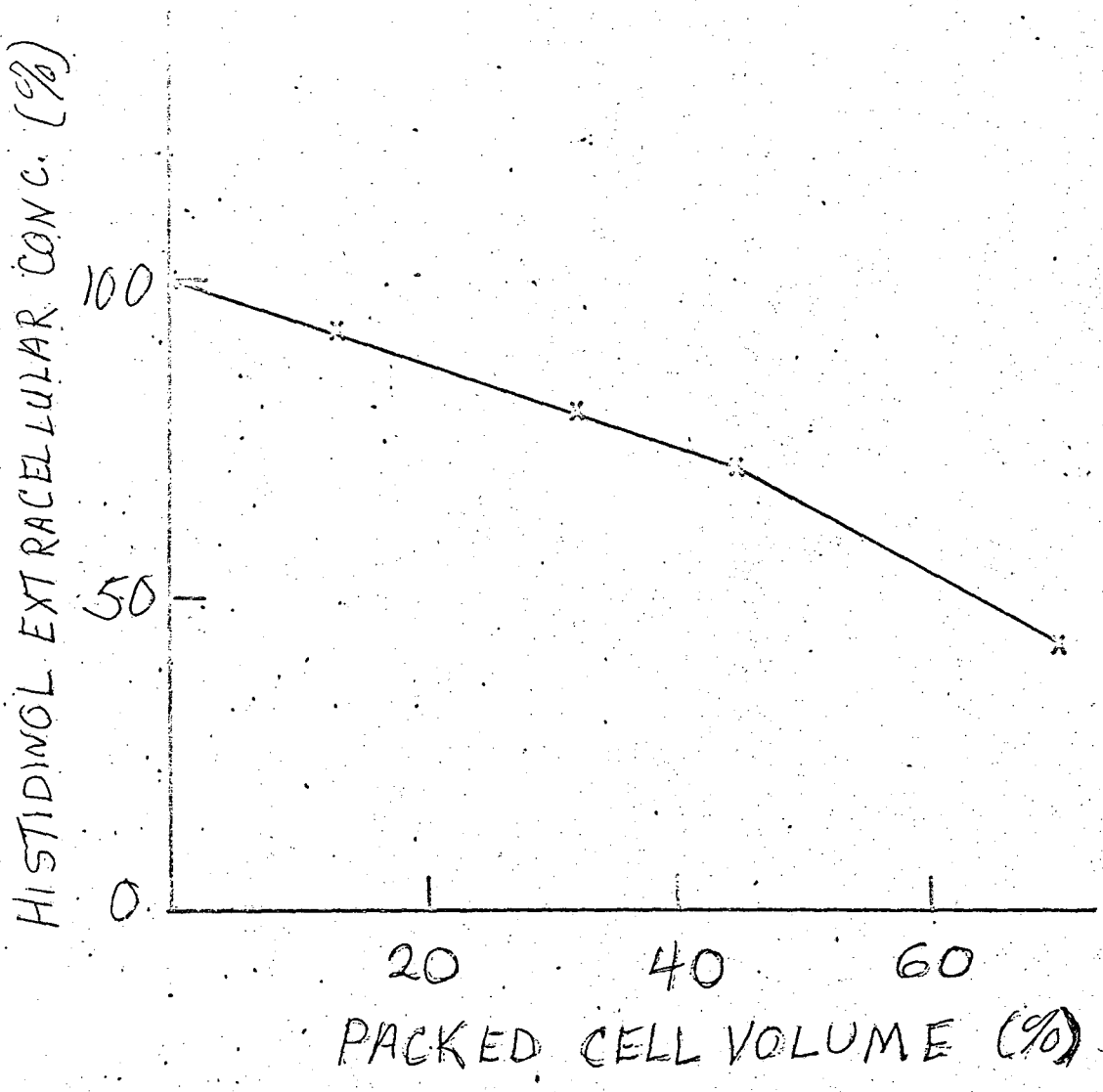

HISTIDINOL EXTRACELLULAR CONC. (%)
100
50
0
20
40
60
PACKED CELL VOLUME (%)

Fig. 6

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