C H A P T E R II

INHIBITION OF DEOXYRIBONUCLEIC ACID SYNTHESIS IN ISOLATED PLANT MITOCHONDRIA BY CERTAIN POTENT INHIBITORS AND ENVIRONMENTAL CARCINOGENS

INTRODUCTION

Modification of the biosynthesis of Nucleic acids is a universal feature of developing chemical carcinogenesis (157). Such a disorder is also frequently associated with the action of mutagenic agents (158). Carcinogens and certain mutagens profoundly interact with nucleic acid to form stable adducts. Due to such interaction, the DNA molecule would be expected to be rendered functionally unsuitable for normal transcription. Alkylation of DNA may lead to blockage of the mitotic cycle, and consequently to inhibition of DNA replication (159), although additional mechanisms may assume contributory significance to this inhibition (160-162). In addition, alkylation of polynucleotides might make them unsuitable for use as a template, abolishing their amino acid incorporating capacity with resultant inhibition of protein synthesis. In fact, depression of nucleic acid synthesis coupled with defective protein-synthesizing capacity, caused by biological alkylants, has been variously demonstrated both *in vivo* and in cell-free preparations (163, 164). The carcinogenic potential and other detrimental effects of nitrosamines arise out of metabolic decomposition into methylcarbonium ions capable of alkylation of target cell DNA is supported by solid evidences (66, 73-82). With the probable exceptions of NTG and nitrosomethyl urea (NMU), it is presumed that all nitrosamines are precarcinogens requiring prior biotransformation to enable DNA methylation and to exert carcinogenic action. The metabolic conversion of dimethylnitrosamine (DMN)

is known to involve primary ∝-C-hydroxylation with subsequent release of formaldehyde to form an unstable intermediate, this then degrades spontaneously into the alkylating ion, the final proximate carcinogen. Diethylnitrosamine suffers a very similar metabolic attack resulting in formation of acetaldehyde and ethylcarbonium ions (66).

The first nitrosocompound which proved to be able to produce chromosome aberrations was N-nitroso-N-methylurethan (165). The chromosome-breaking effect of NTG was studied by Gichner et al. (166) using root-tips of Vicia faba of experimental material. The aberrations obtained were only of the chromatid type. It also causes a delayed effect. Both the toxic and the chromosome breaking effects of NTG were enhanced by low pH.

NTG is also a well known mutagen for bacteria and has been found to mutate selectively the replicating regions of DNA (167, 168). Both the polymerase and associated exonuclease activity of Pol III, the replicating complex in E. coli, are inactivated by the treatment with nitrosoguanidine in vitro. However, the treatment of the enzyme complex Pol III with nitrosoguanidine does not reduce the accuracy of DNA synthesis by the enzyme (169).

The use of inhibitors as tool in the investigation of cellular processes is well known in biochemical research and has been particularly useful in the characterization of macromolecular biosynthesis. As mentioned earlier NTG is a potent mutagen (167-169) as well as a carcinogen (170), but its use as a tool

in the investigation of cellular biosynthetic processes (123, 125) has so far been very limited because its mode of action is yet poorly understood. It has recently been reported that NTG inhibits the protein synthesis in cell-free preparations of rat liver (123, 125). Strand breaks of mammalian mitochondrial DNA has also recently been reported to be induced by NTG and 4-nitroquinoline 1-oxide (4 NQO). Of the two carcinogens NTG caused more strand scission of mitochondrial DNA than 4 NQO at the same concentration (171).

Inhibition of mitochondrial DNA (mt-DNA) synthesis in mouse liver by a carcinogenic mycotoxin, aflatoxin B_1 and DMN has been reported by Friedman et al. Both these carcinogens induced 50% decrease in mt-DNA synthesis acutely, with a 50% decrease in synthesis of high-molecular weight mt-DNA (126). In vitro and in vivo effects of dimethylnitrosamine (DMN) on mouse liver mitochondrial function was also investigated earlier by these workers (172).

A variety of agents bind to the template and thus can function as inhibitors of DNA synthesis. This group includes a wide spectrum of antibiotics e.g. ethidium bromide, actinomycin D, distamycin, neomycin and acridine dyes. Many of these antibiotics intercalate into the DNA double halix rather than interact covalently with DNA. In addition, these drugs may be metabolically activated within the cell. Thus, in common with chemical carcinogens, the active agent may be a cellular metabolite (173). Pyridoxal phosphate may be an inhibitor that can be used to identify the substrate site on the enzyme. Pyridoxal phosphate

inhibits mammalian DNA polymerases α and γ, E. coli, DNA polymerase I and the viral DNA polymerase as reported by Modak (174). I-β-D-arabinofuranosyl cytosine triphosphate is also a well studied inhibitor of DNA polymerase (173). Phosphonoacetate is also a highly specific inhibitor of Herpes simplex virus DNA polymerase (175).

Absence of information related to the effects of carcinogens on plant mitochondria prompted us to initiate studies on the in vitro effects of dimethylnitrosamine (DMN), diethylnitrosamine (DENA) and N-methyl N'-nitro-N-nitrosoguanidine (NTG) on DNA synthesis by isolated mitochondria from germinating seeds of Vigna sinensis (Linn.) Savi. In addition, effects of some well known inhibitors of DNA synthesis were also investigated in this plant system.

MATERIALS

Seed :

Vigna sinensis (Linn.) Savi seeds were purchased from the local market.

Chemicals :

Cold deoxyribonucleoside triphosphate (dNTP) were obtained from P-L. Biochemicals, U.S.A. Radioactive deoxynucleotides were purchased from New England Nuclear Corp. or Amersham, Inc. ATP, PEP, DTT, N-methyl-N'-nitro-N-nitrosoguanidine (NTG), dimethyl-

sodium pyrophosphate was added to it for reprecipitation. The cold acid insoluble material was taken out onto glass fibre filter paper discs and radioactivity was determined in a Liquid Scintillation spectrometer as described elsewhere (112-115).

Uptake of [^{3}H]-dNTP by the isolated intact Plant Mitochondria :-

The incubation mixture and reaction conditions were same as described above. The reaction was stopped by the addition of excess cold dNTP followed by the addition of 5 ml ice-cold buffer (0.05 M Tris-HCl pH 7.4 containing 0.25 M sucrose). The incubation mixture was rapidly filtered through the discs of millipore filter (0.45 μ) and washed further with 15 ml of the same buffer. The millipore discs were then dried and radioactivity was determined in a Liquid Scintillation spectrometer (112, 115).

Determination of Protein and DNA :-

Protein was estimated by the method of Lowry *et al.* (176) and DNA was determined by the diphenyl amine method (177).

RESULTS AND DISCUSSION

That protein and DNA synthesis by isolated plant mitochondria is not due to bacterial contamination, was earlier reported from this laboratory and also by several group of workers (36-37, 178). Although artifact arising out of bacterial contamination in mitochondrial protein synthesis has been completely ruled out by

independent group of workers long ago, nucleotide incorporation into DNA by isolated plant mitochondria was once again reinvestigated based on the selective permeability of radioactive precursors through bacterial and mitochondrial membrane.

Plant mitochondrial DNA synthesis was investigated by allowing the seeds to germinate, after surface sterilization, in presence of 0.0001% chloramphenicol, followed by maximum possible aseptic condition during the entire process. Mitochondria were isolated using buffer prepared by sterile distilled water in sterile containers.

Incorporation of [^{3}H]-deoxythymidine monophosphate (dTMP) into plant mitochondrial DNA was therefore analysed to differentiate it with bacterial DNA synthesis, if any, using the same plant mitochondrial preparation, [^{3}H]-dTTP and [^{3}H]-thymidine as precursors, in two separate experiments. It is clear from Figure 11 that in comparison to [^{3}H]-dTTP, there is three times lesser incorporation of radioactivity into acid insoluble material (mitochondrial DNA) when [^{3}H]-thymidine is used as DNA synthesis precursor, inspite of the fact that the specific activity of [^{3}H]-thymidine used was higher than that of [^{3}H]-dTTP. It should be noted that the distinguishing feature of the precursors used were based on the fact that [^{3}H]-dTTP penetrate bacterial cell membrane even more slowly than [^{3}H]-thymidine due to their catabolism during entrance within the cell. Therefore, [^{3}H]-thymidine is more easily incorporated into the bacterial DNA (179). On the other hand, the rate of incorporation into acid insoluble material

is higher when [^{3}H]-dTTP is used as precursor rather than [^{3}H]--thymidine in case of plant mitochondria (Fig. 11). It is therefore indicative that the observed three times lesser incorporation into DNA when [^{3}H]-thymidine is used as precursor, is due to plant mitochondrial DNA synthesis and not due to any bacterial contamination in the mitochondrial preparation.

Secondly, 1-β-D-arabinofuranosyl cytosine triphosphate (Ara-CTP) which is a potent antileukaemic agent is also known to specifically inhibit DNA synthesis in bacterial and animal cells (180). Inhibition of DNA synthesis in mitochondrial preparation was therefore analyzed in presence of Ara-CTP. It is interesting to note that ^{3}H-dGMP incorporation into DNA by plant mitochondrial preparation is not inhibited by Ara-CTP (Table 13), also indicating thereby that the incorporation represents true mitochondrial DNA synthesis.

Thirdly, during isolation of plant mitochondria if the temperature is not strictly maintained between 0-4^{o}C, the DNA synthesizing capacity of the preparation, measured using both [^{3}H]-dNTP and [^{3}H]-thymidine as precursors, is completely impaired. Had this been due to bacterial contamination, some DNA synthesis would have been expected. Since mitochondrial DNA polymerase is highly sensitive to temperature, this data on temperature sensitivity could also be taken as an additional proof to establish that the DNA synthesizing capacity of plant mitochondria is indeed its intrinsic activity and is not due to any resultant bacterial contamination.

Isolated mitochondria from 48 h etiolated seeds of Vigna sinensis when incubated with radioactive deoxyribonucleoside triphosphate (dNTP) can incorporate radioactivity in acid insoluble material which has been found to be resistant to alkaline hydrolysis, but hydrolyzed into acid soluble product by hot trichloroacetic acid or perchloric acid and pancreatic deoxyribonuclease, as suggested in Figure 12. The incorporation of radioactive dNTP into acid-insoluble material is increased with the increase in time of incubation (Figure 13). Hence, this incorporation of radioactive dNTP in acid-insoluble product truly represents the in vitro DNA synthesis by isolated plant mitochondria.

Table 12 represents the basic requirement for [^{3}H]-dNMP incorporation into mitochondrial DNA by isolated plant mitochondria. These results indicate that for optimum incorporation of [^{3}H]-dTMP, external addition of other deoxynucleoside triphosphate and Mg^{++} are not essential, suggesting the presence of an intramitochondrial pool of these precursors and cofactors. Plant mitochondrial DNA synthesis was further characterized by some potent inhibitors of DNA synthesis (Table 13). It is evident from the results obtained that the incorporation of ^{3}H -dGMP into mitochondrial DNA is inhibited by the addition of ethidium bromide, an intercalating agent which inhibits the DNA strand separation during replicative DNA synthesis (181). Among the compounds belonging to this group, ethidium bromide is of special interest. Unlike its effect on rat liver mitochondrial DNA synthesis, the concentration of ethidium bromide required to

inhibit plant mitochondrial DNA synthesis is relatively high. This interclating agent has been known to cause cytoplasmic mutation and appears to selectively inhibit mitochondrial DNA synthesis in eukaryotic cells (182). It is possible that its effect is via the mitochondrial DNA polymerase. Reactions catalyzed by isolated rat liver mitochondrial DNA polymerase have been shown to be inhibited by this drug under conditions at which the nuclear enzyme is resistant (183).

Pyridoxal phosphate (PALPO) an inhibitor of <u>in vitro</u> DNA synthesis catalyzed by various DNA polymerases (174) has got practically no effect on mitochondrial DNA synthesis. At about 2 mM concentration, PALPO inhibits mitochondrial DNA synthesis by only 17% (Table 13). Pyridoxal phosphate may be an inhibitor that can be used to identify the substrate site on the enzyme. The effect of this compound appears to be mediated through formation of a classical Schiff base with lysine or arginine residues on or around the active site. The inhibitory effect of pyridoxal phosphate is competitive with the complementary substrate as well as partially competitive with non-complementary substrate. This Schiff base can be stabilized by $NaBH_4$ and thus provides a probe for designating the amino acids at the active site (174).

Phosphonoacetic acid (PAA) is a highly specific inhibitor for Herpes simplex virus induced DNA polymerase (175, 184) but at similar concentration it does not inhibit cellular DNA polymerase. It has been hypothesized that the inhibitory effect of phosphonoacetic acid is primarily due to inhibition of the

elongation step by the Herpes induced polymerase (175). Phosphonoacetic acid (0.2 mM) has no effect or plant mitochondrial DNA synthesis (Table 13). However, at higher concentration (0.5 mM) only 42% inhibition of mitochondrial DNA synthesis was observed.

1-β-D-arabinofuranosyl cytosine triphosphate (Ara-CTP) an antileukemic drug as well as inhibitor of DNA synthesis (180) appears to have no effect on plant mitochondrial DNA synthesis *in vitro* (Table 13). Fox *et al.* compared the effect of ara-CTP on the activities of DNA polymerase α and β from a diploid cell line of cultured human lymphocytes. They observed that DNA polymerase-α is more readily inhibited by ara-CTP (185). This preferential inhibition has been confirmed by different groups of workers using DNA polymerase-α and β from a variety of sources (186, 187). Since the plant mitochondrial DNA synthesis is insensitive to Ara-CTP, possibly plant mitochondrial DNA polymerase is resistant to this drug. Unfortunately, no work appears to have been done on the effect of Ara-CTP on mitochondrial polymerase (184).

From Table 14 it is evident that [^{3}H]-dGMP incorporation into mitochondrial DNA is inhibited by NTG even at a very low concentration; at 0.4 mM concentration there is about 87% inhibition of DNA synthesis. This inhibition by NTG can be explained at two levels - firstly the uptake of dNTP by plant mitochondria is perhaps inhibited and secondly inhibition of the mitochondrial DNA polymerase may take place or a cumulative effect of both the

processes. Further investigation was therefore carried out to explain this particular point. The effect of NTG on the uptake of radioactive dGTP by isolated plant mitochondria (Figure 14) revealed that indeed in presence of NTG there is an inhibition in the uptake of radioactive dGTP. Moreover, the incorporation of dGMP into mitochondrial DNA is also inhibited from the early stage of incubation (Figure 14). Hence, the apparent inhibition of plant mitochondrial DNA synthesis by NTG could be due to the alteration in the permeability of the mitochondrial membrane.

In an attempt to elucidate further the mode of action of NTG the isolated plant mitochondria were pretreated with 0.4 mM NTG, washed and used for incorporation studies, as discussed in the earlier Chapter. Similar to previously obtained results, these results (Figure 15) also suggested that NTG somehow makes loose complex with mitochondrial membrane component, possibly with lipopolysaccharide part (121, 122).

It is well known that a decrease of respiratory activity and mitochondrial protein content is observed concomitantly with carcinogenesis, and some atypical mitochondria are observed in cancer tissue. NTG and 4 NQO which are powerful carcinogens and mutagens, produce non-chromosomal mutations resulting in the loss of ability to form chloroplast in Euglena (188) and the respiratory-deficient mutant of Saccharomyces (189, 190). Strand scission of nuclear DNA in mammalian cells and bacteria by NTG and 4 NQO has been described (191-193) but the effect of these chemicals on mammalian mitochondrial DNA is not clear. An inducer of

Table 13

Incorporation of [^{3}H]-dGMP and [^{3}H]-dAMP into mitochondrial DNA in isolated plant mitochondria : effect of inhibitors of deoxyribonucleic acid synthesis

Assay system	cpm/assay system
Complete (a)*	15,209
" + EtBr (25 µgm)	1,181
" + EtBr (50 µgm)	233
Complete (a)*	12,032
" + PALPO (1 mM)	12,437
" + PALPO (2 mM)	9,981
Complete (a)*	3,550
" + PAA (0.1 mM)	3,454
" + PAA (0.2 mM)	3,511
" + PAA (0.5 mM)	2,057
Complete (b)**	15.8† pmoles
" + Ara CTP (5 mM)	19.4† pmoles
" + Ara CTP (10 mM)	18.3† pmoles

Complete incubation system and determination of ^{3}H dNMP incorporation are same as described in "Methods".

(a)* Represent [^{3}H]-dGMP incorporation. Specific radioactivity of [^{3}H]-dGTP used was 7000-8000 cpm/pmole.

(b)** Represent [^{3}H]-dAMP incorporation. Specific radioactivity of [^{3}H]-dATP used was 14,300 cpm/pmole.

† Data expressed in pmoles incorporated per assay system.

Table 14

Effect of different concentration of N-methyl-N'-nitro-N-nitrosoguanidine (NTG) on [^{3}H]-dGMP incorporation by isolated plant mitochondria

Assay system	pmoles of [^{3}H]-dGMP incorporated/mg DNA	% Inhibition
Complete	3.25	0
" + NTG (0.4 mM)	0.41	87
" + NTG (0.8 mM)	0.314	90
" + NTG (1 mM)	0.266	92

Reaction mixture, incubation condition and measurement of radioactivity incorporated were same as described in 'Methods'. Each assay was performed in presence of 0.25 μM [^{3}H]-dGTP (specific activity 7000-8000 cpm/pmole) and NTG was added to each of the assay system in specified amount.

Table 16

[^{3}H]-dGMP incorporation into mitochondrial DNA by isolated plant mitochondria : effect of varying concentration of dimethylnitrosamine (DMN)

Assay system	pmoles of [^{3}H]-dGMP incorporated/mg DNA	% Inhibition
Complete	3.14	0
" + DMN (1 µg)	2.9	6
" + DMN (2 µg)	1.18	63
" + DMN (5 µg)	0.32	89
" + DMN (10 µg)	0	100

Experiment was carried out as indicated in Table 14 except that DMN was added.

Figure- 11

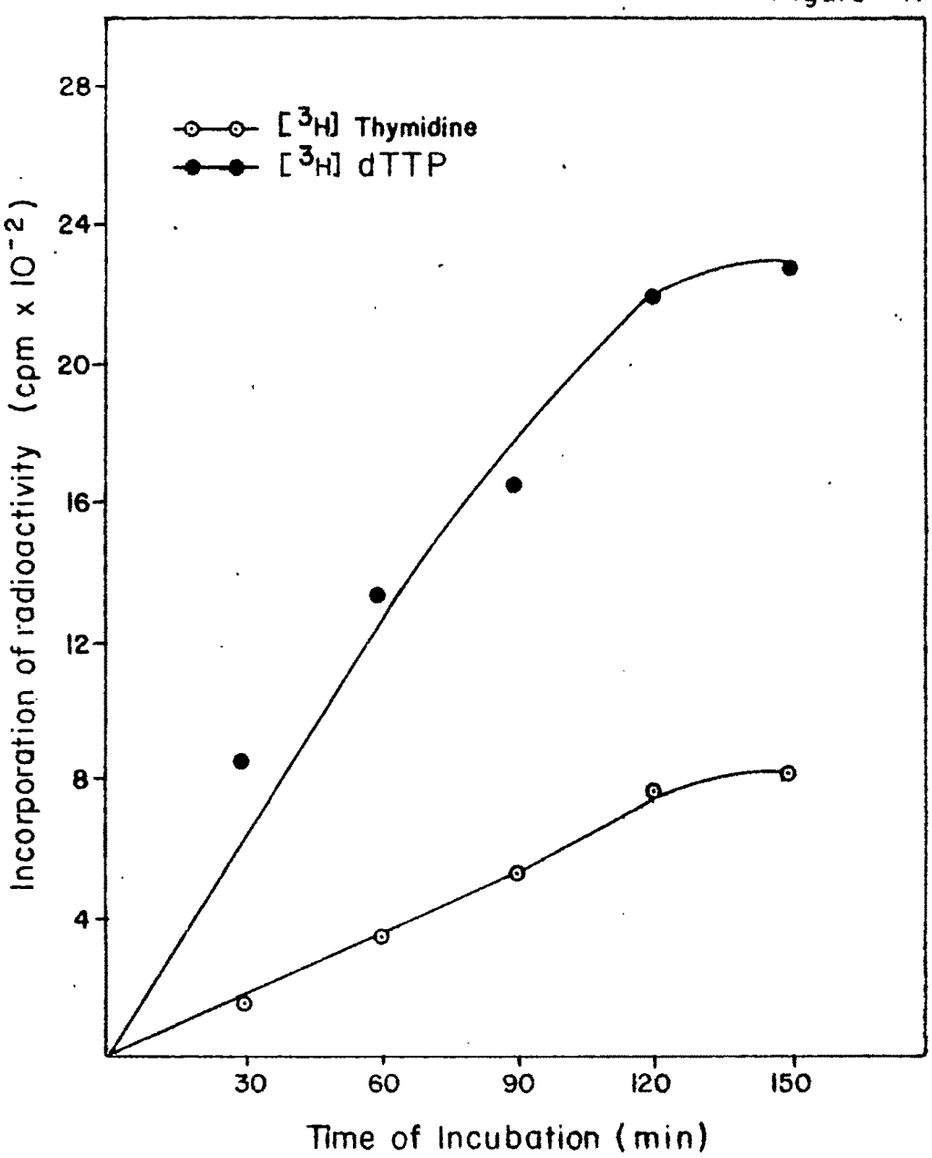

[3H] Thymidine
[3H] dTTP
Incorporation of radioactivity (cpm x 10-2)
28
24
20
16
12
8
4
30
60
90
120
150
Time of Incubation (min)

Figure-12

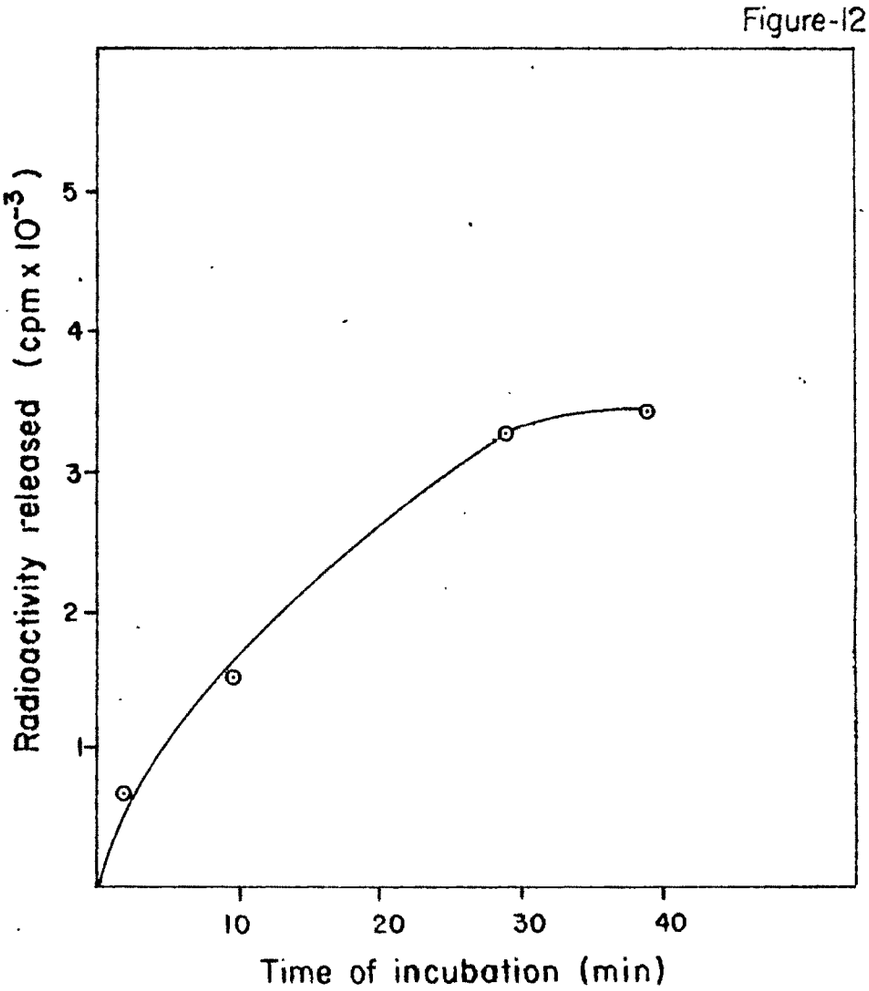

Radioactivity released (cpm x 10^{-3})
5
4
3
2
1
10
20
30
40
Time of incubation (min)

Figure- 13

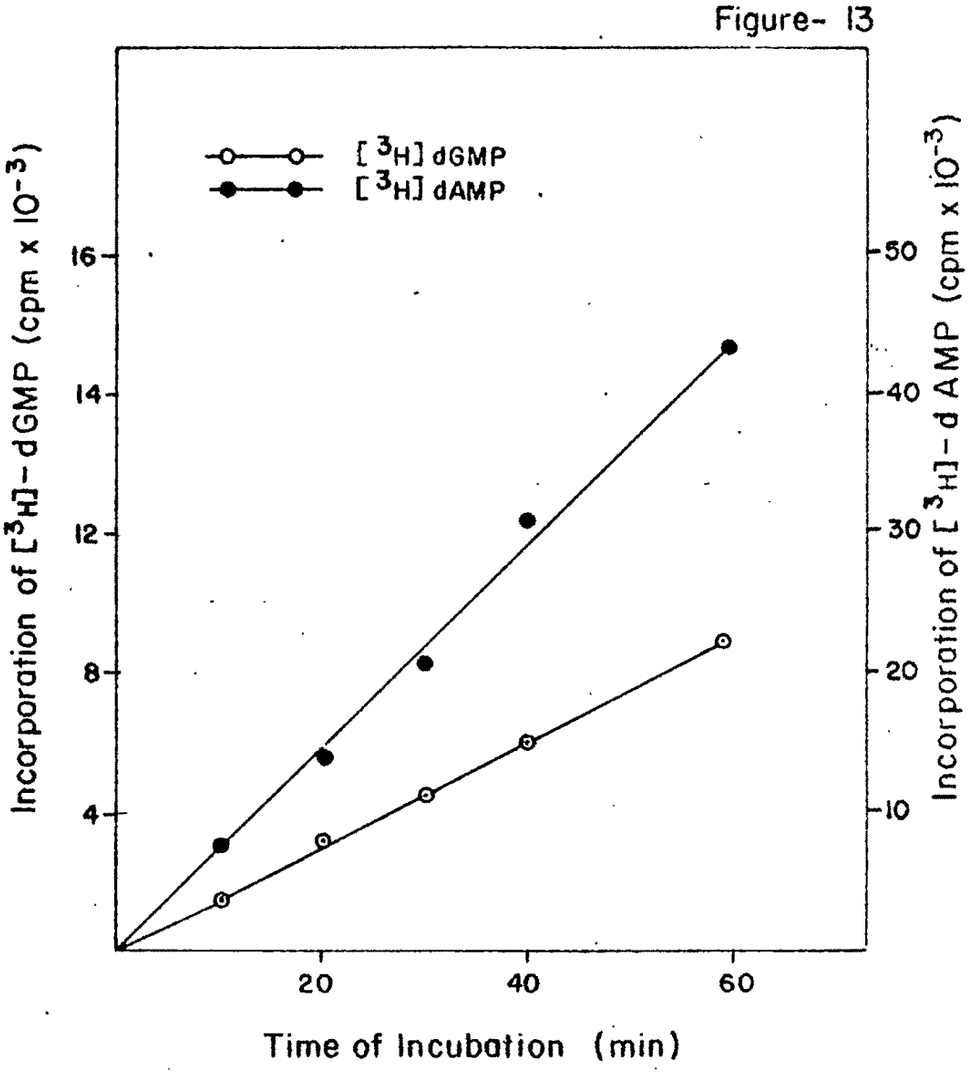

[3H] dGMP
[3H] dAMP
Incorporation of [3H]-dGMP (cpm x 10-3)
Incorporation of [3H]-d AMP (cpm x 10-3)
16
14
12
8
4
50
40
30
20
10
20
40
60
Time of Incubation (min)

Figure-14

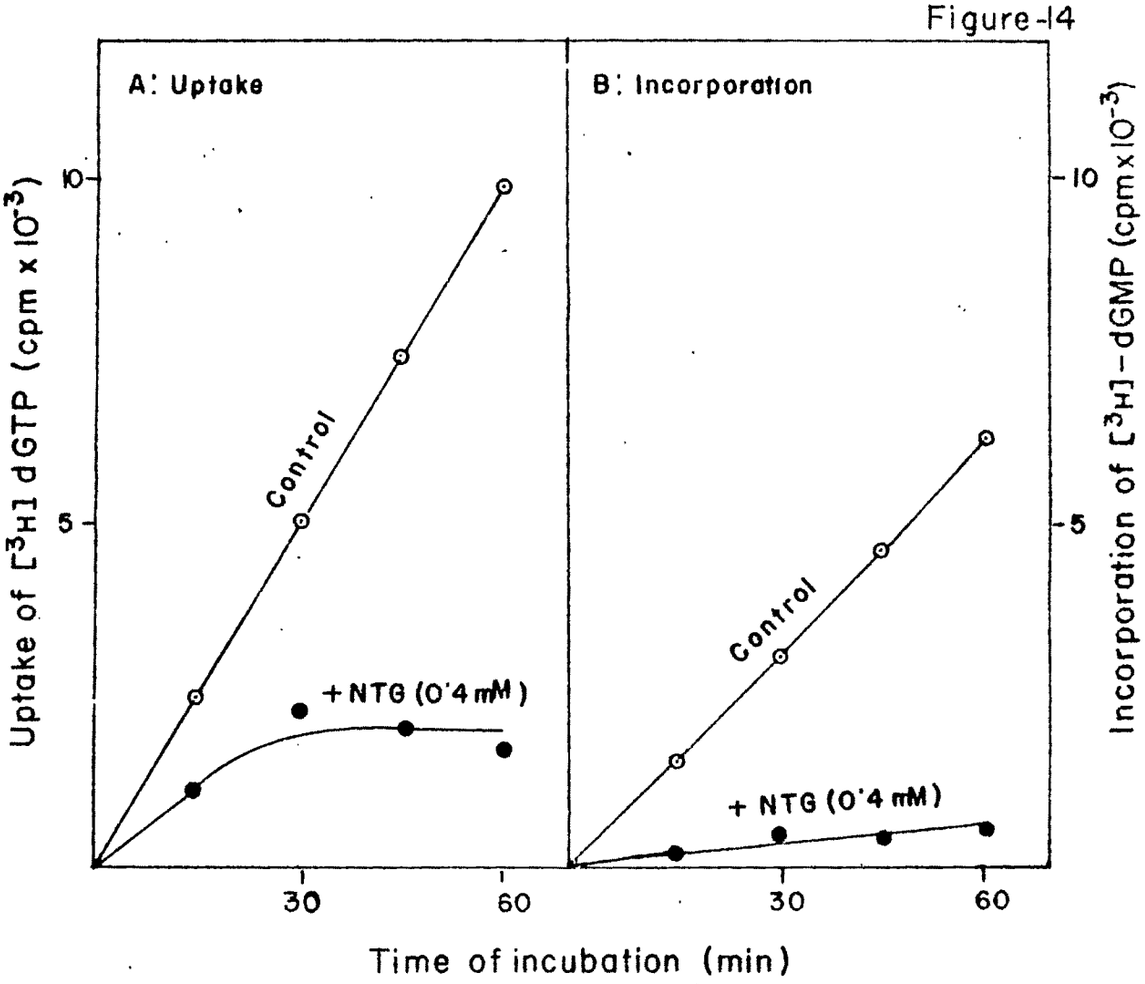

A: Uptake
B: Incorporation
Uptake of [3H] dGTP (cpm x 10-3)
Incorporation of [3H]-dGMP (cpm x 10-3)
10
5
Control
+ NTG (0·4 mM)
30
60
Time of incubation (min)

Figure-15

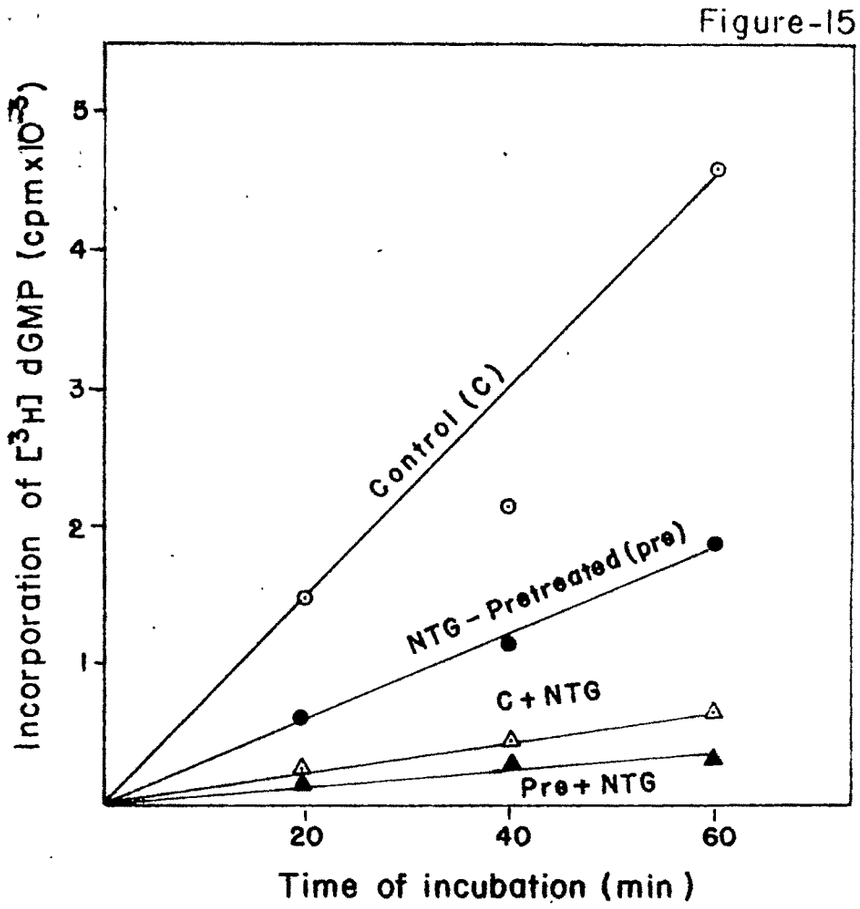

Incorporation of [3H] dGMP (cpm x 10-3)
5
4
3
2
1
Control (C)
NTG - Pretreated (pre)
C + NTG
Pre + NTG
20
40
60
Time of incubation (min)

Figure-16

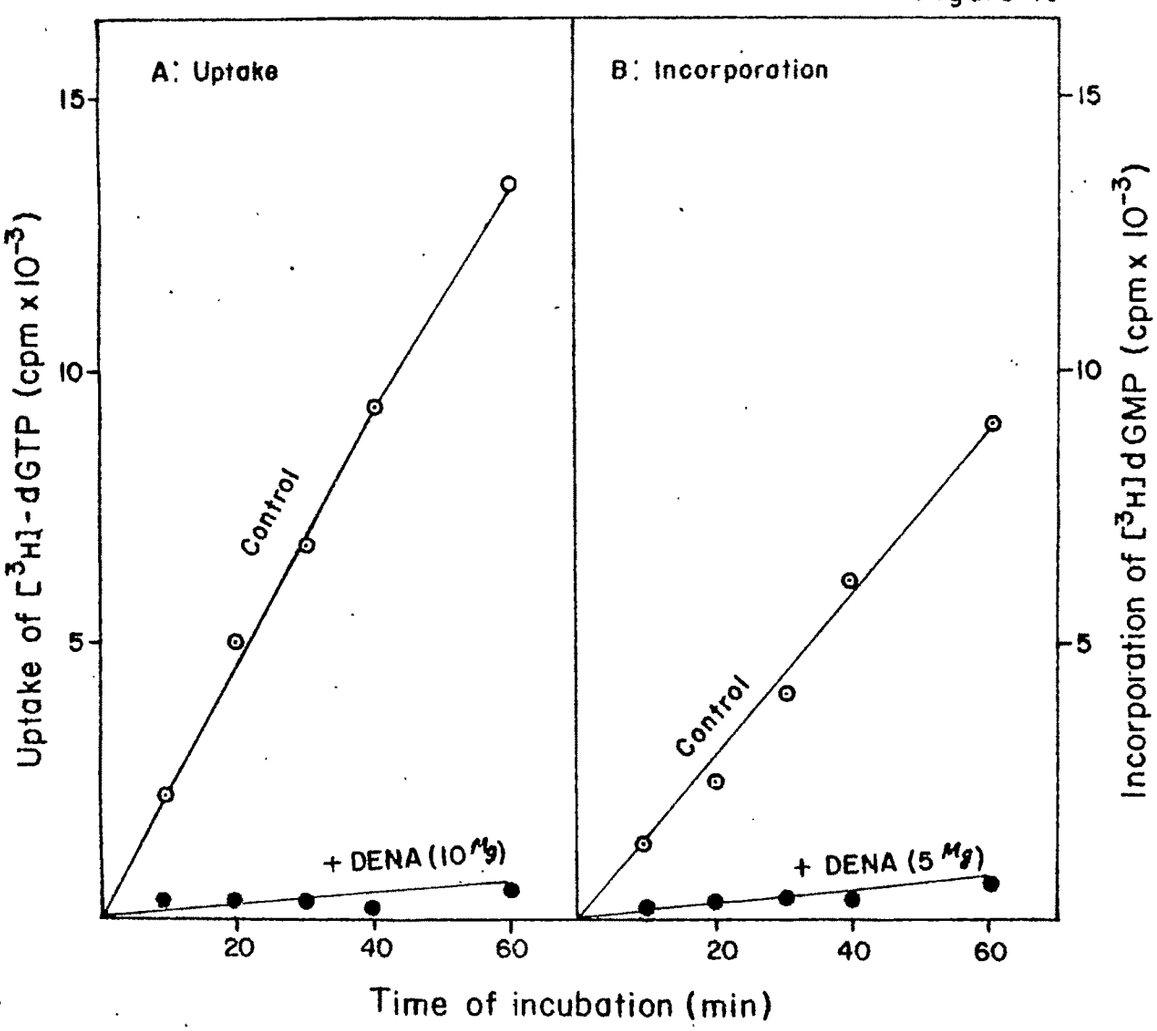

A: Uptake
B: Incorporation
Control
+ DENA (10 Mg)
Control
+ DENA (5 Mg)
Uptake of [3H]-dGTP (cpm x 10-3)
Incorporation of [3H]dGMP (cpm x 10-3)
Time of incubation (min)
15
10
5
20
40
60

Figure - 17

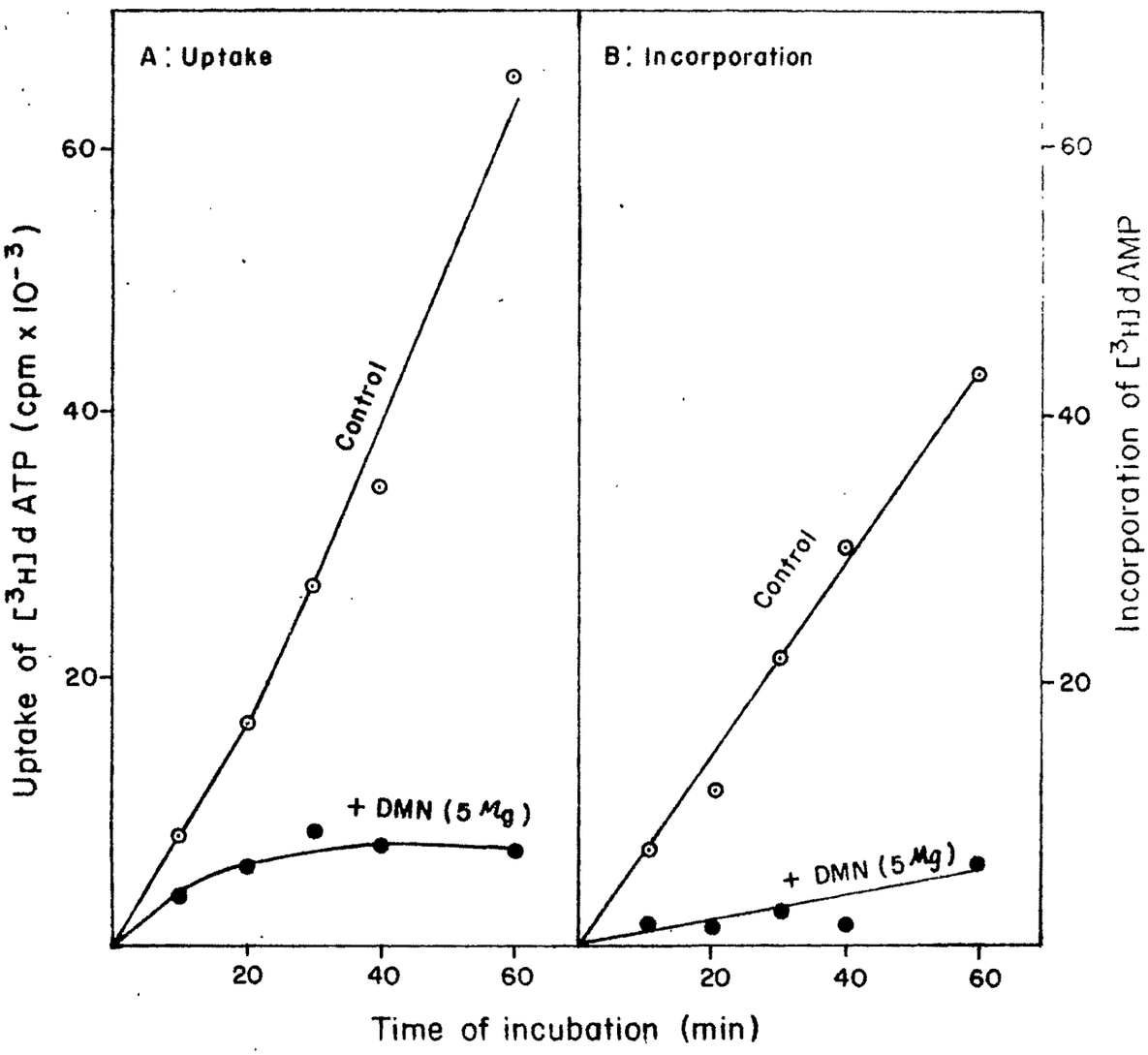
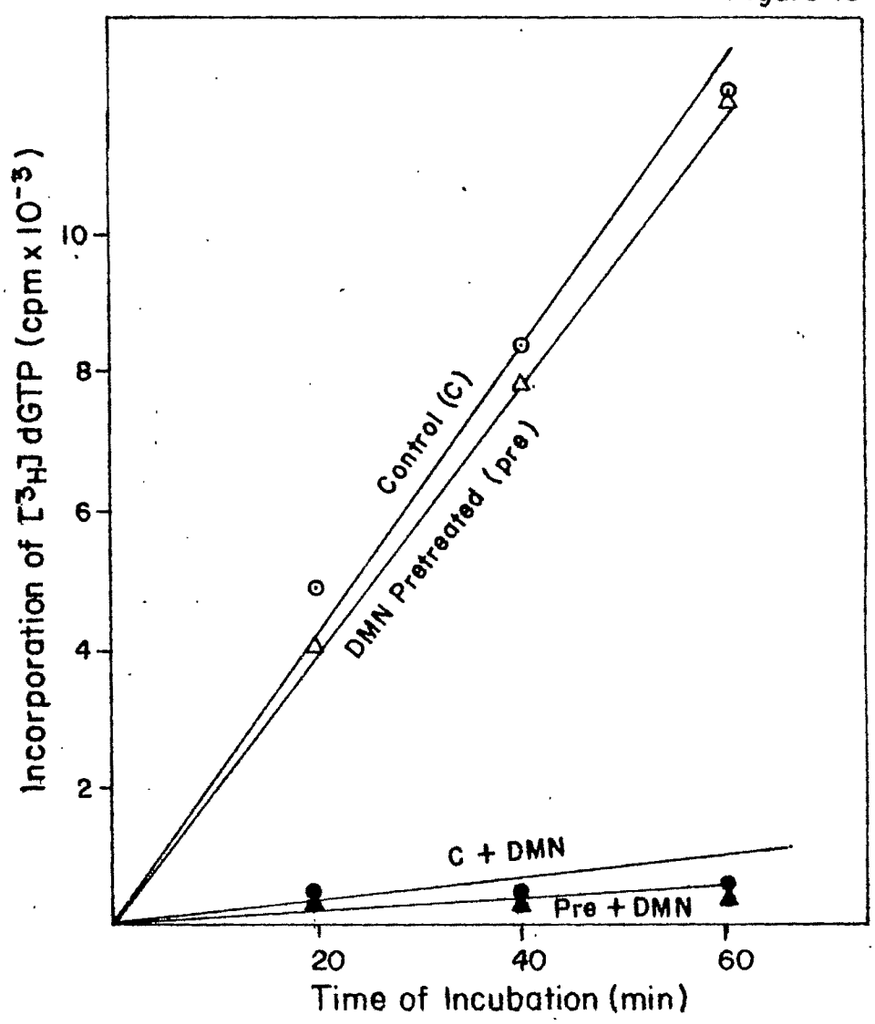

A : Uptake
B : Incorporation
Control
+ DMN (5 Mg)
Control
+ DMN (5 Mg)
Uptake of [3H] d ATP (cpm x 10-3)
Incorporation of [3H] dAMP
Time of incubation (min)
20
40
60
20
40
60
20
40
60
20
40
60

Figure-18


Incorporation of [3H] dGTP (cpm x 10-3)
10
8
6
4
2
Control (C)
DMN Pretreated (pre)
C + DMN
Pre + DMN
20
40
60
Time of Incubation (min)

LEGEND TO FIGURE

Fig. 11 : Time course for the incorporation of [^{3}H]-thymidine and [^{3}H]-dTTP into DNA by isolated plant mitochondria. Complete incubation system for [^{3}H]-thymidine incorporation contained in a total volume 0.3 ml, 0.25 M sucrose, 0.05 M Tris-HCl pH 7.4, 5 mM ATP, 8 mM $MgCl_2$, [^{3}H]-thymidine of specific activity 13875 cpm/pmole. Complete incubation system for [^{3}H]-dTTP was same as described earlier. Specific activity of [^{3}H]-dTTP was 11000 cpm/pmole.
Incubation was carried out for different time as indicated in Fig. 11 and incubation condition and radioactivity were same as described under 'Methods'.

Fig. 12 : Digestion of prepared mitochondrial DNA labelled with [^{3}H]-dATP by pancreatic DNase I. Incorporation of [^{3}H]-dATP into plant mitochondrial DNA was carried out using reaction mixture (total volume 0.8 ml) containing 50 mM Tris-HCl buffer pH 7.4, 5 mM $MgCl_2$, 0.25 mM PEP, 0.25 µM ATP, 12.5 µM dGTP, dCTP and dTTP and 0.25 µM [^{3}H]-dATP (16,000 - 20,000 cpm/pmole) and 2 mg mitochondrial protein. Reaction was stopped by chilling the assay tubes and adding 2 ml cold 10% PCA. Then PCA insoluble material was centrifuged and pellet dissolved in small volume of 2(N) NaOH and neutralized with HCl. Effect of pancreatic DNase on this labelled

mitochondrial DNA was carried out using in the incubation system half of the prepared labelled DNA (150 λ), 5 μg pancreatic DNase, 50 mM Tris-HCl buffer pH 8.0, 5 mM $MgCl_2$. Incubations were carried out at 37°C and at different time intervals from these mixture, 100 λ was taken in 0.5 ml cold 10% PCA. For the blank 100 λ was taken in 0.5 ml cold 10% PCA before addition of pancreatic DNase. To each system 0.2 ml calf thymus DNA (1 mg/ml) was added to each tube to ensure co-precipitation. Each tube was centrifuged and 100 λ acid soluble material was taken into vials containing aquasol for Liquid Scintillation Counting.

Fig. 13 : Time course for the incorporation of [^{3}H]-dNMP into DNA by isolated plant mitochondria. Isolated mitochondria were incubated with either [^{3}H]-dGTP (specific activity 7000 - 8000 cpm/pmole) or [^{3}H]-dATP (specific activity 18000 - 20000 cpm/pmole). Reaction mixture, incubation conditions, and determination of radioactivity into acid insoluble material were same as described in 'Methods'.

Fig. 14 : Uptake and incorporation of [^{3}H]-dGTP and [^{3}H]-dGMP respectively by isolated intact plant mitochondria : effect of N-methyl-N'-nitro-N-nitrosoguanidine (NTG) on the process. The complete incubation system and radioactive processing for uptake and incorporation study have been described in 'Methods'. Specific activity

of [^{3}H]-dGTP was 7000 - 8000 cpm/pmole.

Symbols : Control (—o—o—) and Control + 0.4 mM NTG (—●—●—).

Fig. 15 : Partial reversal of N-methyl-N'-nitro-N-nitrosoguanidine (NTG) induced inhibition of [^{3}H]-dGMP incorporation by NTG pretreated mitochondria after thorough washing. Isolated plant mitochondria (10 mg protein) were preincubated with 0.25 M sucrose, 10 mM Tris-HCl pH 7.4 and 200λ NTG (8 mM) for 15 min at 37°C. The pretreated mitochondria thus obtained were washed twice with isotonic buffer by centrifugation at 10,000 x g in cold and subsequently 500 µg protein used for incorporation studies using [^{3}H]-dGTP (7000 - 8000 cpm/pmole). Mitochondria preincubated without NTG and processed similarly were used as control in the experiment.

Symbols : Control (—o—o—), NTG-pretreated (—●—●—), Control + 0.4 mM NTG (—▲—▲—) and NTG--pretreated + 0.4 mM NTG (—▲—▲—). Incubation system and determination of radioactivity were same as described in 'Methods'.

Fig. 16 : Effect of diethylnitrosoamine on uptake and incorporation of [^{3}H]-dGTP and [^{3}H]-dGMP respectively by isolated plant mitochondria. The complete incubation system and radioactive processing for uptake and incorporation study were described in 'Methods'. Specific activity

of [^{3}H]-dGTP was 7000 - 8000 cpm/pmole.
Symbols : Control (—○——○—) and Control + DENA (—●——●—)

Fig. 17 : Effect of dimethylnitrosøamine on uptake and incorporation of [^{3}H]-dATP and [^{3}H]-dAMP respectively by isolated plant mitochondria. The complete incubation system and radioactive processing for uptake and incorporation study were described in 'Methods'. Specific activity of [^{3}H]-dATP was 18,000 - 20,000 cpm/pmole.
Symbols : Control (—○——○—) and Control + DMN (—●——●—).

Fig. 18 : Reversal of dimethylnitrosøamine (DMN) induced inhibition of [^{3}H]-dGMP incorporation by DMN (5 µg) pretreated mitochondria after thorough washing. For pretreatment experiment isolated mitochondria (10 mg protein) were preincubated with 0.25 M sucrose, 10 mM Tris-HCl pH 7.4 and 20 µg DMN for 15 min at 37°C and washed twice with isotonic buffer by centrifugation at 10,000 x g in cold and from these 500 µg protein were subsequently used for incorporation of [^{3}H]-dGMP (specific activity 7000 - 8000 cpm/pmole). Mitochondria preincubated without DMN and processed similarly were used as control in the experiment. Control (—○—○—), 10 µg DMN--pretreated (—△—△—), Control + 5 µg DMN (—●——●—) and DMN pretreated + 5 µg DMN (—▲—▲—). Assay system and determination of radioactivity were same as described earlier.