UPTAKE OF SYNTHETIC POLYNUCLEOTIDES

A survey was made of the capacity of competent cells of Bacillus subtilis to uptake the synthetic polynucleotides reported in Table I. None of the ribo-polynucleotides tested was taken up to a significant extent by the cells under any of the tested conditions. When radioactive polynucleotides were used, a certain amount of radioactivity was recovered with the cells; however, the reaction did not show any time dependency so that it could be ascribed to binding of the polymers to cells and/or to the uptake of a certain amount of contaminating oligomers present in the preparation of polynucleotides. Indeed, in the case of rU, treatment of the cells with RNase after exposure to radioactive rU resulted in almost complete loss of the radioactivity which was bound to the same cells. Similar results were obtained in the case of hybrids between ribo-polynucleotide and one deoxyribo-polynucleotide such as rAdT or rU:DNA. On the contrary, dAT and dCdG appear to be taken up significantly with a kinetic which is time dependent, (Fig. 1).

Attempts were made to alter cell permeability by pretreating the cells with 5% dimethylsulfoxide (DMSO) for sixty minutes or EDTA or by incubating cells with lysozyme (10 μg/ml). No significant uptake under such conditions were observed as well as in experiments in which protein sythesis was inhibited by pretreating the cells with puromycin, and chloramphenical or polysome depolymerization was favored by depriving cells of Magnesium or by treating with Sodium Fluoride.

The same type of experiments employing radioactive rU and rA was performed with equally unsuccessful results with the following strains

of bacteria: Escherichia coli, Aerobacter aerogenes, Pseudomonas aeruginosa, Klebsiella pneumomiae, Staphylococcus aureus, Proteus vulgaris, Alcaligenes foecalis, and Corynebacterium sp..

SECTION II -STIMULATION OF AMINO ACID INCORPORATION

Notwithstanding the negative results reported in the previous section, it could be argued that some of the tested polynucleotides were taken up by the cells in a very small amount. If such were the case, and if such polynucleotides in the cell could be used for protein synthesis, as it could be expected since the cells of Bacillus subtilis were deprived of the compounds for which they are auxotrophs, then stimulation of the incorporation of the specific amino acids which are coded for by the synthetic polynucleotides employed, could be expected. In the case of rU, stimulation of phenylalanine incorporation should be evident while stimulation of lysine incorporation should be brought about by the addition of rA. Stimulation of the incorporation of both amino acids should be noticed when rUrA is employed. An obvious control was the study of the incorporation of a different amino acid which is not coded for by the synthetic polynucleotides employed. Such is the case of arginine whose codons are rich in C and G and poor in U and A.

As Figure 2 shows, the addition of rU stimulates, as compared to the control containing no polynucleotides, the incorporation of phenylalanine in TCA insoluble material, but stimulation of incorporation of lysine was also observed. The same results were obtained in the case of the incorporation of lysine and phenylalanine in the presence of rA

and for the incorporation of phenylalanine and arginine in the case of rArU. Such results would suggest that the addition of polynucleotides brings about a somewhat general stimulation of amino acid incorporation, possibly for the presence of small oligomers or mononucleotides in the preparations of polynucleotides and that such oligomers or mononucleotides are used by the cell and they stimulate the synthesis of protein. Such a contention appears to be substantiated by the finding that sheared polynucleotides stimulate much more than intact polynucleotides the incorporation of amino acids. Also in the case of the hybrid rAdT, stimulation of amino acid incorporation was observed but not of the specific amino acids which are coded for by either rA or dT. Similar results were also obtained in the case of the hybrid formed between single-stranded DNA and rU.

Such data further strengthens contention that such polynucleotides are not taken up to a significant extent by the cells or else that if they are taken up they are not utilized as specific messenger RNA for protein synthesis. The stimulation of protein synthesis brought about by all the tested polynucleotides should then be described to a general stimulation of protein synthesis caused by either these polynucleotides or by the products of their degradation.

In the case of dAT, a polynucleotide that is taken up by competent cells of Bacillus subtilis, no specific stimulation of the

incorporation of tyrosine was observed. It may be remembered that one of the codons assigned to tyrosine is UAU. In this case, however, it may be postulated that transcription of the synthetic deoxypolynucleotide did not take place.

SECTION III COMPETITION BETWEEN DNA AND SYNTHETIC POLYNUCLEOTIDES

A further control on the possible uptake of the synthetic polynucleotides was made by studying the competition between these polymers and transforming DNA. If synthetic polynucleotides are taken up by the same mechanism by which DNA is incorporated during transformation, then competition should be observed between natural and synthetic polynucleotides. Likewise, competition between synthetic polynucleotides and DNA should be observed if the binding of synthetic polynucleotides to cells takes place an the same sites through which DNA enters the cells.

The experiments of Table 2 show that addition of rU, rA and rUrA at the same time of DNA does not decrease transformation even when the synthetic polynucleotides are employed at a concentration 100 fold higher than that of the transforming DNA. The data of Table 3 show that the addition of rUrA to the suspension of competent cells before the addition of DNA has little if any effect on transformation. Indeed a one thousand fold excess of hybrid even when added

ten minutes before DNA does not significantly decrease transformation. It may be added that no competition with DNA for transformation was observed by employing the following polynucleotides at concentrations that were in certain experiments one thousand fold higher than that of DNA: rA, rU, rArU, rC, rI, rCrI, rAdT:dAT, sRNA, and ribosomal RNA. On the contrary, addition of different concentrations of heterologous DNA reduces stoichiometrically the percentage of transformants. It remains to be explained why dCdG and dAT which are taken up by competent cells (Fig. 1) do not compete with DNA for transformation (Fig. 2). The phenomenon may perhaps be explained by the presence of different sites of entrance for the deoxypolyribonucleotides and for DNA or by a difference in the uptake of these polynucleotides by competent and non-competent cells; against this last hypothesis is the finding that the uptake of dAT and dCdG is greater when the competence is higher, indicating that only the competent cells are responsible for their uptake. Since there is apparently no substantial variation in the structure of the synthetic and natural poly-deoxyribonucleotides, the higher molecular weight of DNA (approximately 100 times) may be one of the discriminating factors.

In conclusion, the reported data indicate that natural and synthetic deoxyribopolynucleotides are taken up by cells of Bacillus-subtilis while such is not the case for ribopolynucleotides either in single or in double stranded structure. In any event, no relation appears to exist between uptake of synthetic polynucleotides and transformation.

TABLE I

Labeled polynucleotides Used

Polynucleotide	Abbreviation
Poly A	rA
Poly U	rU
Poly U: Poly A	UrA[1]
Poly U: DNA	rU:DNA[2]
Poly A: Poly dT	rAdT[3]
Poly dAT	dAT[4]
Poly dC: dG	dCdG[5]

1. Prepared by mixing equimolecular amounts (≈500 γml of each) of the ribopolynucleotides in 0.1M nacl for 15' at 70°C and cooling slowly to room temperature. Tm in 0.1M phosphate buffer pH 7.8 was estimated to be 52°C. The label was either in rU or rA.

2. Prepared as described by . Formation of the hybrid was assessed by the decrease in transforming activity as compared to that of renaturated DNA in the absence of polyribonucleotide. Label was in the rU.

3. Prepared by mixing 0.1 ml of H^3 rA (1 μc) in 0.4 ml 0.2 M phosphate buffer pH 7.8 with 0.5 ml of 10 γ/ml dT in 10^{-2} M Tris buffer pH 7 at 28°C. dT was generously supplied by Dr. R. Lehman. Label in 2A.

4., 5. From Biopolymers Inc.(5.5 and 3.4 μc/ml specific capacity). The molecular weight was estimated in sucrose density gradient and found to be about 2-3 x 10^5.

(Experiments on the uptake of radioactive polynucleotides were performed as reported in Fig. 1).

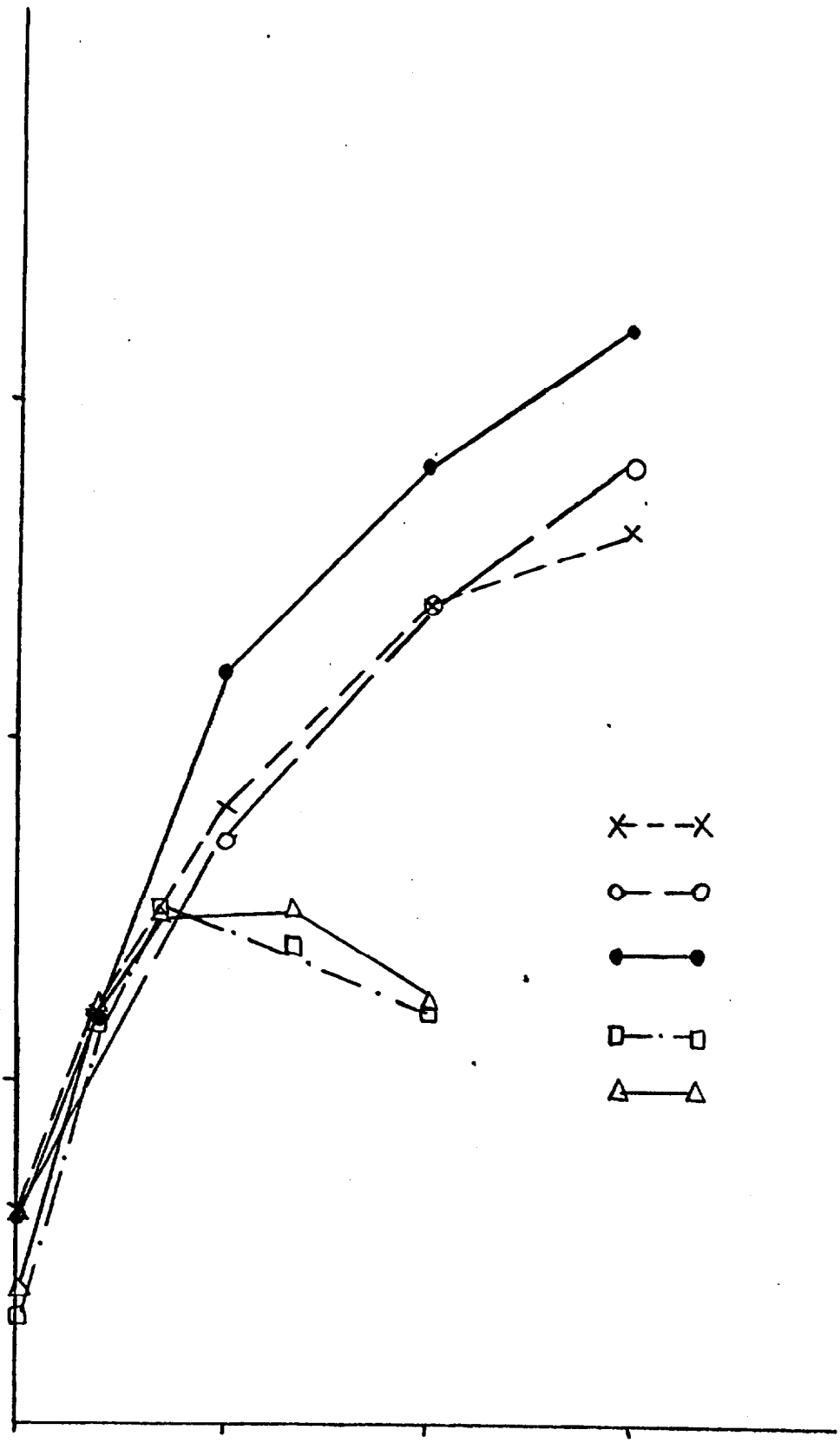

Fig. 1

Uptake of Synthetic Deoxyribopolynucleotides by Competent Cells of B. subtilis

Competent cells of B. subtilis SB893 (xantine$^-$, arginine$^-$, tryptophon$^-$) were prepared, stored and utilized as reported by . H^3 dAT or H^3 dGdC was added at a concentration of 0.1 γ/ml 8.5×10^7 cells to a cell suspension containing -

1. A = adenine, T = Thymidine added at 20 γ/ml.
2. Transforming DNA from wild tipe SB850 was used at a concentration of 3.5 ml of 0.1 γ/ml. 3.5 ml of competent cells were added to the mixtures of polynucleotides and incubated at 37°C in a rotary shaker. At the shown time intervals, 0.4 ml aliquots were filtered on Millipore filters and extensively washed with cold Spizizen medium. The filters were then dried and the radioactivity determined.

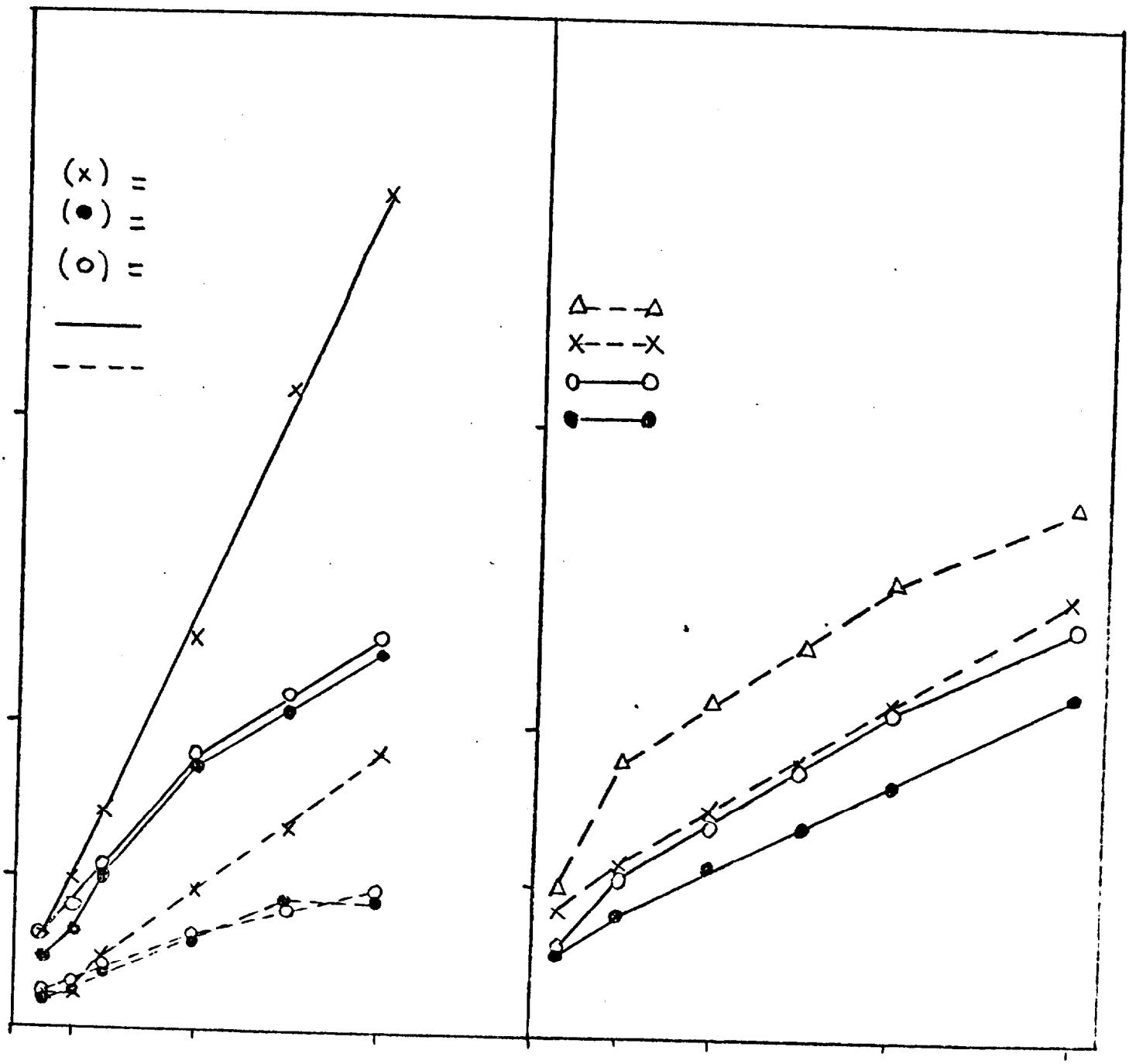
(x) =
(●) =
(o) =

Fig. 2

Stimulation of C^{14} Amino Acid Incorporation in the Presence of Synthetic Polyribonucleotides

Cells prepared as reported in Fig. 1 were washed and resuspended in Spizizen medium containing glucose without supplements.

xμc/ml of C^{14} amino acids (spec. act.) were added and at the shown time intervals 0.2 ml aliquots were withdrawn and pipetted in 1 ml of cold 10% TCA. After standing in the cold at least 30', samples were filted on Millipore, washed with 5% TCA, dried and counted. rU, rA and rU2A were added at a final concentration of 100 γ/ml.

TABLE II

Effect of the Addition of Synthetic Polyribonucleotides on Transformation

Polyribonucleotide concentration	% Transformation(try+)		
g/ml	rU	rA	rUrA
0	4.0	4.0	4.0
1	4.2	4.5	3.6
10	4.0	4.1	3.9
100	3.9	4.0	4.9

The competent cells were prepared as reported in Fig. 1). DNA was added at a concentration of 1γ/ml and the cells incubated at 37°C for 20'. The reaction was stopped by addition of 10 γ/ml of DNase and incubating for 10' at 37°C. Transformation was started by adding the cells to the mixture of the two nucleic acids.

TABLE III

Effect of the Order of Addition of rUrA on Transformation

rUrA Concentration γ/ml	% Transformation (try+) rUrA at -10 min.*	0 min.	+10 min.
0	2.28	1.25	1.12
10	2.50	0.93	0.74
100	2.05	1.22	0.67
500	1.84	0.94	0.87
1000	2.15	0.92	0.92

Experimental conditions are the same as those reported in Table II.

*Respect to the time of addition of DNA.

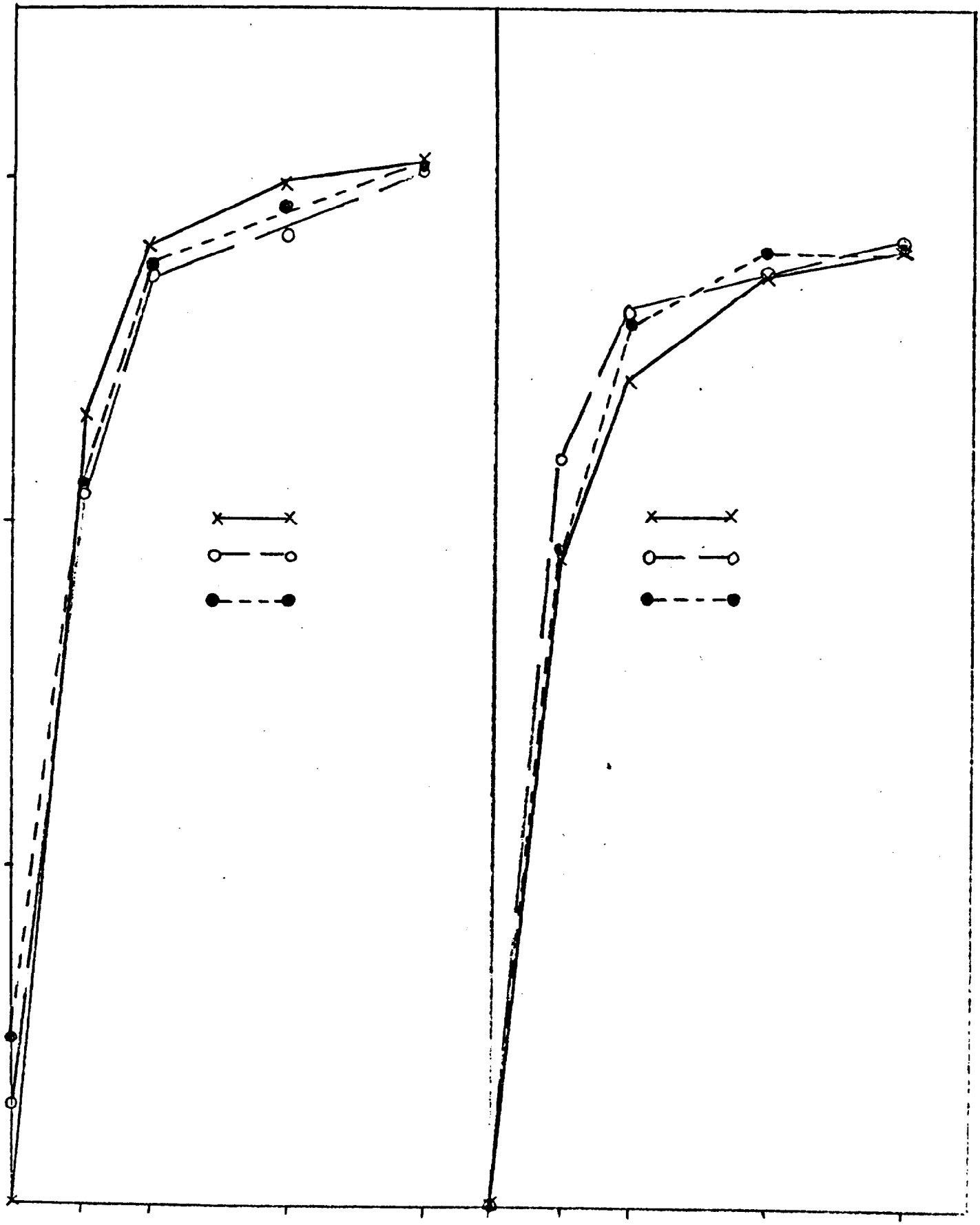

Fig. 3

Effect of the Addition of dCdG and DAT on Transformation

The competent cells were prepared as reported in Fig. 1). The concentration of DNA was 0.1 γ/ml of cell suspension. At the shown time intervals, 0.1 ml of cell suspension were withdrawn, incubated for 10' at 37°C in 0.4 ml Spizizen medium + 10^{-2}M Mg^{++} +5 γ/ml DNase. Tryptophan transformants were scored.