# THE EFFECT OF 4-NITR0QUIN0LINE 1-OXIDE ON THE UPTAKE AND INCORPORATION OF SELECTED AMINO ACIDS BY LACTOBACILLUS CASEI

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## ABSTRACT

Utilizing Lactobacilling casei cells an experimental teat system was developed to measure the effect of varying concentrations of the potent, water-soluble, oncogen 4-nitroquinoline 1-oxide (4-4Q0) on amino acid uptake and Incorporation by the bacteria. The rates of uptake, incorporation, and removal of tritium labeled 4-NQ0 were measured. Clycine-$^{14}$C, L-alauine-$^{14}$C, L-methionine-$^{14}$C, and L-tyrosine-$^{14}$C standard uptake and Incorporration measurements wore also taken.

The labeled aaiino acids all displayed a rapid Initial uptake with saturation being almost completed after 180 seconds. The 4-UQ0-$^{3}$H uptake was initially rapid, reaching a maximum limit after approximately 60 seconds, after which an equally rapid renoval of tritium label was experienced. After 180 seconds from the time of oncogen introduction the bacteria had returned to a bound oncogen label approximately one sixth of the maximum level.

The standard incorporated amino acid curves showed a time dependency with maximum levels being reached after 10 minutes. Incorporated oncogen showed an initial level that was approximately twice as high as the level measured after 180 or 600 seconds.

Of the amino acids tested L-alanine-$^{14}$C was the most Sensitive to 4-NQ0 levels. When C.0167 mM 4-NQ0 was introduced

Into the test system reduction of L-alanine-$^{14}$C uptake was noted. Incorporation of the same amino acid was stimulated.

In general, the same results were shown for the other amino acids though they we re less sensitive to the presence of oncogen. An alteration in the amino acid incorporation curves, deaonst rating an apparent dependence on the amino acid concentration in the cell and not on time , was noted in all of the data.

From the data presented a mechanists for the alteration of biological systems by 4-nitroquinollne l-oxide was given. The reasoning suggests that an alteration of the messenger ribonucleic acid may be the point of initial metabolic change.

Other known oncogens we re also introduced into the experimental system. They were N-cicrosodimethylamine and X-nethyl-N'nitro-N'-nitrosoguanidine. Both chemicals induced a simllar aIteration of the standard L-alanine-$^{14}$C incorporation demonstrated by 4-NQ0. These data suggest that this system may be used to screen potential oncogens.

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## INTRODUCTION

The discovery of many pure chemical oncogens may allow scientists to elucidate the mechanism by which normal cells undergo cancerous transformation. Whereas, the complexity of spontaneous cancer formation prohibits a mechanism from being developed, some of the simpler chemical oncogens offer the opportunity to explore oncogenesis on a molecular level. 4-nitroquinoline 1-oxide (4-NQO) is such a chemical oncogen.

### A. History

4-Nitroquinoline 1-oxide was first synthesized by Ochiai and his co-workers in 1942 (1). The group of organic chemists, lead by Ochiai, specialized in quinoline compound chemistry. Utilizing their chemical knowledge, they not only synthesized 4-NQO but many of its derivatives.

The carcinogenicity of 4-NQO was established by Nakahara, et al (2) in 1957 at the Cancer Institute, Japanese Foundation for Cancer Research. Nakahara's first publication reported squamous cell carcinoma and sometimes fibrosarcoma of mice skin after painting with 4-NQO (2). Prior to the establishment of the carcinogenicity of 4-NQO, the compound had been shown to have mutagenic (3,4,5,6) and tumoricidal properties (7,8). Making use of the knowledge of the mutagenic and turmoricidal properties of 4-NQO as well as Haddow's postulate (9), which

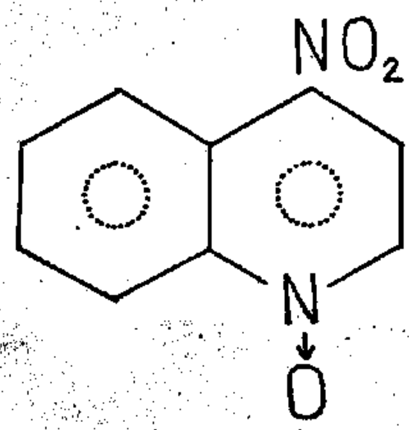


Figure 1. 4-Nitroquinoline 1-oxide

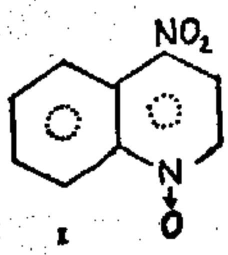
can be stated as certain types of carcinogens exhibit anticancer action and *vice versa*, led Nakahara to presume that 4-NQO might be carcinogenic.

Nakahara, Fukuoka, and Sakai (10), while testing 4-NQO and related compounds found 4-NQO, 2-methyl-4-NQO, 2-ethyl-4-NQO and 6-chloro-4-NQO to be oncogenic in character; whereas, quinoline N-oxide, 3 methyl-quinoline N-oxide, 4-nitroquinoline gave negative oncogenic tests. These findings, which have been confirmed by other laboratories (11), led to the hypothesis that the nitro group at position 4 on the quinoline ring, as well as the oxygenated nitrogen on position 1 was prerequisite to oncogenic behavior.

4-Nitroquinoline 1-oxide has the interesting distinction of having two strong polar groups in the molecule, the N-oxide and the nitro group. The molecule is susceptible to nucleophilic attack and may also function as an electron acceptor (12). Moreover, 4-NQO is photodynamically active (13,14).

One of the more interesting discoveries about 4-NQO chemistry concerns its reduction products: 4-hydroxyamino-quinoline 1-oxide (4-HAQO) and 4-aminoquinoline 1-oxide (4-AQO). 4-HAQO was found to have potent oncogenic character. Some researchers have suggested that 4-HAQO is the proximate oncogen of 4-NQO (15,16,17,18). Both the chemical and enzymatic reduction of 4-NQO to 4-HAQO has been reported (19,20,21).

4-NQO and its related compounds have been shown to react with native macromolecules, both *in vivo* and *in vitro*. These molecules include RNA, DNA, and proteins, as well as selected



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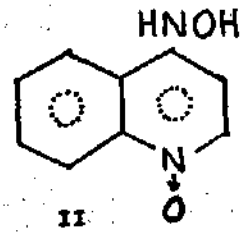

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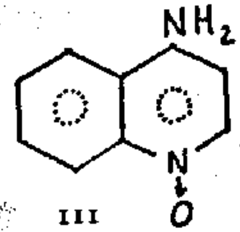

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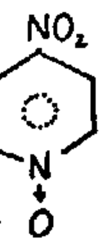


IV

Figure 2. 4-Nitroquinoline 1-oxide and related compounds.
(I) 4-Nitroquinoline 1-oxide
(II) 4-Hydroxyaminoquinoline 1-oxide
(III) 4-Aminoquinoline 1-oxide
(IV) 4-Nitropyridine 1-oxide

free amino acids.

4-NQO and 4-HAQO have been used to induce the malignant transformation of normal cells in tissue culture. However, further studies are still needed to determine the point at which chemical interactions become oncogenic initiation and development.

B. Chemical Properties

A review of the chemical properties of 4-NQO and other quinoline derivatives should disclose any structural features and properties common to the oncogenic derivatives that could be used to develop model systems with which to study the mechanism of action of the chemical carcinogen.

Upon examination of the quinoline molecule one would expect the 2 and 4 positions to be highly resistant to electrophilic attack. This would be due to the electron withdrawing character of the ring nitrogen. In 1943 Ochiai (1), who had already established himself in the field of aromatic amine chemistry, proposed that the oxygenation of the ring nitrogen would alter the reactivities of the aromatic ring system due to the electron donating ability of the N-oxide. To proof this speculation Ochiai and co-workers demonstrated that the 4 position of quinoline 1-oxide was more easily nitrated than the unsubstituted quinoline (1,22). This marked the beginning of the synthesis of many 4-nitroquinoline 1-oxide derivatives. Some of these derivatives are listed in Table 1.

TABLE 1

4-NITROQUINOLINE 1-OXIDE DERIVATIVES

| 4-nitroquinoline 1-oxide | m.p.(°C) | References |
|---|---|---|
| 4-nitroquinoline 1-oxide | 153~154 | (1,22) |
| 2-methyl | 157 | (23,24) |
| 3-methyl | 179~180 | (25,26) |
| 5-methyl | 174~175 | (25,26) |
| 6-methyl | 184~186 | (23,27,28) |
| 7-methyl | 164~165 | (25,26) |
| 8-methyl | 153~154 | (25,26) |
| 2-ethyl | 144~146 | (29) |
| 2-n-propyl | 156~160 | (29) |
| 2-hydroxymethyl | 120 | (30) |
| 2-piperidinomethyl | 92~95 | (30) |
| 2-phenyl | 135 | (12) |
| 2-carboxyethyl | 155 | (30) |
| 3-diethylmalonyl | 101~102 | (31) |
| 6-n-butyl | 96~97 | (32) |
| 6-tert-butyl | 126~129 | (32) |
| 6-n-hexyl | 102 | (32) |
| 6-cyclohexyl | 161~162 | (32) |
| 6-carboxy(-COOH) | 241 | (30) |
| 6-sulfoxy(-$SO_3H$) | 230~235 | (30) |
| 2-chloro | 154~156 | (33) |
| 2-bromo | 183 | (33) |

TABLE 1
(Continued)

| 4-nitroquinoline 1-oxide | m.p.(°C) | References |
|---|---|---|
| 3-fluoro | 164~165 | (34) |
| 3-chloro | 159~160 | (25,26) |
| 3-bromo | 159 | (35) |
| 5-chloro | 145~146 | (25,26) |
| 6-chloro | 193~195 | (28,36) |
| 6-bromo | 200~202 | (28,36) |
| 2-chloro | 219 | (37) |
| 6,7-dichloro | 191 | (25,26) |
| 2-hydroxy | 205~206 | (33) |
| 2-phenoxy | 107.5 | (29) |
| 3-methoxy | 196 | (26) |
| 6-methoxy | 204~205 | (38) |
| 5-nitro | 260~262 | (39,40) |
| 6-nitro | 218 | (36,39) |
| 7-nitro | 195~196 | (29) |
| 8-nitro | 222~223 | (40) |
| 6,8-dinitro | 189~191 | (23,40) |
| 2-morpholino | 162 | (29) |
| 2-piperidino | 118 | (29) |
| 3-amino | 218 | (34) |
| 3-piperidino | 167.5 | (34) |
| 3-anilino | 164~165 | (34) |
| 3-hydroxyethylamino | 211 | (34) |

TABLE 1
(Continued)

| 4-nitroquinoline 1-oxide | m.p.(°C) | References |
|---|---|---|
| 3-carboxymethylamino | 205 | (34) |
| 3-phenylhydrazino | 138 | (34) |
| (4,4'-dinitro-2,'-biquinoline 1,1'-dioxide) | 285~290 | (41) |

## C. Nucleophilic Substitutions

Resonance properties of substituents at the 4 positions of the quinoline 1-oxide ring lead to a high reactivity towards nucleophilic reagents involved in substitution reactions (42, 43). 4-NQO has been shown to react readily at ice temperatures with acid chlorides (39) acetyl chloride, (44,45) and sulfuryl chloride (22,44,45) to give 4-chloroquinoline 1-oxide in excellent yield. At temperatures above 40°C 4-NQO reacts with the acid chlorides to yield 2,4-dichloroquinoline (1,44,45). Acid bromides can be utilized to give 4-bromoquinoline 1-oxide as product. The synthesis of 2,4-dibromoquinoline and 2-bromo-4-nitroquinoline can be accomplished through the use of phosphoryl bromide (46). This synthesis shows evidence that the phosphoryl bromide mechanism proceeds first by attacking the N-oxide then the nitro group at the 4-position.

Almost complete conversion of 4-NQO to 4-chloroquinoline 1-oxide is accomplished by boiling with concentrated hydrochloric acid (27). It has also been shown that many of the derivatives of 4-NQO undergo a similar replacement reaction in the presence of hot hydrochloric acid (27). Under more vigorous conditions concentrated hydrobromic acid can be reacted with 4-NQO to yield 4-bromoquinoline 1-oxide (47,48).

The nitro group of 4-NQO can be readily replaced with alkoxides i.e. methoxide, ethoxide (1,49) and benzyloxide, (50) to form 4-alkoxyquinoline 1-oxides. The replacement can also be accomplished with alkanethiols such as cysteine, (29, 51) thioglycolic acid and ethanethiol (51,52) even under the relatively mild state of physiological conditions.

4-Chloroquinoline 1-oxide can be treated directly with sodium hydrogen sulfide to yield 4-mercaptoquinoline 1-oxide. It can also be prepared by the treatment of 4-chloroquinoline 1-oxide with thiourea followed by alkali decomposition of the intermediate 4-thiouronium chloride (22,45,53).

Attachment of alkyl and aryl groups to 4-mercaptoquinoline 1-oxide to yield alkyl- and aryl-thio-quinoline 1-oxides can be accomplished with alkyl and aryl halides (54). The alkyl and arylthioquinoline 1-oxides can also be prepared from the reaction of 4-halogenoquinoline 1-oxides with alkyl- and aryl - thiols (54). These derivatives can be oxidized with hydrogen peroxide in acetic acid or with chlorine gas in methanol to yield sulfonyl derivatives (54). 4-Hydroxyquinoline 1-oxide is prepared by refluxing 40% sulfuric acid with 4-NQO or 4-chloroquinoline 1-oxide (27).

Alternatively, 4-HQO can be prepared by warming 4-NQO (70 -80°C) in acetic anhydride in the presence of dimethylaniline (55). A third method for the production of 4-hydroxyquinoline 1-oxide is the catalytic reduction of 4-benzyloxyquinoline 1-oxide with palladium-charcoal (50).

Direct replacement of the nitro group with an amino group is not the synthetic method of choice for preparing 4-AQO. Instead, 4-chloroquinoline 1-oxides reacted with ammonia and amines produce 4-amino-, alkylamino-, and arylaminoquinoline 1-oxides.

4-Hydroxyaminoquinoline 1-oxide, 4-hydrazinoquinoline 1-oxide and 4-azidoquinoline 1-oxide can be prepared by reacting 4-halogenoquinoline 1-oxides with hydroxylamine (56) hydrazine (57) or sodium azide (57) respectively.

D. Reduction of the Nitro Group

The reduction products of 4-NQO and their related compounds can be found in Table 2. When 4-NQO is catalytically reduced, utilizing palladium-charcoal in alcohol, 4-hydroxyaminoquinoline 1-oxide is generated. Further reduction to 4-aminoquinoline 1-oxide is limited due to the lack of solubility of 4-HAQO in alcohol (56). Utilization of a nickel catalyst for the reduction of 4-NQO yields 4-aminoquinoline 1-oxide (58).

It is only with great difficulty and a multitude of exceptions that comprehensive statements concerning the catalytic reduction of 4-NQO derivatives could be given. It would be more appropriate for the interested researcher to discern the characteristics of the particular derivative.

Among the most important methods for the chemical reduction of 4-NQO is reaction with phenylhydrazine. 4-NQO and its derivatives, with few exceptions, are converted in good yield to the appropriate 4-hydroxyaminoquinoline 1-oxides (59,60,61, 62,63). The most general method for the reduction of 4-NQO and its derivatives to 4-aminoquinolines is with iron powder and acetic acid at 60°C (64). It should be noted, however, that 3- and 8- substituted 4-nitroquinoline 1-oxides do not react in the manner characteristic of 4-NQO or most of its derivatives.

TABLE 2

THE REDUCTION PRODUCTS OF 4-NITROQUINOLINE 1-OXIDES AND THEIR RELATED COMPOUNDS

| Compound | Melting point | | References |
|---|---|---|---|
| | Free bases | HCl salts | |
| 4-NHOH-quinoline 1-oxide | 198.5(dec.) | 198 | (56,59,60) |
| 2-$CH_3$-4-NHOH-quinoline 1-oxide | 189 | 232 | (60) |
| 5-$CH_3$-4-NHOH-quinoline 1-oxide | 173-180 | 195-198 | (25) |
| 6-$CH_3$-4-NHOH-quinoline 1-oxide | 212-213 | 221-223 | (25) |
| 7-$CH_3$-4-NHOH-quinoline 1-oxide | 205 | 243-245 | (25) |
| 8-$CH_3$-4-NHOH-quinoline 1-oxide | 146 | 189-190 | (25) |
| 6-Cl-4-NHOH-quinoline 1-oxide | 199-200 | 223 | (25) |
| 7-Cl-4-NHOH-quinoline 1-oxide | 233 | 207 | (25) |
| 6,7-di-Cl-4-NHOH-quinoline 1-oxide | 233 | 219-220 | (25) |
| 6-$NO_2$-4-NHOH-quinoline 1-oxide | 210 | 202-203 | (25) |
| 7-$NO_2$-4-NHOH-quinoline 1-oxide | 207- | 197-198 | (25) |
| 4-NHOH-quinoline | 201-202(dec.) | 262-263(dec.) | (65) |
| 3-$CH_3$-4-NHOH-quinoline | 155-156 | 255-260 | (63) |
| 8-$CH_3$-4-NHOH-quinoline | 128 | 274-275 | (63) |
| 8-F-4-NHOH-quinoline | 126 | 281 | (63) |

TABLE 2
(Continued)

| Compound | Melting point | | References |
|---|---|---|---|
| | Free bases | HCl salts | |
| 3-$OCH_3$-4-NHOH-quinoline | 105 | | (63) |
| 4-$NH_2$-quinoline 1-oxide | 272 | 275 | (59) |
| 4-$NH_2$-quinoline | 154 | | (59) |
| 4-NHOH-pyridine 1-oxide | 237(dec.) | 157 | (59) |
| 3-NHOH-quinoline 1-oxide | 174 | | (60) |
| 5-NHOH-quinoline 1-oxide | 190 | | (61) |
| 4-$NO_2$-quinoline | 87-89 | | (66) |

These derivatives when subjected to the same chemical reducing agents are reduced further than those substituted on the 2, 5, 6 and 7 positions. These derivatives are probably not stabilized in the presence of reducing agents.

Reduction of 4-NQO compounds substituted at the 3 position leads to products that are deoxygenated at the N-oxide. The mechanism involved in this deoxygenation orocess has not yet been elucidated.

Deoxygenation of the N-oxide of 4-NQO, or its derivatives, is usually carried out with phosphorus trichloride or phosphorus tribromide (66,68,69,70). Chloroform is usually the solvent of choice but is not used exclusively. For example, 4-NQO can be converted to 4-nitroquinoline in quantitative yield by utilizing 1.5 equivalents of phosphorus tribromide in chloroform (below 15°C) for fifteen minutes (66,67). The mechanism for this reaction involves the transfer of oxygen from the nitrogen to phosphorus.

If the reaction is carried out above 30°C 4-bromoquinoline is produced. The same product is formed if the reaction is allowed to react for an extended time.

## E. Oncogenicity

The oncogenic character of 4-nitroquinoline 1-oxide was first demonstrated in the work of Nakahara, Fukuoka and Sugimura in 1957 (2). There has been an impressive number of reports on the oncogenic action of 4-NQO and its derivatives. Some of these data are summarized in Table 3.

TABLE 3

WORK DEMONSTRATING THE ONCOGENIC ACTION OF 4-NITROQUINOLINE 1-OXIDE

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| 20 mice | mixed | 20 M | 0.02 ml of 0.25%, 3 times a week |
| mice | mixed | -- | total 0.5--2.0 mg in 5 or 6 ij. in 50 to 60 days |
| 213 mice | dd | 147 M 66F | 0.02 ml of 0.25--1.9% |
| 40 mice | XVIInc | 24 M 16 F | 0.6 mg in 0.2 ml, 3 times a month |
| 48 mice | XVIInc/Z | 24 M 24 F | one drop of 0.5 or 0.3%, twice a week |
| 8 mice | $C_{57}$bl/Z | 8 F | |
| 50 mice | dd | 50 F | 0.5 or 1.0 mg |
| 12 mice | $C_{57}$bl | 12 F | |
| 20 mice | dd | 20 F | 0.1 ml of 25%, once a week, 10 times |
| rats | albino | M and F | 0.05 ml of 0.5%, twice a week for 6 months |
| 20 rats | Buffalo | 10 M | 0.2--0.3 ml of 2.05%, once a week, 16 times |
| rats | Long-Evance | 10 M | 28 times |

TABLE 3
(CONTINUED)

| Vehicle | Site and route | Animals with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| 4-nitroquinoline 1-oxide | | | | |
| benzene | painting | 14/14 papilloma 12/14 squamous cell carcinoma | over 200 days | (2) |
| propylene glycol | s.c. | 12/29 fibro-sarcoma | over 300 days | (71) |
| acetone | single painting | 15 papilloma 2 carcinoma | 200 days | (72) |
| olive oil | s.c. | M 4/9 F 4/7 sarcoma | over 177 days | (73) |
| benzene | painting | none papilloma | 300 days | (74) |
| | | epithelioma | | |
| olive oil-cholesterol | single s.c. | 25/27 pulmonary tumor | 3 or 4 months | (75) |
| (7:1) | | 2/11 pulmonary tumor | | |
| olive oil-cholesterol (100:5) | s.c. | 2/10 adeno-carcinoma 10/10 adenoma | 400 days | (76) |
| acetone | painting | 4/11 fibrosarcoma | 195 days | (77) |
| olive oil-cholesterol (100:5) | s.c. | 7/7 adenoma 1/7 squamous cell carcinoma | 400 days | (78) |
| | | 6/8 adenoma 2/8 adenocar-cinoma 1/8 squamous cell carcinoma | 344 days | (78) |

TABLE 3
(CONTINUED)

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| 33 rats | Wistar | 11 M<br>6 F | 1 ml of 0.1%, twice a week, 6--29 times |
| | | 12 M<br>4 F | 2--30 times+painting a few drops of 0.3% of 20 MC in acetone, 2--96 times |
| 10 guinea pig | albino | 5 M<br>5 F | 0.1 ml of 0.25 or 0.5%, once a week, total 5--10 mg |
| 10 hamster | golden | -- | 0.5 ml of 0.5%, twice a week |
| mice | ICR | -- | 0.5 mg |
| | | | 0.1 mg, weekly |
| | | | 0.03 mg, weekly |
| | | | 0.03 mg |
| 31 mice | $C_{57}$bl | -- | 0.01 mg, weekly<br>0.01 mg |
| 18 mice (newborn) | A/Jax | -- | 0.05 ml in 0.05 ml |
| 44 mice (newborn) | AKR | -- | 0.025 mg |
| 30 rats | Buffalo | F | 0.1--0.2 ml of 0.25% weekly, total 10 mg |

TABLE 3
*(CONTINUED)*

| Vehicle | Site and route | Animal with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| ethanol | stomach through tube | 1/17 squamous carcinoma<br>1/17 sarcoma | 407 days | (79) |
| | | 1/16 adeno-carcinoma<br>4/16 squamous carcinoma<br>1/16 papilloma | 548 days | |
| propylene glycol | s.c. | 1/19 fibrosarcoma | 2.5 years | (17) |
| acetone | painting | 5/7 squamous cell carcinoma<br>2/7 melanoma | 36--48 weeks | (80) |
| propylene glycol | i.p. | 6/14 generalized lymphoma | 304 days | (81) |
| | i.v. | 1/20 lymphoma | | |
| | i.p. | 1/20 *breast cancer* | | |
| gelatine-saline solution | single i.p. | 9/27 lymphoma<br>2/27 spindle cell sarcoma | 318 days | |
| cotton seed oil | i.p. | 12/16 spindle cell carcinoma | over 300 days | (81) |
| propylene glycol | i.p. | 0/15 | | |
| 2% gelatine solution | single s.c. | 7/18 lung tumor | 12 weeks | (82) |
| -- | single s.c. | 3/36 lymphogenic leukemia | 5 months | (83) |
| olive oil-lecithin (100:5) | s.c. | 7/25 uterine tumor<br>22/25 adenoma<br>22/25 sarcoma | 360 days | (84) |

TABLE 3
(CONTINUED)

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| 191 mice | A/Jax | M and F | 0.04--0.3 mg |
| 20 Uroiancha domestica | albino | -- | 0.1 ml of 0.2%, once in 2--3 weeks for 4 times |
| 95 mice (newborn) | Swiss | M and F | 50--200 μg |
| 20 mice | ddN | M | 0.15 mg in 0.05 ml, once a week, total 0.45 mg |
| 113 mice | ddN | M | 0.006--0.009 ml of 0.25%, until death |
| 168 mice | CF # 1 | M | 0.04 ml of 0.24%, weekly for 23 weeks 0.24% |
| 20 mice | Swiss | F | 0.1 ml of 0.25%, weekly, total 2.5 mg |
| 260 mice | 4 pure strains $F_1$ and random breeding | 220 M 40 F | 0.1--0.3 ml of 0.1 or 0.3%, weekly |
| 8 guinea pigs | -- | 2 M 6 F | 0.5 ml of 0.5 or 1%, twice a week |

TABLE 3
(CONTINUED)

| Vehicle | Site and route | Animals with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| 1% gelatine solution | single s.c. | 154/160 pulmonary tumor<br>37/160 leukemia | 1 year | (85) |
| olive oil | i.m. | 1/3 fibrosarcoma | 230 days | (86) |
| olive oil-cholesterol (100:5) | single s.c. | 16/46 adenocarcinoma | 6 months | (87) |
| propylene glycol | ij. into lingual mucosa | 2/20 early neoplastic lesion | 137 days | (88) |
| propylene glycol | painting on lower lip given injury | 16/34 squamous cell carcinoma<br>61/79 squamous cell carcimp,a | over 180 days | (89) |
| 95% ethanol<br>70% ethanol | esophageal infusion<br>forced drinking | 40/105 papilloma<br>14/105 carcinoma<br>62/63 papilloma<br>8/63 carcinoma<br>1/63 adenoma | 22 months | (90) |
| olive oil-lecithin (100:5) | s.c. | 4/16 sarcoma<br>7/16 lung cancer<br>4/16 uterine cancer<br>11/16 leukemia | 357 days | (91) |
| acetone or benzene | painting | 23/260 connective tissue tumor<br>28/260 squamous cell carcinoma<br>21/260 epithelial tumor | 80 weeks | (92) |
| acetone | painting | a variety of tumors including melanomas | 134 weeks | (93) |

TABLE 3
(CONTINUED)

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| 11 hamster | golden | M | 0.5 ml of 0.5% twice a week |
| 50 mice | dd | F | 0.1 ml of 0.25% weekly, total 2.5 mg |
| 25 rats | Buffalo | F | 0.1 ml of 0.25%, weekly, total 10 mg |
| 176 mice (newborn) | dd | M and F | 0.05 mg |
| 20 rats | S.D. | M | 1 mg in 1 ml, 3 times a week for 14 weeks |
| 210 mice | dd | F | 0.06--0.5 mg, once in 7--16 days, 10--12 times |
| 25 rabbits | -- | 11 M | mixture of 20 mg of 3-MC and 0.2 mg of 4NQO in rabbit plasma in combination with ij. of 0.4 mg of 4NQO in 0.1 ml olive oil and cholesterol, 4--41 times |
| | | | 4-Hydroxyaminoquinoline 1-oxide |
| 40 rats | Wistar (Oosama) | F | 0.1 ml of 0.1%, Every 10 days for 10 times |

TABLE 3
(CONTINUED)

| Vehicle | Site and route | Animals with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| acetone | painting | 8/11 a variety of tumors including melanotic tumor | | (94) |
| 10% lecithin | s.c. | 36/36 lung tumor<br>1/36 leukemia<br>1/36 skin cancer | 296 days | (95) |
| 10% lecithin | s.c. | 19/21 ling cancer<br>2/21 endometrial sarcoma<br>11/21 subcutaneous sarcoma | 329 days | (95) |
| 10% lecithin | s.c. | 6/10 adenoma<br>6/14 ling cancer | 180 days | (96) |
| Tween 80-olive oil | stomach through tube | 3/10 squamous cell carcinoma<br>2/10 sarcoma | over 5 months | |
| 50% ethanol | stomach through tube | 2/42 adenocarcinoma<br>11/42 carcinoma | 302 days | (97) |
| | intrabronchial infusion and s.c. | 12/25 adenocarcinoma<br>2/25 squamous cell carcinoma<br>1/25 undifferentiated cell carcinoma | 397 days | (97) |
| 4-Hydroxyaminoquinoline 1-oxide | | | | |
| peanut oil-cholesterol (100:5) | s.c. each in different site<br>same site | 8/15 fibrosarcoma<br>16/17 fibrosarcoma | 230 days | (98) |

TABLE 3
(CONTINUED)

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| 30 mice | ddOM | F | 0.1 ml of 0.2%, weekly, 3 times |
| | | | 0.03 ml of 0.25%, twice a week |
| 26 mice | ddOM | F | 0.1 ml of 0.05 or 0.1%, weekly for 15 times |
| 10 rats | Wistar | F | 1.0 ml of 0.1%, weekly, for 3 times |
| 10 rats | Wistar (Oosama) | F | 2 mg |
| 60 mice | $C_3H$/Hen | 10 M | 0.1 ml of 0.1%, |
| | $C_{57}$bl/6JN | 10 F | weekly for 10 times |
| | Noninbred | 10 M | 0.1 ml of 0.1% |
| | albino | 10 F | weekly for 10 times |
| | | 10 M | 0.1 ml of 0.1% |
| | | 10 F | weekly for 10 times |
| 20 mice | ddN | -- | 0.5 ml in 0.1 ml, 6 times at intervals of 10 days |
| 60 mice | dd | F | 0.25 mg in 0.1 ml weekly, total 2.5 mg |
| 70 mice | dd | F | 0.25 mg of the hydrochloride in 0.1 ml |
| mice | -- | -- | 0.325 mg, weekly, for 10 times |

TABLE 3
(CONTINUED)

| Vehicle | Site and route | Animals with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| 0.5% Tween 80 in saline solution | s.c. | 4/15 papilloma | over 8 months | (99) |
| absolute ethanol | painting | none | 6 months | |
| 0.5% Tween 80 in saline solution | s.c. | 11/26 sarcoma<br>5/26 carcinoma<br>6/25 papilloma | 8--9 months | (17) |
| 0.5% Tween 80 in saline solution | s.c. | 5/10 sarcoma | over 8 months | (17) |
| peanut oil-cholesterol (100:5) | single s.c. | 2/8 fibrosarcoma | 1 year | (98) |
| saline solution | s.c. | 6/20 sarcoma<br>1/20 papilloma<br>2/20 sarcoma<br>2/20 papilloma<br>2/20 sarcoma<br>1/20 papilloma<br>5/20 leukemia | 32 weeks | (18) |
| propylene glycol | s.c. | tumors at the site of ij. | 300 days | (25) |
| 10% lecithin | s.c. | 5/26 lung cancer<br>23/26 adenoma<br><br>5/21 lung cancer<br>17/21 adenoma | 180 days | (100) |
| ethanol | stomach | 1/18 adeno-carcinoma | 262-302 days | (101) |

TABLE 3
(CONTINUED)

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| 200 mice | dd | F | 0.25 or 0.5 mg in 0.1 ml, every 7--10 days |
| Methyl derivatives of 4-nitroquinoline 1-oxide, 2-methyl derivative | | | |
| 7 mice | mixed | M | 0.04--0.02 ml of 0.25%, 3 times a week, until tumor production |
| 40 mice | XVIInc | 24 M<br>16 F | 0.6 mg in 0.2 ml, 3 times a month |
| 40 mice | $C_{57}bl$ | 8 F | one drop of 0.3% twice a week |
| | XVIInc/Z | 16 M<br>16 F | one drop of 0.3% twice a week |
| 15 mice | ddO | F | 0.02 ml of 0.27% 3 times a week for 123 days |
| | | | 6-methyl derivative |
| 28 mice | XVIInc/Z | 14 M | 0.6 mg in 0.2 ml, 3 times at intervals of one month |
| | | | 6,7 dimethyl |
| 28 mice | XVIInc/Z | 14 M | 0.6 mg in 0.2 ml, 3 times at intervals of one month |
| | | | 5-methyl derivative |
| 20 mice | ddN | -- | 0.5 mg in 0.1 ml, 6 6imes at intervals of 10 days |

TABLE 3
(CONTINUED)

| Vehicle | Site and route | Animals with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| 50--70% ethanol | stomach | 13/63 carcinoma in glandular stomach<br>1/63 in duodenum | 341 days | (102) |
| Methyl derivatives of 4-nitroquinoline 1-oxide, 2-methyl derivative | | | | |
| benzene | painting | none | 192 days | (10) |
| olive oil | s.c. | M1/11 sarcoma<br>F3/16 papilloma, acanthoma, pulmonary carcinoma | 232 days<br>300 days | (73) |
| olive oil | painting | none | 300 days | (74) |
| | | none | | |
| acetone | painting | 2/10 papilloma | 270 days | (103) |
| 6-methyl derivative | | | | |
| olive oil | s.c. | M2/14 sarcoma<br>F2/14 sarcoma | 342 days | (104) |
| 6,7-dimethyl derivative | | | | |
| olive oil | s.c. | M5/14 sarcoma<br>F4/14 sarcoma | 342 days | (104) |
| 5-methyl derivative | | | | |
| propylene glycol | s.c. | tumors at the site of ij. (fibrosarcoma) | 300 days | (25) |

TABLE 3
(CONTINUED)

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| | | | 7-methyl derivative |
| 20 mice | ddN | -- | 0.5 mg in 0.1 ml, 6 times at intervals of 10 days |
| | | | 8-methyl derivative |
| 20 mice | ddN | -- | 0.5 mg in 0.1 ml, 6 times at intervals of 10 days |
| Chlor derivatives of 4-nitroquinoline 1-oxide, 6-chlor derivative | | | |
| 8 mice | mixed | M | 0.04--0.02 ml of 2.5%, 3 times a week until tumor production |
| 20 rats | Wistar | 10 M<br>10 F | 0.05 ml of 0.5%, 3 times a week, 12--24 times |
| 15 mice | ddO | F | 0.02 ml of 0.29%, 3 times a week for 123 days |
| | | | 6,7-dichlor derivative |
| 20 mice | ddN | -- | 0.5 mg in 0.1 ml, 6 times at intervals of 10 days |

TABLE 3
(CONTINUED)

| Vehicle | Site and route | Animals with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| 7-methyl derivative | | | | |
| propylene glycol | s.c. | tumors at the site of ij. (fibrosarcoma) | 300 days | (25) |
| 8-methyl derivative | | | | |
| propylene glycol | s.c. | tumors at the site of ij. (fibrosarcoma) | 300 days | (25) |
| Chlor derivatives of 4-nitroquinoline 1-oxide, 6-chlor derivative | | | | |
| benzene | painting | 2/8 carcinoma<br>6/8 papilloma | over 150 days | (10) |
| acetone | painting | 12/20 fibro-sarcoma<br>5/20 fibro-sarcoma asso-ciated with basal cell car-cinoma<br>1/20 fibrosar-coma associated with sweat gland adenoma | 320 days | (105) |
| acetone | painting | 1/11 papilloma<br>6/11 squamous cell carcinoma | 270 days | (103) |
| 6,7-dichlor derivative | | | | |
| propylene glycol | s.c. | tumors at the site of ij. | 300 days | (25) |

TABLE 3
(CONTINUED)

| Animal | Strain or type | Sex | Preparation and dose |
|---|---|---|---|
| | | | 5-chlor derivative |
| 20 mice | ddN | -- | 0.5 mg in 0.1 ml, 6 times at intervals of 10 days |
| | | | 7-chlor derivative |
| 20 mice | ddN | -- | 0.5 mg in 0.1 ml, 6 times at intervals of 10 days |
| *Miscellaneous derivatives of 4-nitroquinoline 1-oxide, 2-ethyl* derivative | | | |
| 7 mice | mixed | M | 0.04--0.02 ml of 0.25%, 3 times a week until tumor production |
| | | | 6-carboxyderivative |
| 6 rats | Wistar | M and F | 1 mg in 0.1 ml, once a week for 20 weeks |
| 21 rats | Wistar | M and F | 1 mg in 0.1 ml, once a week for 20 weeks |
| | | | 3-brom derivative |
| 20 mice | ddN | -- | 0.5 mg in 0.1 ml, 6 times at intervals of 10 days |

TABLE 3
(CONTINUED)

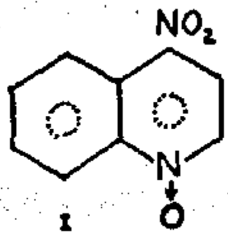
| Vehicle | Site and route | Animals with tumors | Duration of experiment | Ref. |
|---|---|---|---|---|
| 5-chlor derivative | | | | |
| propylene glycol | s.c. | tumors at the site of ij. | 300 days | (25) |
| 7-chlor derivative | | | | |
| propylene glycol | s.c. | tumors at the site of ij. | 300 days | (25) |
| Miscellaneous derivatives of 4-nitroquinoline 1-oxide, 2-ethyl derivative | | | | |
| benzene | painting | 3/3 carcinoma | 190--200 days | (10) |
| 6-carboxy derivative | | | | |
| saline solution | s.c. | 3/13 tumor | 400 days | (67) |
| olive oil | s.c. | 8/21 tumor | 400 days | (106) |
| 3-brom derivative | | | | |
| propylene glycol | s.c. | tumors at the site of ij. | 300 days | (25) |

Abbreviations ij., injection; i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous; MC, methylcholanthrene.

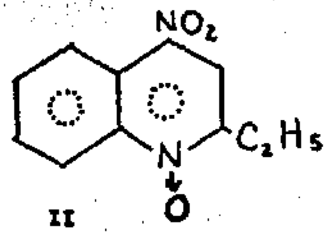
In a later publication Nakahara, et al (10) utilized 4-NQO (I), 4-nitro-2-ethyl-quinoline 1-oxide (II), 4-nitroquinaldine 1-oxide (III), 6-chloro-4-nitroquinoline 1-oxide (IV), quinoline 1-oxide (V), 3-methylquinoline 1-oxide (VI), 4-nitroquinoline (VII), and 6-nitroquinoline (VIII). The compounds are illustrated in Figure 3. By applying 4-NQO and these derivatives to mouse skin they were able to show that compounds (I), (II), (III), and (IV) were oncogenic. Compounds (V), (VI), (VII), and (VIII) were not oncogenic. Thus the conclusion, both the $NO_2$ group at position four and the oxide group at position one were fundamental to the oncogenicity of 4-NQO.

F. Reaction with -SH Groups

The effect of the N-oxide on the nitro group causes one to be particularly interested in the nucleophilic reactions that the $-NO_2$ group may undergo. Sulfhydryl groups undergo such reactions (107,108). Therefore, the importance of -SH groups in cellular metabolism and the reaction of 4-NQO with these compounds under physiological conditions generated interest in exploring this reaction both _in vivo_ and _in vitro_. Okabayashi (51) found that the 4-nitro group of 4-NQO could be substituted by the -SH group of both cysteine, and thioglycolic acid under physiological conditions. Compounds containing -SH groups lost their antifungal activity to _Aspergillus niger_ when reacted with 4-NQO (109). Endo showed that 4-NQO reacted with cysteine and glutathione (29). Bond, et al (110) showed that 4-NQO inhibited



I



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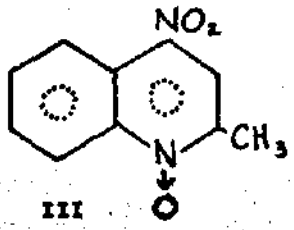

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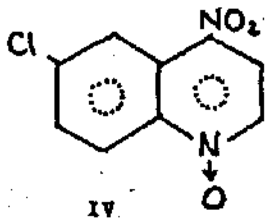

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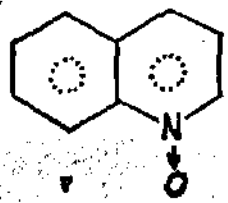
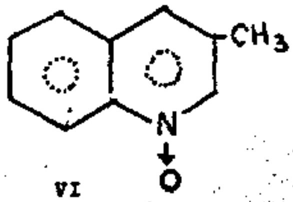


V



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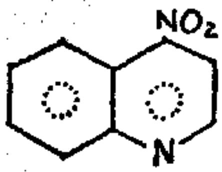
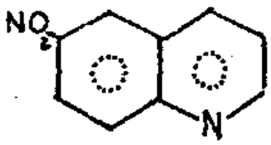


VII



VIII

Figure 3. (I) 4-Nitroquinoline 1-oxide,
(II) 4-nitro-2-ethylquinoline 1-oxide,
(III) 4-nitroquinaline 1-oxide,
(IV) 6-chlor-4-nitroquinoline 1-oxide,
(V) quinoline 1-oxide,
(VI) 3-methylquinoline 1-oxide,
(VII) 4-nitroquinoline,
(VIII) 6-nitroquinoline.

the growth of Lactobacillus casei, but that normal growth curves could be restored with cysteine, glutathione, and Cleland's Reagent (dithiothreitol).

Nakahara and Fukuoka (71) reported that 4-NQO incubated with human serum for 30 minutes totally lost its carcinogenic activity. Takayama (72) demonstrated that a single application of 4-NQO to mouse skin could induce carcinoma. Kamahora and Kakunaga (111, 112) reported that treatment of tissue culture cells with 4-NQO gives rise to severe necrosis and population reduction followed by an increase in cell number. Twenty days after oncogen application polynucleated giant cells and spindle cells appeared. These data suggest that cancer initiation by 4-NQO must occur within a very short time after application. Other workers have also suggested a quick initiation reaction (72,113,114).

Lacassagne, et al (74) reported that 4-NQO painted on mouse skin would result in tumors in one strain but not in others. Whereas, 3,4-benzpyrene showed no such selectivity. Horikawa, et al (115) used tissue culture to show a difference in strain sensitivity to 4-NQO induced change and that caused by ultra-violet irradiation. This data lends further support to the hypothesis of a unique cancer initiation mechanism of 4-NQO and its derivatives.

Besides mice, the oncogenic action of 4-NQO has been demonstrated using rats (116), birds (86), guinea pigs (80,93). It is unique that 4-NQO can induce malignant tumor formation in the guinea pig since they are usually resistant to oncogenic actions of all kinds.

It appears that tumor formation in the lungs of laboratory animals may be induced with very minute amounts of 4-NQO (76,78,84,91,95,96). Even though it is more difficult to induce cancer artifically in the glandular stomach, this too has been accomplished with 4-NQO by Baba, et al (79) and Mori, et al (97,117).

The ability of 4-NQO to induce cancers is also influenced by the solvent-carrier. Mori (84) found that the oncogenic ability of 4-NQO was enhanced when the vehicle was changed from olive oil and cholesterol to olive oil and lecithin.

An even higher incidence of lung cancer was observed when the 4-NQO was dissolved in 10:90 lecithin: water (95). Tanaka, et al (81) in an earlier investigation, observed that mouse sarcomas could be induced utilizing 4-NQO in cotton seed oil but when dissolved in propylene glycol produced no tumors in the test animals.

### G. Enzymatic Induction

After the initial proof of the oncogenicity of 4-NQO where one found most suggested mechanism were based upon the reaction of the $NO_2$ with SH groups, a second set of possible mechanisms arose from the fact that 4-NQO can be enzymatically reduced to 4-hydroxyaminoquinoline 1-oxide, 4-aminoquinoline 1-oxide and 4-aminoquinoline (19,109).

4-NQO can exert biological alteration such as mutation induction (118,119), phase induction in lysogenic E. coli (11), and formation of nuclear inclusion bodies in tissue culture cells (98). These facts along with the indictment of 4-HAQO as a possible proximate oncogen caused investigators to research the 4-NQO derivative, 4-HAQO. Endo and Kume (98) showed 4-HAQO to be the more potent oncogen when treating rats with single injections. These results were confirmed by Shirasu (17,18, 99). Kawazoe, et al (25,26) in their study of structure versus oncogenic relationship suggested that the oncogenicity of 4-NQO and its derivatives is directly proportional to their ability to form 4-HAQO or a 4-HAQO derivative.

Non-carcinogenic derivatives of 4-NQO are given in Table 4.

## E. Syncarcinogenic Effect

The theory that cancerization results from the summation of multiple irreversible alterations of critical cell substituents was first proposed by Druckrey and Küpfmüller (120). The theory was based on their quantitative feeding of laboratory animals 4-dimethylaminoazobenzene. Their data showed the final tumor yield reflected the total dose of the oncogen.

Nakahara and Fukuoka (121) used 20-methylcholanthrene and 4-nitroquinoline 1-oxide to test the proposal of Druckrey and Küpfmüller. These two known oncogens were chosen because they appear to have the same target tissue. Submanifestational doses of each oncogen were applied to mice with results of no tumor incidence. When the same size doses of both oncogens were applied in sequence to mice some tumors were induced. The order of application did not cause a change in cancer incidence.

TABLE 4

NONCARCINOGENIC DERIVATIVES OF 4-NITROQUINOLINE 1-OXIDE

| Derivative | Animal | Preparation and dose | site and route | Duration of experiment | Reference |
|---|---|---|---|---|---|
| 4-Nitropyridine 1-oxide<br>4-Nitroquinoline 1-oxide<br>Quinoline 1-oxide<br>4-Aminoquinoline 1-oxide<br>4-Hydroxyquinoline 1-oxide<br>4-Bromoquinoline 1-oxide<br>4-Chloroquinoline 1-oxide<br>3-Nitroquinoline 1-oxide<br>4-Thioglycolylquinoline<br>9-Nitroacridine 1-oxide<br>4,6-dinitroquinoline 1-oxide<br>4,6,8-trinitroquinoline 1-oxide | mice | 0.1 ml of 2.5 mg/ml in propylene glycol, 6 times in each 10 da days | s.c. | 300 days | (86) |
| Trinitroquinoline 1-oxide<br>Dinitroquinoline 1-oxide<br>4-Nitroquinoline | mice<br>ddO | 0.02 ml of 0.23~0.37% in acetone, 3 times in a week for 123 days | painting | 270 days | (103) |
| 4-Hydroxyaminoquinoline 1-oxide acetate | mice<br>ddOM | 0.25% in ethanol, twice a week for 3 months | painting | 6-10 months | (17) |
| | | 0.1 ml of 0.2% in saline suspension, 10 times | s.c. | 6-10 months | (17) |
| 4-Azidoquinoline 1-oxide<br>4,4'-azoquinoline 1,1'-dioxide | | 0.1 ml of 0.2% in saline suspension, 3 times | s.c. | 8 months | (17) |
| | | 0.1 ml of 0.2% in 10% ethanol-saline solution | i.v. | 8 months | (17) |
| 3-Nitroquinoline 1-oxide | | 0.25% in ethanol, twice a week for 6 months | painting | 6-10 months | (17) |
| | | 0.1 ml of 2.5% in olive oil, 3 times | s.c. | 6-10 months | (17) |

TABLE 4
(Continued)

| Derivative | Animal | Preparation and dose | Site and route | Duration of | Reference |
|---|---|---|---|---|---|
| 4-Chloroquinoline 1-oxide | | 0.2 ml of 2.5% in saline suspension, 5 times. | s.c. | 6-10 months | (17) |
| 4-Aminoquinoline 1-oxide | | 0.1 ml of 3% in saline suspension, 5 times | s.c. | 6-10 months | (17) |
| 4-Nitropyridine 1-oxide | | 0.1 ml of 0.6% in saline solution, 5 times | s.c. | 6-10 months | (17) |
| 4-Hydroxyaminopyridine 1-oxide | | 0.1 ml of 0.2% in saline solution, 5 times | s.c. | 6-10 months | (17) |
| 5-Nitro-4-nitroquinoline 1-oxide<br>7-Nitro-4-nitroquinoline 1-oxide<br>8-Nitro-4-nitroquinoline 1-oxide<br>3-Chloro-4-nitroquinoline 1-oxide<br>3-Methyl-4-nitroquinoline 1-oxide<br>3-Methoxy-4-nitroquinoline 1-oxide<br>3-C-Diethylmalonyl-4-nitroquinoline 1-oxide | mice<br>ddN | 0.1 ml of 5 mg/ml in propylene glycol,<br>6 times at intervals of 10 days | s.c. | 300 days | (25) |

Muta (122) showed that the summation effect can be achieved even if the method of introduction is different. He fed rats and mice o-aminoazotoluene and followed this with skin painting using arsenic trioxide, 3,4-benzpyrene, and coal tar.

Although the results are not completely quantitative Hoshino et al (123) have shown cancer can be induced utilizing 4-NQO and β-radiation. Even though syncarcinogenism has been useful in 4-NQO work (124), not all oncogen combinations have been shown to follow a syncarcinogenic route. Searle and Woodhouse (80,125) showed that the rate of occurrence of mouse skin papillomas treated with 3,4-benzpyrene is markedly inhibited by 4-NQO. 4-NQO also retards the induction of oncogenesis by 1,2,5,6-dibenzanthracene. This data has not yet been adequately explained.

I. Metabolism

Proximate oncogens are those active compounds that are formed in vivo from possible oncogens. These compounds are the true oncogens.

4-Nitroquinoline 1-oxide can be chemically reduced to a more potent oncogen 4-hydroxyaminoquinoline 1-oxide. Further reduction yields 4-aminoquinoline 1-oxide. Okabayashi, et al (104, 118) utilizing growing culture of *Escherichia coli* and *Aspergellus niger* were first to take cognizance of the metabolic reduction of 4-NQO to 4-HAQO or 4-AQO. These early findings also showed that *A. niger* was more susceptible to yielding mutants than *E. coli* as well as being less able to continue the reduction of 4-NQO from 4-HAQO to 4-AQO.

Endo's group (19) demonstrated the reduction of 4-NQO to 4-AQO by mamalian systems utilizing rat liver homogenate. Required for the reduction was a hydrogen donor, the corresponding reductase, and NAD (nicotinamide-adenine dinucleotide) or NADP (nicotinamide-adenine dinucleotide phosphate). Qualitative proof for the presence of 4-AQO was made by thin layer chromatography showing an exact correspondence to authenic 4-AQO.

Aerobic incubation markedly inhibited the reaction. This has been attributed to the formation of azoxy or azohydrazoquinoline derivatives from 4-HAQO.

Sugimura, Okabe, and Nagao (20) were the first to report the isolation of the enzyme catalyzing the reaction 4-NQO to 4-HAQO. It was isolated from various homogenized rat organs after centrifugation for one hour at 105,000 X g. Early enzyme assays were followed by the decrease in optical activity caused by conversion of NADH + $H^+$ (reduced NAD) or NADPH + $H^+$ (reduced NADP). The enzyme was further purified and identified as DT-diaphorase by Ernster, Danielson and Ljungren (126). The enzyme was found to be a metalloflavoprotein containing FAD and iron and had an optimum pH of 5.8.

The highest enzyme activities were found in rat liver, stomach, and lung. Whereas the majority of activity from enzymes catalyzing reduction of 4-NQO were noticed in the above organs, small amounts of activity could be found in other organs.

Based on the premise that the injection of 4-NQO will produce fibrosarcoma at the injected loci, Hoshino, et al (15) and Matsushima, et al (127) determined the in vivo fate of 4-NQO.

The products were again, 4-HAQO and 4-AQO. It is interesting to note that unconverted 4-NQO was rapidly removed from the site of injection while the reduction product, 4-HAQO, was retained for a comparably longer time.

After 4-NQO was shown to be somewhat inert under physiological conditions, researchers found that alteration of metabolic processes could be attributed to 4-HAQO even in trace quantities (128). Hozumi, et al (129) not only found catalytic amounts of 4-HAQO caused oxidation of sulfhydryl groups of cysteine and glutathione but also that hydrogen peroxide could be produced by passing air through a 4-HAQO solution (pH 7.5). It has been shown that the presence of hydrogen peroxide can cause alteration of DNA and thus mutation.

Matsushima, et al (127) has found that 4-NQO reduces cytochrome. They have also reported incubation with 4-HAQO inactivates 3-phosphoglyceraldehyde dehydrogenase. Their work has also shown methionine and tyrosine were converted into ninhydrin positive compounds with $R_f$ values differing from the original amino acids.

## J. Interaction with DNA

Ono (130) first reported the inactivation of transforming <u>B. subtilis</u> DNA with 4-HAQO. He also reported that the DNA is not affected by the 4-NQO. His work was confirmed by other researchers (131). Ishizawa and Endo (132) have shown 4-HAQO to be a phage inactivator <u>in vitro</u> and attribute this phenomenona to an oxidation product, 4-nitrosoquinoline 1-oxide, or perhaps a free radical which was finally converted to 4,4'-azoxyquinoline 1,1'-dioxide.

In 1967 Nagata, et al (133) found that by use of flow dichroism and equilibrium dialysis, 4-NQO and its derivatives bind with calf thymus DNA. Malkins and Zahalsky (134) confirmed the DNA - 4-NQO binding with thin-layer chromatography. Matsushima, et al (127) as well as Tada, et al (135) have detected stable 4-HAQO-DNA complexes.

K. RNA Synthesis

RNA synthesis of whole cells is inhibited by 4-NQO and its derivatives. Tada, et al (135) reported that RNA synthesized from DNA isolated from 4-HAQO treated cells were approximately 1/10 the length of standard RNA's. Nonetheless, an increase in the number of RNA chains synthesized was noted. Sugimura et al (136) have explained this increase in the number of transcription initiating points may be due to the increased number of scissions in the DNA sugar phosphate chain.

Kuwano, et al (137) found that tRNA irradiated with visible light along with 4-NQO ($10^{-4}$ to $10^{-3}$M) decreased the amount of guanylic acid in proportion to the time irradiated. Other nucleotides were not affected. This conformational change inhibited the ability of the tRNA to accept proline. It should be stressed that no such effect occurred without the irradiation.

L. Protein Synthesis

Protein synthesis of the whole cell is inhibited by 4-NQO. This is usually explained in terms of the previously noted transcription process inhibition. Nagasaki and Naora (138) found evidence that the incorporation of $^{14}C$-leucine as well as $^{14}C$-arginine incorporation was inhibited by the oncogen. These data suggest that the 4-NQO does not just cause a simple misreading. Earlier work by the same authors reveals that 4-NQO or 4-HAQO does not affect in vitro protein synthesis. Nagasaki and Naora (138) have reported that 4-NQO gives a higher $^{14}C$-leucine uptake by the reticulocyte ribosome.

## METHODS

### A. Bacteria Growth and Preparation - A Typical Experiment

Freeze-dried Lactobacillus casei subsp. rhamnosus (ATCC 7469) was purchased from American Type Culture Collection, Rockville, Maryland. The bacteria was kept viable in nutrient agar medium (Table 5) with stab-culture transfers made biweekly.

The methods of Leach and Snell (139) for measuring the uptake and incorporation of amino acids were modified to allow the addition of water soluble oncogen and eventual determination of carbon-14 activity in a liquid scintillation system.

To insure the luxuriant growth of Lactobacillus casei, (L. casei) the bacteria for each experiment was transferred from the stock culture in a conventional manner to double strength liquid culture medium (Table 6) and incubated for 18-24 hours at 37°C.

After incubation the bacteria cells were harvested by centrifugation and resuspended in sterile 0.15 M NaCl solution. After the saline wash, the bacteria was once again separated by centrifugation and diluted with balanced salts solution (Table 7) to a turbidity reading of 125 (5.56 mg, dry weight/ml), taken on a Klett-Summerson photoelectric colorimeter.

### B. Standard Uptake of Amino Acids, General Method

One ml of bacterial cell suspension in balanced salts solution (5.56 mg/ml) was diluted with 3 ml saline and incubated

TABLE 5

NUTRIENT AGAR MEDIUM

| | |
|---|---|
| Bactopeptone | 5 g |
| Glucose | 5 g |
| Bacto-agar | 10 g |
| Water | 500 ml |

TABLE 6

DOUBLE STRENGTH LIQUID CULTURE MEDIUM

| Amino Acids | mg | Vitamine Supplement | mg |
|---|---|---|---|
| DL-alanine | 1900 | Riboflavin | 6.4 |
| L-arginine·HCl | 484 | Nicotinic acid | 6.4 |
| L-asparagine | 800 | Ca pantothenate | 6.4 |
| L-aspartic acid | 200 | Thiamine HCl | 3.2 |
| L-cysteine | 100 | p-aminobenzoic acid | 3.2 |
| L-cystine | 200 | Folic acid | 0.160 |
| L-glutamic acid | 600 | Pyridoxine·HCl | 0.160 |
| Glycine | 50 | Biotin | 0.032 |
| L-histidine HCl | 124 | | |
| DL-isoleucine | 500 | | |
| DL-leucine | 500 | | |
| L-lysine·HCl | 500 | | |
| DL-methionine | 700 | | |
| DL-phenylalanine | 200 | | |
| L-proline | 200 | | |
| DL-serine | 900 | | |
| DL-threonine | 400 | | |
| DL-tryptophan | 80 | | |
| L-tyrosine | 200 | | |
| DL-valine | 500 | | |

| Purines and Pyrimides | mg | Salts and Glucose | mg |
|---|---|---|---|
| Adenine | 10 | $MgSO_4 \cdot 7H_2O$ | 400 |
| Guanine·HCl | 10 | NaCl | 20 |
| Uracil | 10 | $FeSO_4 \cdot 7H_2O$ | 20 |
| Xanthine | 10 | $MnSO_4 \cdot H_2O$ | 29 |
| | | | g |
| | | Glucose | 10 |
| | | Sodium acetate·$3H_2O$ | 6 |
| | | $KH_2PO_4$ | 1 |
| | | $K_2HPO_4$ | 1 |

TABLE 7

BALANCED SALTS SOLUTION

| | g/liter |
|---|---|
| $K_2HPO_4$ | 0.25 |
| $KH_2PO_4$ | 0.25 |
| $MgSO_4$ | 0.10 |
| $NaCl$ | 0.005 |
| $FeSO_4 \cdot 7H_2O$ | 0.005 |
| $MnSO_4$ | 0.005 |

at 37.5°C for 15 min. After the 15 minute interval, one ml of 1% glucose solution was added and the incubation was continued for an additional 5 minutes. One ml of the selected carbon-14 labeled amino acid, (33 μCi) (New England Nuclear Corp.) was added without further purification.

Aliquots of 0.5 ml were taken from the uniformly suspended cell suspension by means of an Eppendorf Autopipette (Brinkman Instruments, Inc.). The addition of labeled amino acid was marked as zero time.

The cells in each aliquot were collected by suction filtration on a glass fiber filter disc (Reeve Angel). After two saline washes utilizing the same autopipette tip, the filter discs containing the washed cells were placed in scintillation vials and dried at 80-90°C.

Two ml of toluene scintillation cocktail (toluene: Beckman; Fluoralloy TLA: Beckman) were added to the scintillation vial. The determination of carbon-14 activity was carried out in a Beckman LS-100 liquid scintillation system.

### C. Standard Incorporation of Amino Acids

The bacteria were cultured, harvested and resuspended in the manner described above. Glucose and labeled amino acid were also added as before. Aliquots of 0.5 ml were drawn from the uniformly suspended bacterial cell suspensions prepared as previously described and expelled into 1 ml of 12% trichloroacetic acid (TCA). The TCA cell suspensions were incubated at 37.5°C for 15 minutes. After incubation, the cells were collected and washed as before in the millipore filter apparatus, utilizing glass fiber filters. The discs were placed in scintillation vials, dried and counted as described previously.

### D. The Uptake of Amino Acids in the Presence of 4-Nitroquinoline-1-Oxide and Other Selected Oncogens

The L. casei was treated in the usual experimental manner and a one ml cell suspension (5.56 mg/ml) was incubated with varying amounts of 1 mM 4-nitroquinoline 1-oxide (4-NQO) purchased from Daiichi Pure Chemicals Co., Tokyo, Japan. The oncogen was previously dissolved in physiological saline. Variance in volumes was corrected by the addition of physiological saline so that the total oncogen-saline solution volume was 3 ml.

Oncogen-saline solutions were made as follows: Per 100 ml solution; 0.9g NaCl and 19 mg 4-NQO. Other oncogens substituted for 4-NQO were N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), Daiichi Pure Chemicals Co., 14.7 mg; and N-nitrosodimethyl-amine (NDMA), Eastman, 7.4 mg.

The oncogen-saline-cell suspension was incubated for 15 minutes at 37.5°C. One ml of 1% glucose was added and incubation was continued for an additional 300 seconds, with occasional manual swirling. Labeled amino acid was introduced and the time was marked as zero.

Aliquots of 0.5 ml were taken from the uniformly suspended cell suspension and treated as in the standard amino acid uptake study described previously.

### E. The Incorporation of Amino Acids in the Presence of 4-Nitroquinoline-1-oxide and Other Selected Oncogens.

Oncogen-saline-cell suspensions were prepared and incubated as described in the methods for measuring amino acid uptake in the presence of oncogen. Once again 1 mM oncogen-physiological saline solutions were used in varying amounts. After the addition of labeled amino acid aliquots of 0.5 ml were taken from the cell suspension and were expelled into 1 ml of 12% TCA. This suspension was incubated for 15 minutes after which time the acid insoluble fraction was filtered, washed, dried, and counted in the manner previously described.

### F. The Effect of Nononcogenic Compounds on Amino Acid Uptake and Incorporation.

The previously described methods were utilized to determine the effect of selected nononcogenic compounds on amino acid uptake and incorporation in order to compare effects of oncogens and nononcogens. One mM nononcogen solutions in physiological saline were made by the addition of selected nononcogen to 100 ml of 0.9% NaCl solution.

Nononcogens utilized included: Cleland's reagent (dithiothreitol) (Calbiochem), 15.4 mg, and 4-nitropyridine-N oxide (4-NPO) (K and K Laboratories), 14.0 mg. Actinomycin D, (Sigma Chemicals, Inc.) was utilized in a concentration of 0.125 mg/3 ml. The bacteria was treated in the same experimental manner as before with aliquots of 0.5 ml being taken from the suspension and counted directly as well as being subjected to the TCA treatment before counting.

### G. The Uptake and Incorporation of 4-Nitroquinoline 1-oxide-$^3H$.

4-Nitroquinoline 1-oxide-$^3H$ (4-NQO-$^3H$) was prepared in this laboratory utilizing the Skraup reaction adapted by Manske, et al (140). One ml of 1mM 4-NQO-$^3H$ gave a reading of 2,900 CPM in the liquid scintillation system used. The powdered $^3H$-4-NQO was dissolved in physiological saline to a concentration of 1 mM and substituted for 4-NQO in the uptake experiments described in the section on the determination of the effect of 4-nitroquinoline-1-oxide on amino acid uptake and incorporation. However, time zero was taken as the addition of oncogen and saline in contrast of the use of the addition of labeled amino acid as time zero. Sampling, washing, drying, and counting were carried out as previously described. Care was taken to duplicate the same concentrations found in amino acid uptake and incorporation work.

# RESULTS

## A. Bacteria Concentration

Turbidity readings of a known dry weight of Lactobacillus casei were taken on a Klett-Summerson photoelectric colorimeter so that dilutions of freshly harvested bacteria could be made for each experimental determination. These readings are listed in Table 8 and illustrated in Figure 4. A reading of 125 on the Klett-Summerson was routinely used. This is equivalent to a concentration of approximately 5.6 mg of bacteria per ml.

## B. Standard Uptake and Incorporation

Standard uptake and incorporation curves for 4-nitroquinoline 1-oxide-$^{3}H$ are shown in Figures 5 and 6, respectively. The data for these curves are listed in Table 9 and Table 10.

Figures 7 and 8 show uptake by the bacteria when labeled amino acid levels are at concentrations less than saturation. The data for these figures can be found in Tables 11 and 12.

Data for the standard uptake and incorporation of $^{14}C$ glycine are found in Tables 13 and 14. This data is plotted in Figure 9.

The data and figures for standard uptake and incorporation of other amino acids may be found as follows: L-alanine-$^{14}C$, Tables 15 and 16, Figure 10; L-methionine-$^{14}C$, Tables 17 and 18, Figure 11; L-tyrosine-$^{14}C$, Tables 19 and 20, Figure 12.

TABLE 8

KLETT-SUMMERSON PHOTOELECTRIC COLORIMETER STANDARDIZATION
0.0789 MG *LACTOBACILLUS CASEI* (DRY WEIGHT)

| K-S reading | ml suspension | concentration mg/ml |
|---|---|---|
| 150 | 12.7 | 6.2 |
| 140 | 13.3 | 5.9 |
| 131 | 13.9 | 5.8 |
| 123 | 14.6 | 5.5 |
| 115 | 15.0 | 5.3 |
| 107 | 15.5 | 5.1 |
| 101 | 16.1 | 4.9 |

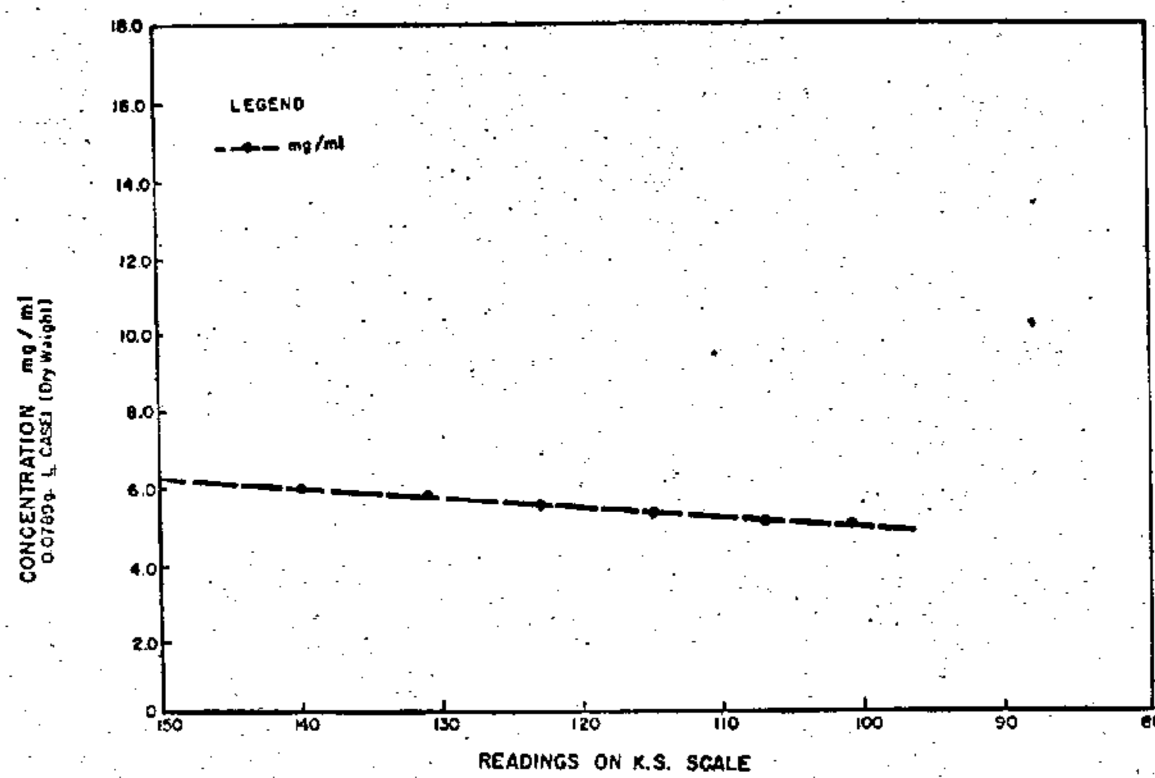



**Figure 6.** **Klett-Summerson Photoelectric Colorimeter standardization utilizing 0.0786 mg** ***Lactobacillus*** *casei*, dry weight.

TABLE 9

TOTAL UPTAKE OF 4-NITROQUINOLINE 1-OXIDE-$^{3}$H BY *LACTOBACILLUS CASEI*

| Time (sec) | Counts per minute |
|---|---|
| 60 | 685 |
| 180 | 192 |
| 300 | 218 |
| 420 | 142 |
| 600 | 132 |
| 1020 | 139 |
| 1140 | 120 |
| 1260 | 92 |
| 1440 | 90 |
| 1620 | 79 |
| 1800 | 72 |

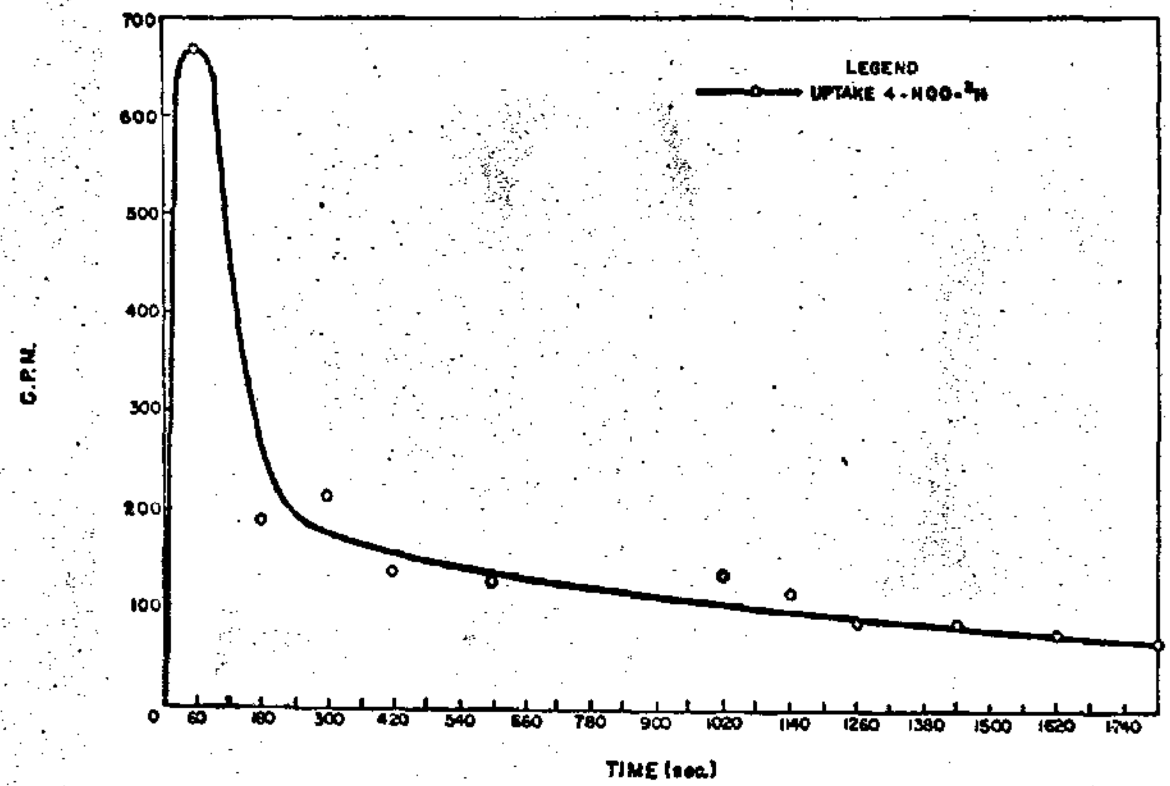



**Figure 5.** Uptake of 4-nitroquinoline 1-oxide-$^3$H by <u>Lactobacillus</u> <u>casei</u>

TABLE 10

INCORPORATION OF 4-NITROQUINOLINE 1-OXIDE-$^{3}H$
BY *LACTOBACILLUS CASEI* IN ACID INSOLUBLE PROTEIN

| Time (sec) | Counts per Minute |
|---|---|
| 60 | 91 |
| 120 | 99 |
| 180 | 72 |
| 300 | 63 |
| 420 | 62 |
| 660 | 60 |
| 960 | 62 |
| 1140 | 53 |
| 1260 | 58 |
| 1380 | 59 |
| 1440 | 52 |
| 1620 | 54 |

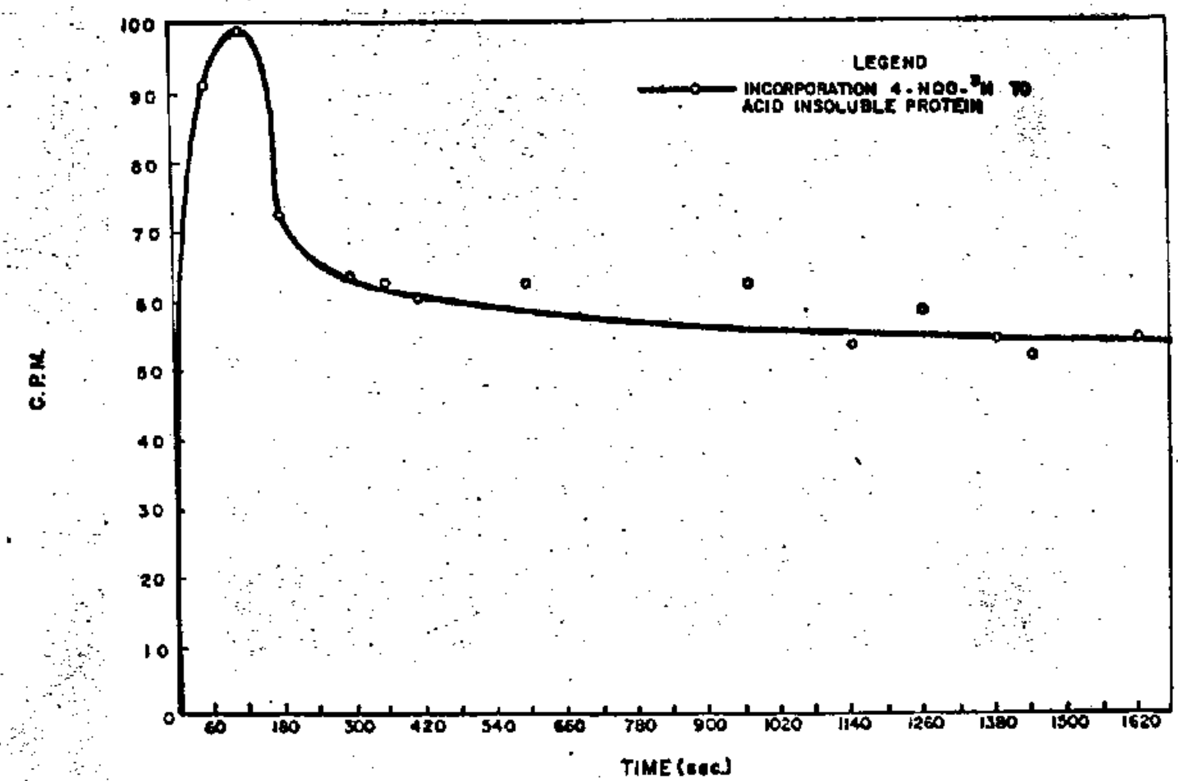



**Figure 6.** Incorporation of 4-nitroquinoline 1-oxide-$^3$H by <u>Lactobacillus casei</u>

TABLE 11

EFFECT ON UPTAKE OF $^{14}C$ AMINO ACID BY LACTOBACILLUS CASEI WHEN CONCENTRATIONS ARE BELOW SATURATION LEVELS. 10μCi GLYCINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 30 | 1490 |
| 120 | 1703 |
| 240 | 1837 |
| 660 | 2437 |
| 1080 | 2854 |
| 1980 | 3419 |
| 3200 | 4343 |

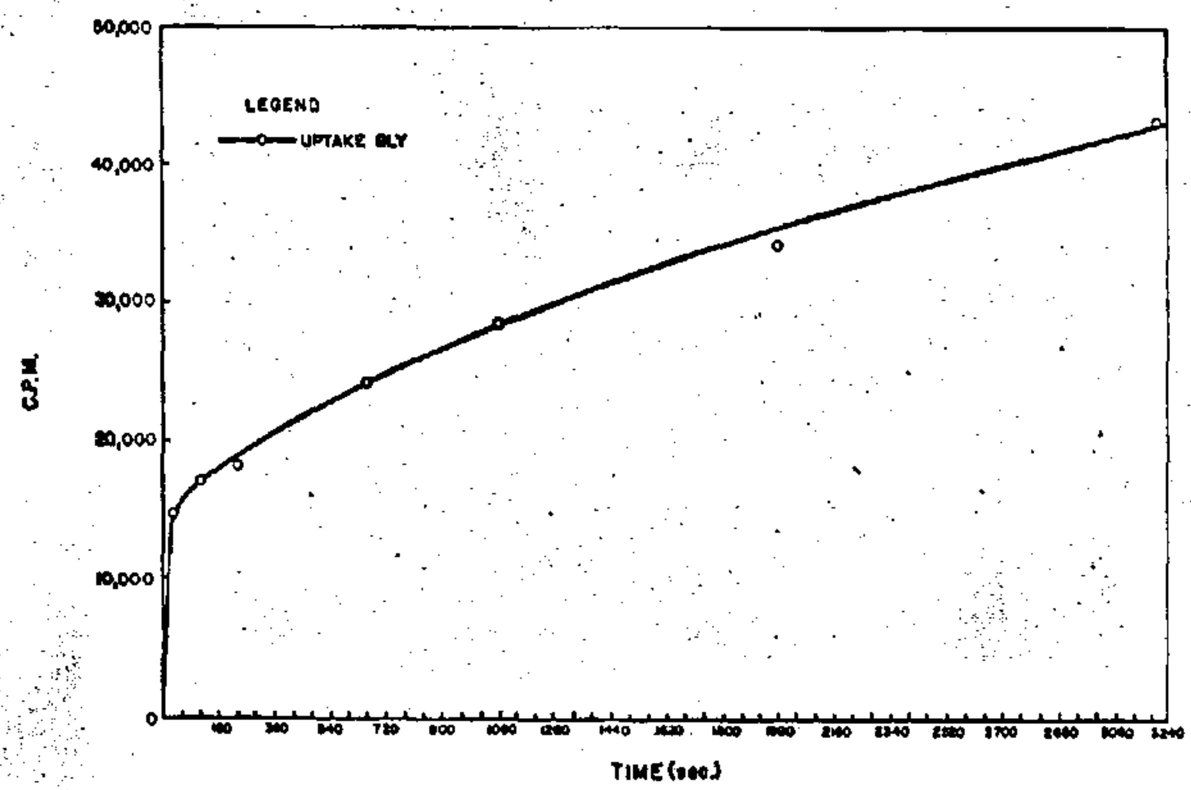



**Figure 7.** Effect on uptake of $^{14}C$ labeled amino acids by Lactobacillus casei when concentrations are below saturation levels. 10μCi glycine-$^{14}C$

TABLE 12

EFFECT ON UPTAKE OF $^{14}C$ AMINO ACIDS BY LACTOBACILLUS CASEI WHEN CONCENTRATIONS ARE BELOW SATURATION LEVELS. 1μCi L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 719 |
| 60 | 871 |
| 120 | 974 |
| 180 | 755 |
| 600 | 1231 |
| 720 | 1312 |
| 780 | 1436 |
| 1140 | 1526 |
| 1200 | 1751 |
| 1320 | 1548 |

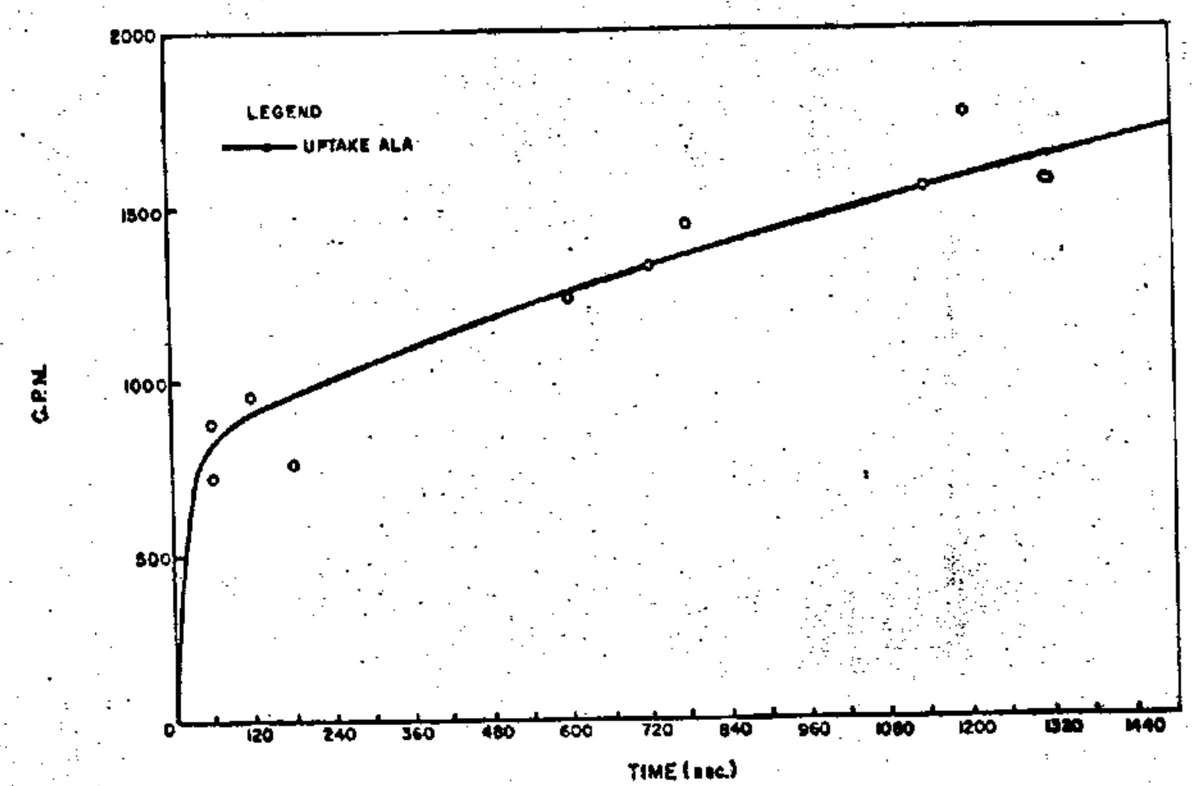



Figure 8. Effect on uptake of $^{14}C$ labeled amino acid by Lactobacillus casei when concentrations are below saturation levels. 1μCi l-alanine-$^{14}C$.

TABLE 13

STANDARD UPTAKE OF GLYCINE-$^{14}C$ BY *LACTOBACILLUS CASEI*, 33μCi GLYCINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 18,509 |
| 60 | 24,440 |
| 180 | 32,173 |
| 180 | 35,841 |
| 220 | 37,280 |
| 220 | 41,722 |
| 420 | 50,000 |
| 660 | 47,998 |
| 780 | 48,955 |

TABLE 14

STANDARD INCORPORATION OF GLYCINE-$^{14}C$ BY LACTOBACILLUS CASEI. 33μCi GLYCINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 220 | 16,569 |
| 220 | 14,338 |
| 360 | 27,121 |
| 360 | 24,316 |
| 480 | 23,500 |
| 600 | 28,434 |
| 600 | 36,538 |

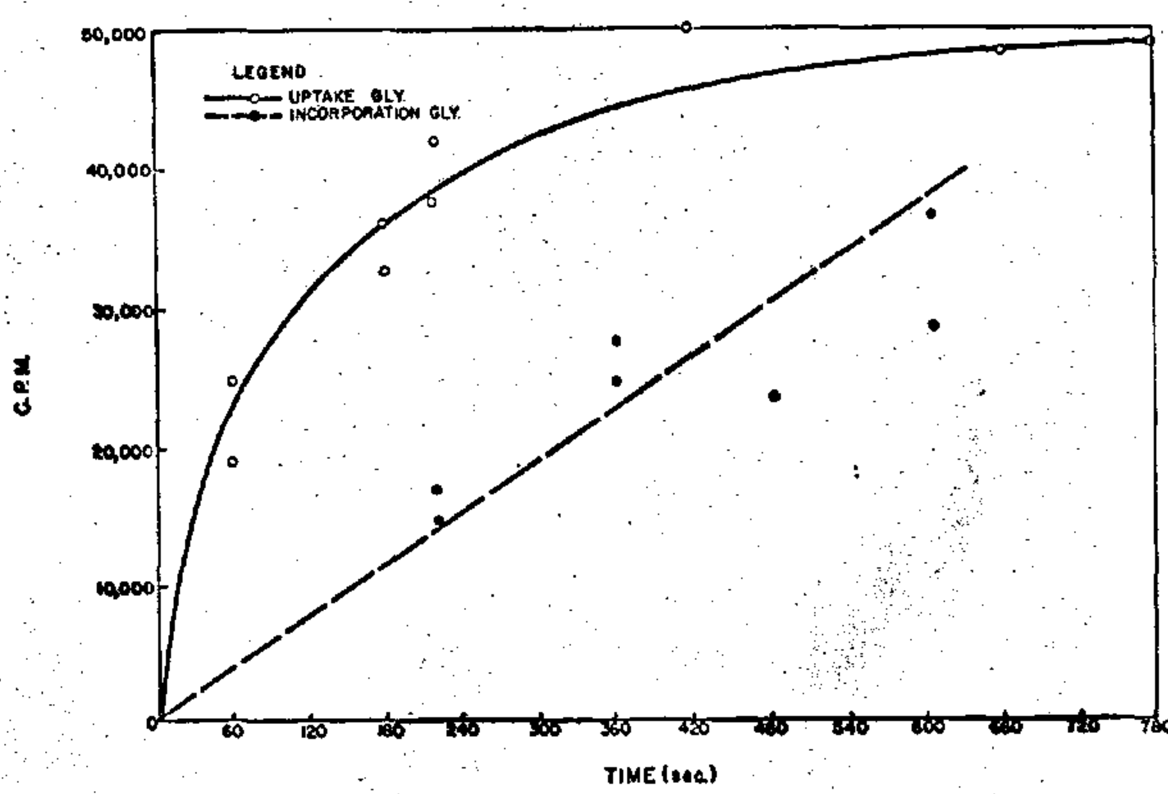



Figure 9. Standard uptake and incorporation of glycine-$^{14}C$ by *Lactobacillus casei*. 33µCi glycine-$^{14}C$.

TABLE 15

STANDARD UPTAKE OF L-ALANINE-$^{14}C$ BY LACTOBACILLUS CASEI.
33μCi L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 12,568 |
| 60 | 20,270 |
| 180 | 29,738 |
| 220 | 42,280 |
| 360 | 46,977 |
| 360 | 60,905 |
| 420 | 60,620 |
| 480 | 60,226 |
| 600 | 63,797 |

TABLE 16

STANDARD INCORPORATION OF L-ALANINE-$^{14}C$ BY *LACTOBACILLUS CASEI*.
33μCi L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 220 | 7,940 |
| 360 | 10,170 |
| 480 | 18,924 |
| 600 | 27,479 |
| 600 | 32,930 |

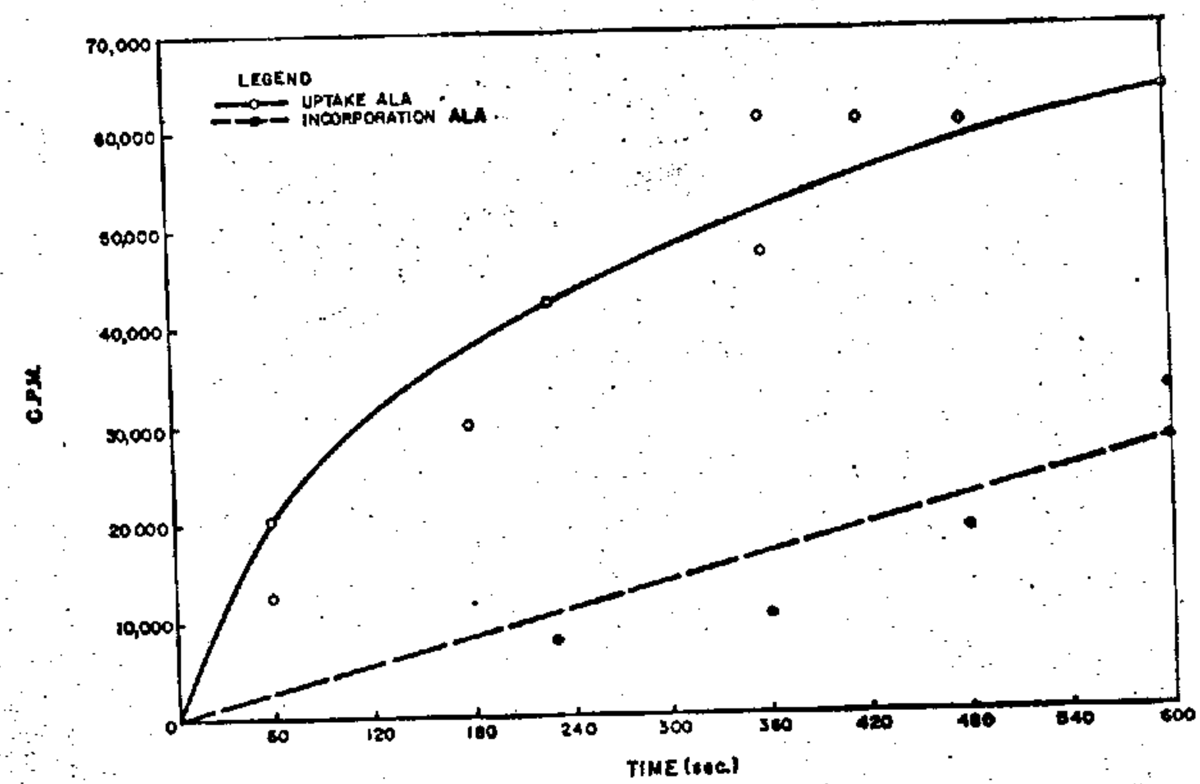



Figure 10. Standard uptake and incorporation of L-alanine-$^{14}C$ by <u>Lactobacillus casei</u>. 33µCi L-alanine-$^{14}C$.

TABLE 17

STANDARD UPTAKE OF L-METHIONINE-$^{14}C$ BY *LACTOBACILLUS CASEI*.
33μCi L-METHIONINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 114,259 |
| 60 | 117,247 |
| 180 | 144,291 |
| 300 | 118,299 |
| 300 | 108,649 |
| 420 | 136,454 |
| 600 | 142,699 |

TABLE 18

STANDARD INCORPORATION OF L-METHIONINE-$^{14}C$ BY LACTOBACILLUS CASEI.
33μCi L-METHIONINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 180 | 31,248 |
| 180 | 23,404 |
| 300 | 28,102 |
| 300 | 33,678 |
| 420 | 39,323 |
| 420 | 41,840 |
| 420 | 48,960 |
| 600 | 53,521 |
| 600 | 56,074 |

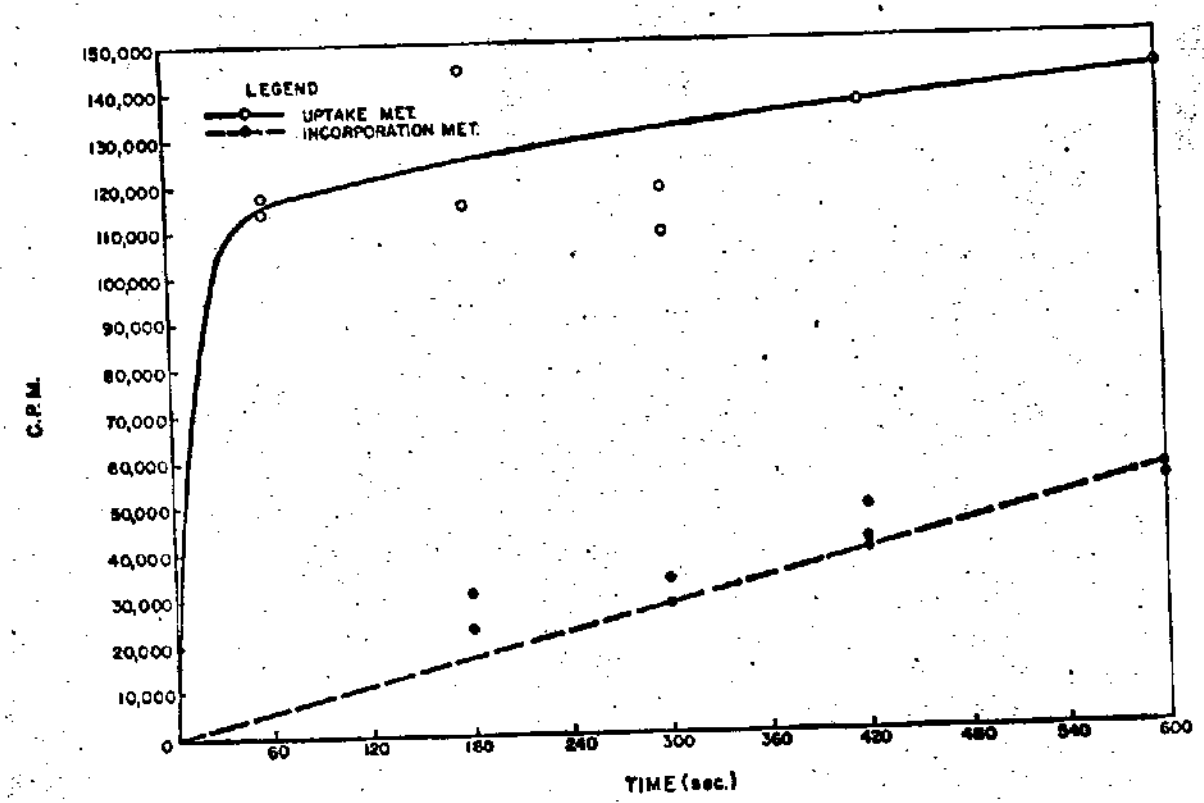



**Figure 11.** Standard uptake and incorporation of L-methionine-$^{14}C$ by *Lactobacillus casei*. 33µCi L-methionine-$^{14}C$.

TABLE 19

STANDARD UPTAKE OF L-TYROSINE-$^{14}C$ BY LACTOBACILLUS CASEI.
33μCi L-TYROSINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 35,210 |
| 60 | 37,577 |
| 180 | 47,816 |
| 180 | 52,400 |
| 300 | 69,815 |
| 300 | 46,119 |
| 420 | 64,815 |
| 420 | 63,637 |
| 600 | 56,089 |
| 600 | 65,344 |

TABLE 20

STANDARD INCORPORATION OF L-TYROSINE-$^{14}C$ BY *LACTOBACILLUS CASEI*. 33μCi L-TYROSINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 15,558 |
| 60 | 15,051 |
| 180 | 14,980 |
| 180 | 13,907 |
| 300 | 19,367 |
| 300 | 44,222 |
| 420 | 24,462 |
| 420 | 32,347 |
| 420 | 49,201 |
| 600 | 51,534 |
| 600 | 31,066 |

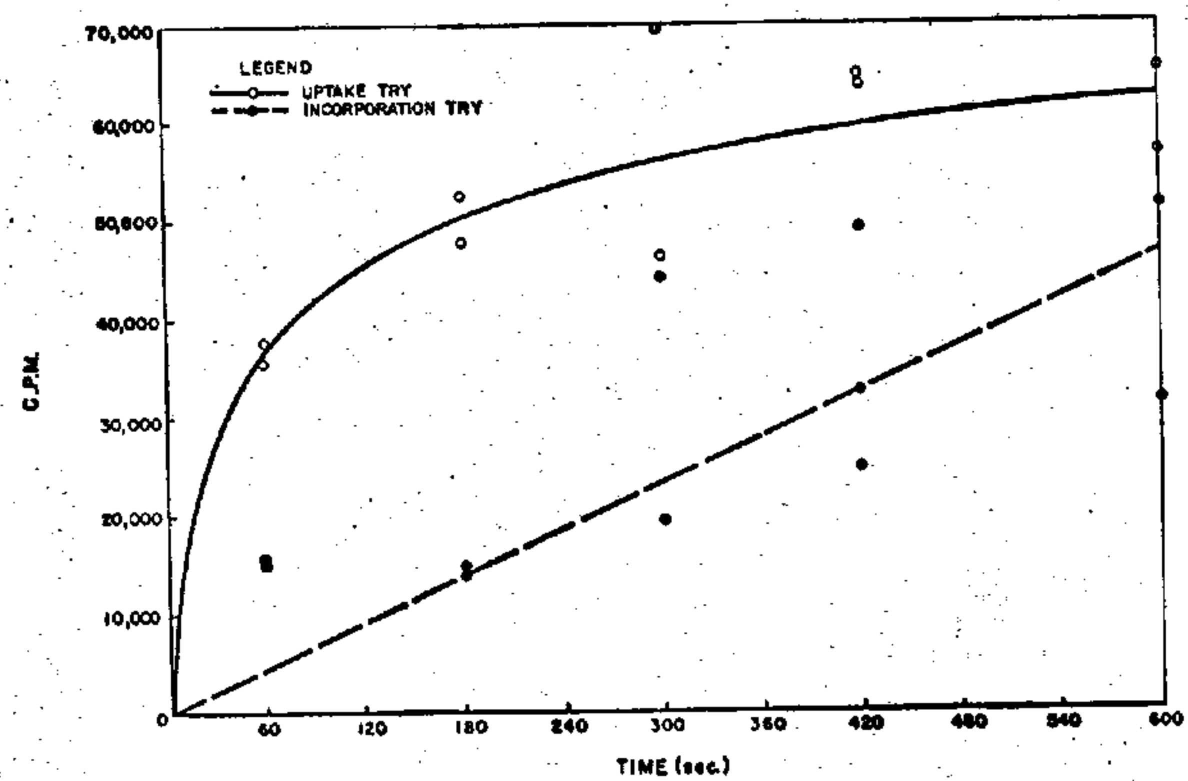



**Figure 12.** Standard uptake and incorporation of L-tyrosine-$^{14}C$ by *Lactobacillus casei*. 33μCi L-tyrosine-$^{14}C$/

### C. Effects of 4-Nitroquinoline 1-Oxide

The effect of 4-NQO concentration on glycine-14C uptake and incorporation can be found in Tables 21 through 24 and in Figures 13 and 14. The uptake values obtained when 0.1 ml/mM 4-NQO was added in the presence of L-alanine-$^{14}C$ are shown in Table 25 and diagramed in Figure 15. Incorporation results for the same experimental procedures are listed in Table 26 and shown in Figure 15.

Other uptake and incorporation data in the presence of 1 ml/mM 4-NQO are as follows: L-methionine-$^{14}C$ Tables 27 and 28, Figure 16; L-tyrosine-$^{14}C$ Tables 29 and 30, Figure 17.

### D. Effects of Other Chemicals

For comparative purposes, the nononcogenic chemical 4-nitropyridine 1-oxide was used to replace 4-NQO in the L-alanine-$^{14}C$ uptake experiments. The results are presented in Table 31 and illustrated in Figure 18. The incorporation of L-alanine-$^{14}C$ is shown in Table 32 and graphed in Figure 19.

Two known oncogenic chemicals were individually tested with this experimental procedure. The effect of 1 ml 1 mM N-nitrosodimethylamine on L-alanine-$^{14}C$ uptake is listed in Table 33 and drawn in Figure 20. The effect of this oncogen on L-alanine-$^{14}C$ incorporation is reflected in Table 34 and illustrated in Figure 21. Tables 35 and 36 record the effect of 1 m./mM of the other oncogen tested, N-methyl-N'nitro-N-nitroso guanidine, on the uptake and incorporation of L-alanine-$^{14}C$, respectively. Figure 22 records the data in Table 35 and Figure 23 records the data in Table 36.

TABLE 21

EFFECT OF 0.3 ml 1mM 4-NQO ON GLYCINE-$^{14}$C UPTAKE BY LACTOBACILLUS CASEI.
33μCi GLYCINE-$^{14}$C

| Time (sec) | Counts per minute |
|---|---|
| 60 | 19,253 |
| 60 | 19,463 |
| 180 | 21,156 |
| 300 | 23,660 |
| 420 | 24,365 |

TABLE 22

EFFECT OF 0.3 ml 1mM 4-NQO ON GLYCINE-$^{14}C$ INCORPORATION BY LACTOBACILLUS CASEI. 33μCi GLYCINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 16,969 |
| 60 | 16,774 |
| 300 | 19,989 |
| 420 | 22,253 |

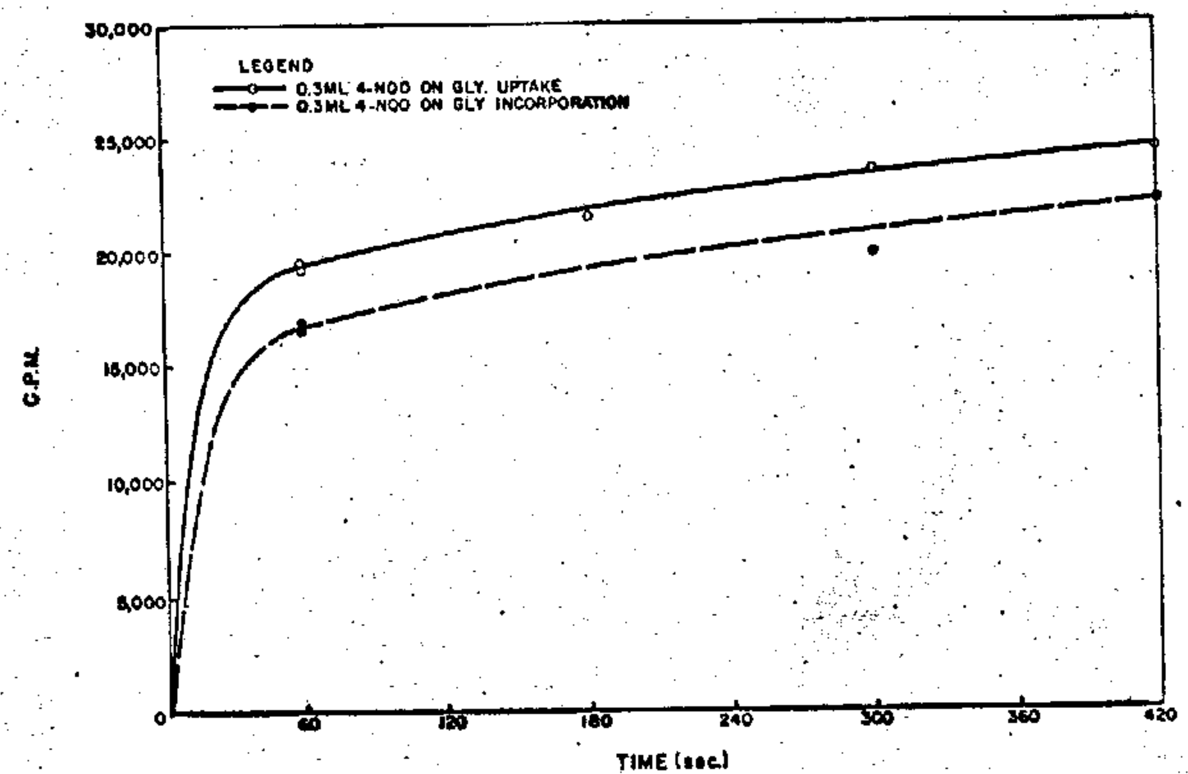



Figure 13. The effect of 0.3 ml 1 mM 4-NQO on the uptake and incorporation of glycine-14C by Lactobacillus casei. 33μCi glycine-$^{14}$C.

TABLE 23

EFFECT OF 1.0 ml 1mM 4-NQO ON GLYCINE-$^{14}C$ UPTAKE BY LACTOBACILLUS CASEI. 33µCi GLYCINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 120 | 13,542 |
| 300 | 18,352 |
| 340 | 17,913 |
| 420 | 21,674 |
| 480 | 20,187 |
| 600 | 19,778 |
| 600 | 18,582 |

TABLE 24

*EFFECT OF 1.0 ml 1mM 4-NQO ON GLYCINE-$^{14}C$*
INCORPORATION BY LACTOBACILLUS CASEI.
33μCi GLYCINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 300 | 12,519 |
| 340 | 11,465 |
| 600 | 18,581 |
| 660 | 18,137 |
| 720 | 17,195 |

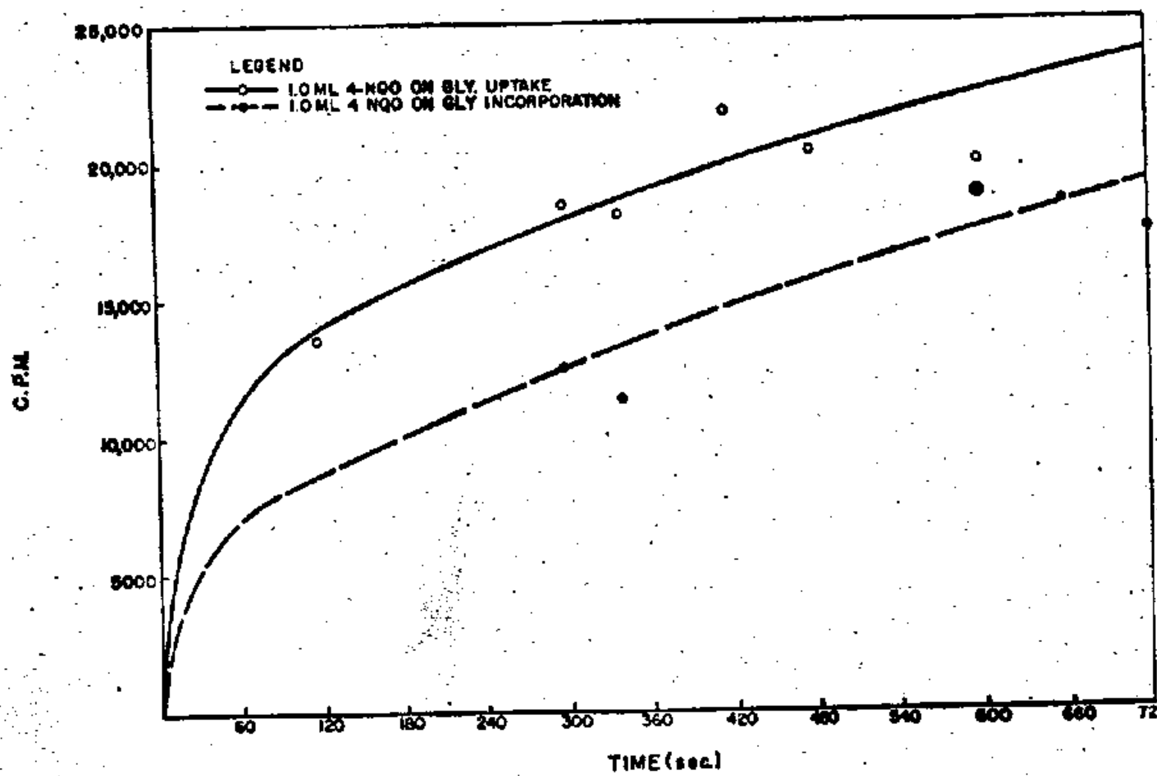



**Figure 14.** The effect of 1.0 ml 1 mM 4-NQO on the uptake and incorporation of glycine-$^{14}C$ by Lactobacillus casei. 33µCi glycine-$^{14}C$.

TABLE 25

EFFECT OF 0.1 ml 1mM 4-NQO ON L-ALANINE-$^{14}C$ UPTAKE BY LACTOBACILLUS CASEI.
33µCi L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 10,344 |
| 60 | 12,721 |
| 180 | 11,712 |
| 300 | 15,098 |
| 300 | 15,132 |
| 420 | 15,274 |
| 600 | 18,429 |
| 600 | 25,250 |

TABLE 26

EFFECT OF 0.1 ml 1mM 4-NQO ON L-ALANINE-$^{14}C$
INCORPORATION BY LACTOBACILLUS CASEI
33$\mu$Ci L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 10,049 |
| 300 | 15,513 |
| 420 | 17,075 |
| 600 | 19,340 |

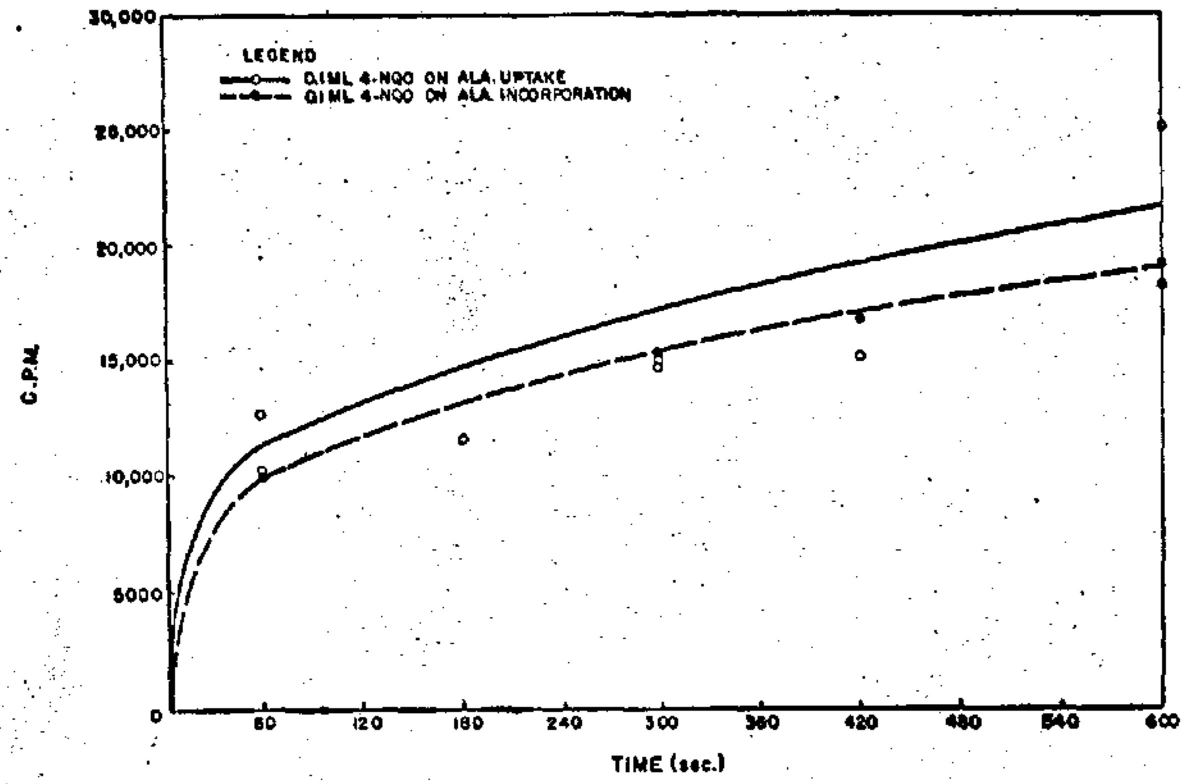



**Figure 15.** The effect of 0.1 ml 1 mM 4-NQO on the uptake and incorporation of L-alanine-$^{14}C$ by Lactobacillus casei. 33μCi L-alanine-$^{14}C$.

TABLE 27

EFFECT OF 1.0 ml 1mM 4-NQO ON L-METHIONINE-$^{14}C$
UPTAKE BY LACTOBACILLUS CASEI.
33μCi L-METHIONINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 66,487 |
| 180 | 74,171 |
| 180 | 73,138 |
| 300 | 96,166 |
| 420 | 92,106 |
| 600 | 115,919 |

TABLE 28

EFFECT OF 1.0 ml 1mM 4-NQO ON L-METHIONINE-$^{14}C$ INCORPORATION BY LACTOBACILLUS CASEI.
33µCi L-METHIONINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 18,751 |
| 60 | 24,195 |
| 180 | 25,618 |
| 360 | 36,829 |
| 360 | 30,760 |
| 600 | 47,552 |

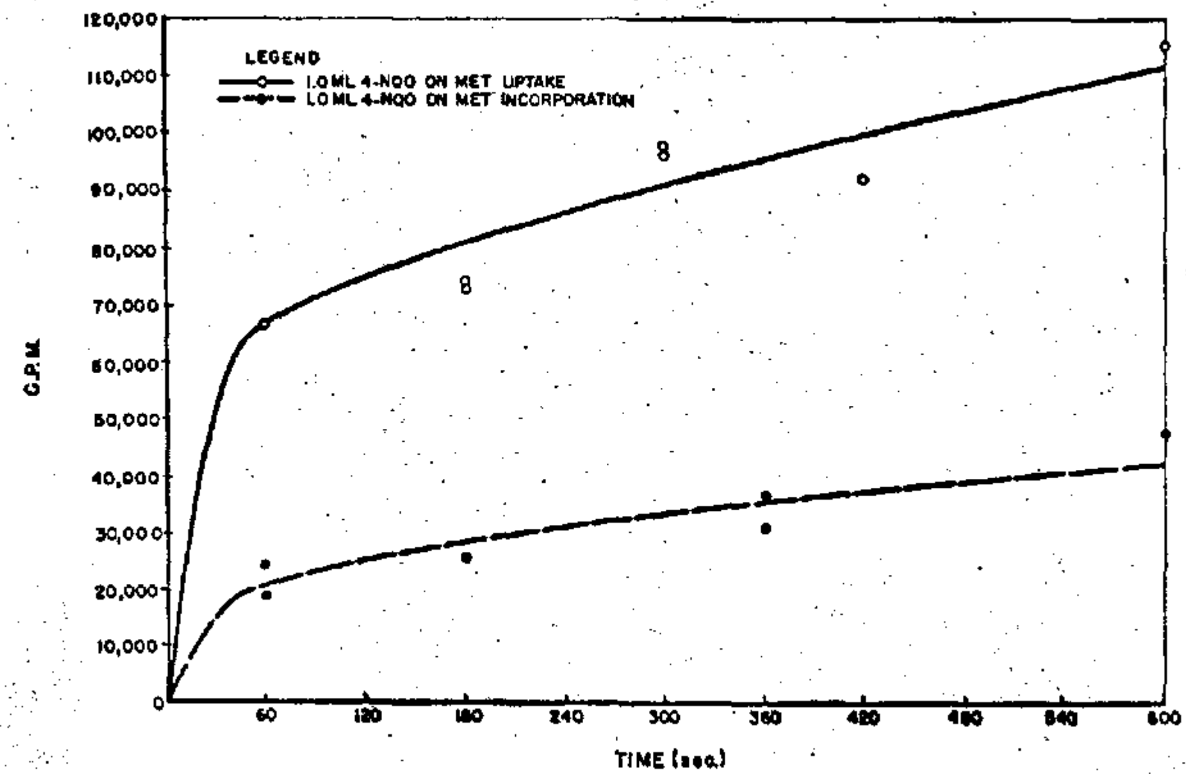



Figure 16. The effect of 1.0 ml 1 mM 4-NQO on the uptake and incorporation of L-methionine-$^{14}C$ by Lactobacillus casei. 33μCi L-methionine-$^{14}C$.

TABLE 29

EFFECT OF 1.0 ml 1mM 4-NQO ON L-TYROSINE-$^{14}C$
UPTAKE BY LACTOBACILLUS CASEI.
33$\mu$Ci L-TYROSINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 26,442 |
| 180 | 44,767 |
| 300 | 68,505 |
| 420 | 62,393 |
| 600 | 95,798 |
| 600 | 76,124 |

TABLE 30

EFFECT OF 1.0 ml 1mM 4-NQO ON L-TYROSINE-$^{14}$C INCORPORATION BY *LACTOBACILLUS CASEI*. 33μCi L-TYROSINE-$^{14}$C

| Time (sec) | Counts per minute |
|---|---|
| 60 | 42,518 |
| 180 | 65,607 |
| 180 | 31,925 |
| 300 | 42,427 |
| 420 | 79,977 |
| 600 | 66,183 |

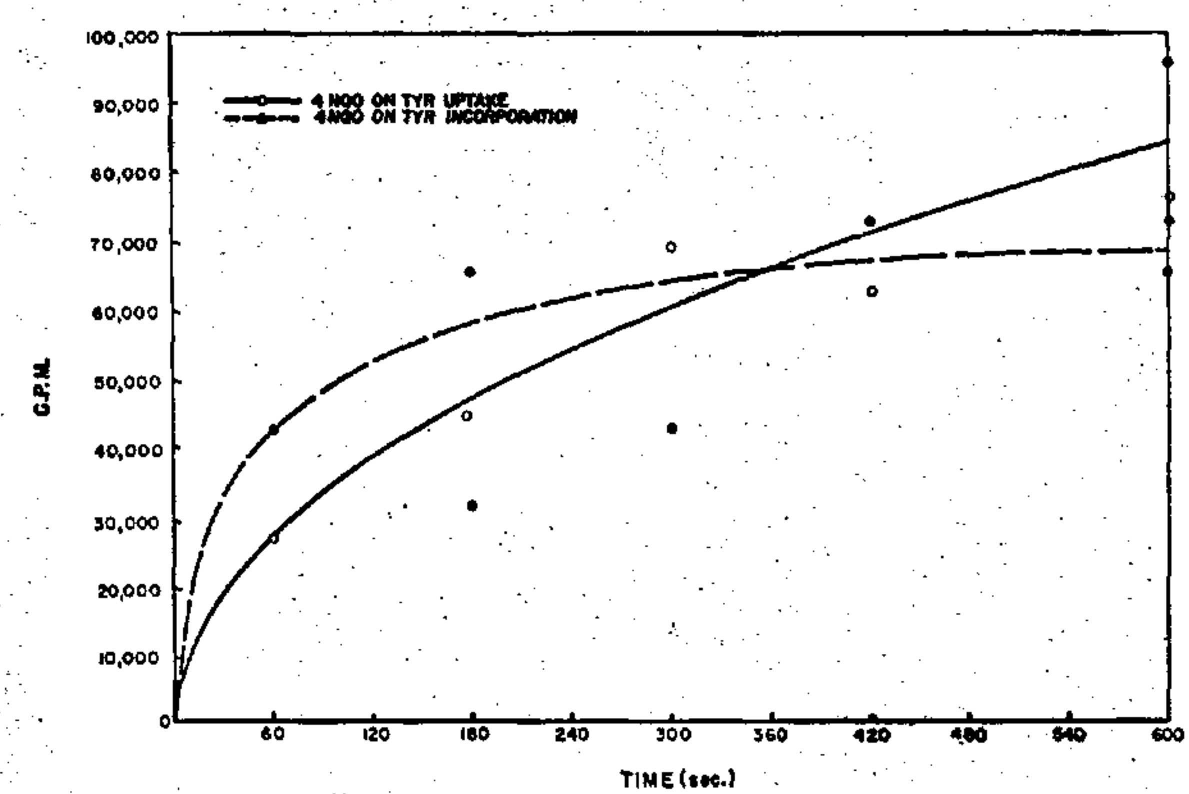



**Figure 17.** The effect of 1.0 ml 1 mM 4-NQO on the uptake and incorporation of L-tyrosine-$^{14}$C by *Lactobacillus casei*. 33µCi L-tyrosine-$^{14}$C.

TABLE 31

EFFECT OF 1.0 ml 1mM 4-NPO ON L-ALANINE-$^{14}C$ UPTAKE BY LACTOBACILLUS CASEI.
33µCi L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 19,371 |
| 60 | 14,988 |
| 60 | 11,268 |
| 180 | 16,175 |
| 180 | 33,016 |
| 180 | 20,230 |
| 300 | 23,897 |
| 300 | 28,591 |
| 300 | 34,720 |
| 420 | 29,284 |
| 420 | 36,331 |
| 420 | 32,936 |
| 600 | 31,749 |
| 600 | 31,200 |

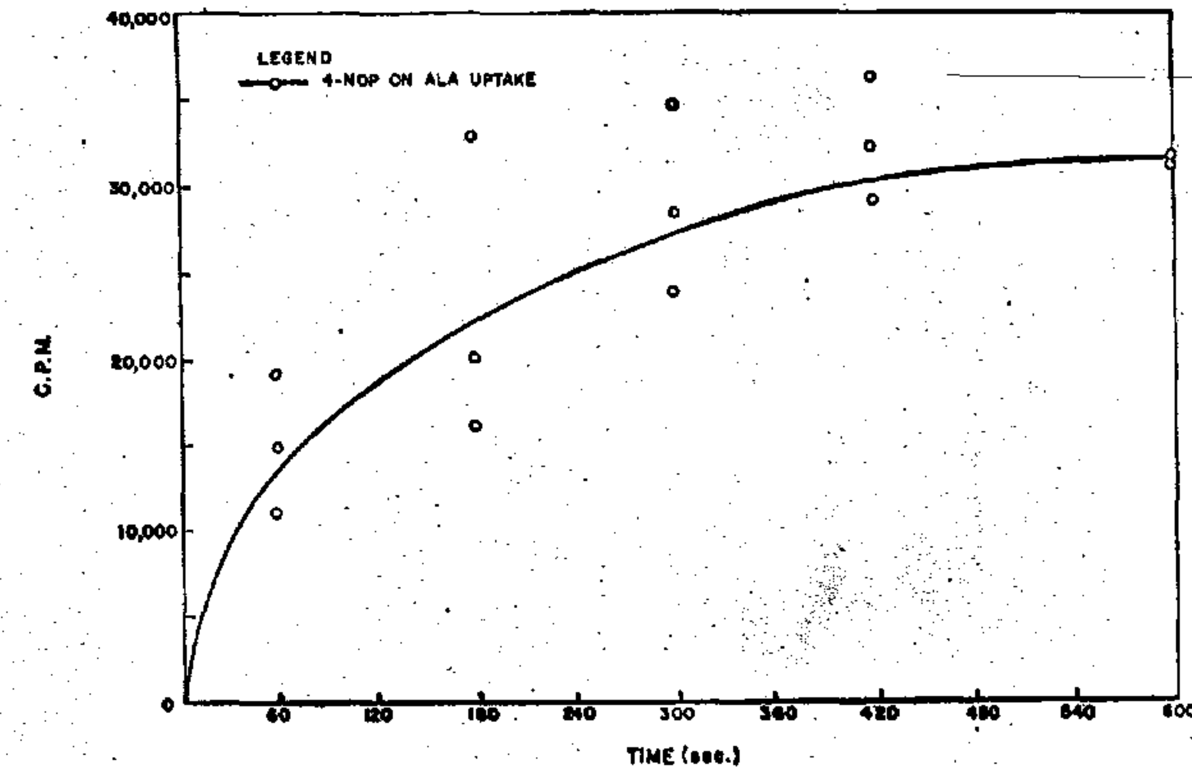



Figure 18. The effect of 1.0 ml 1 mM 4-NPO on the uptake of L-alanine-$^{14}C$ by *Lactobacillus casei*. 33μCi L-alanine-$^{14}C$.

TABLE 32

EFFECT OF 1.0 ml 1mM 4-NPO ON L-ALANINE-$^{14}C$ INCORPORATION BY <u>LACTOBACILLUS CASEI</u>. 33$\mu$Ci L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 12,160 |
| 60 | 11,567 |
| 180 | 14,922 |
| 180 | 10,566 |
| 300 | 13,862 |
| 300 | 21,471 |
| 300 | 10,505 |
| 420 | 11,475 |
| 420 | 25,237 |
| 420 | 17,451 |
| 600 | 30,293 |
| 600 | 24,332 |
| 600 | 18,995 |

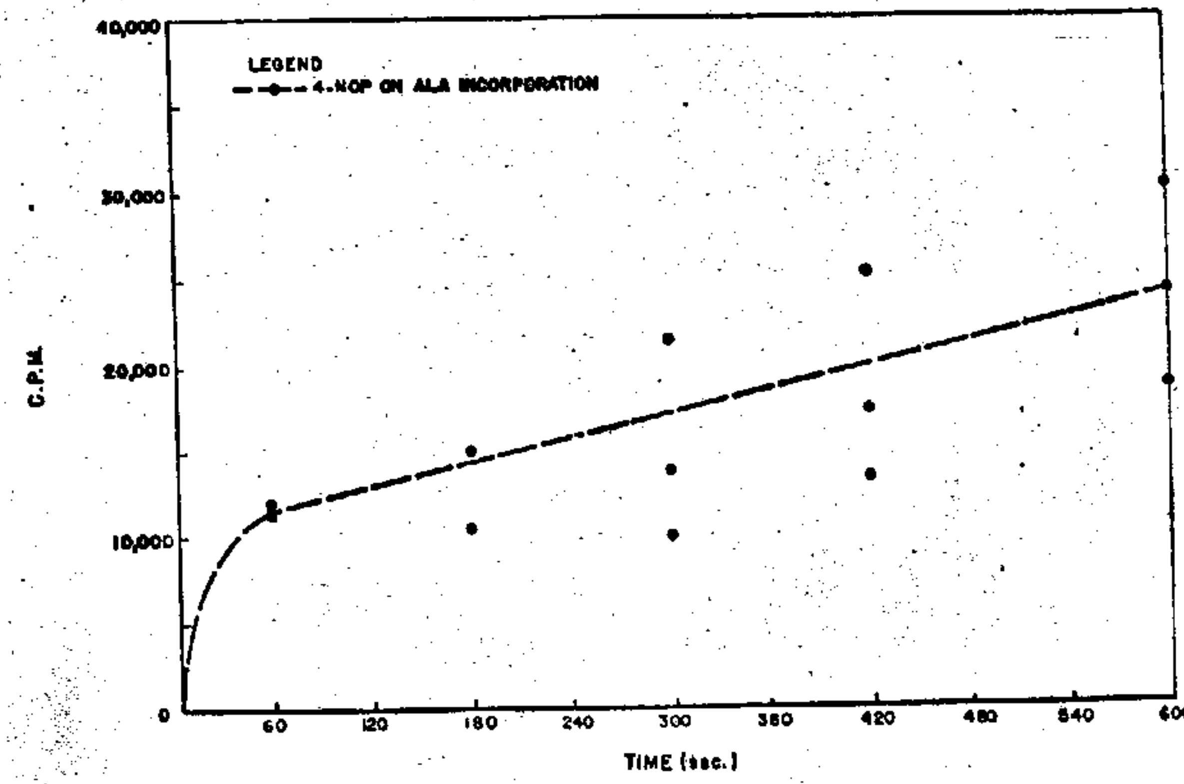



Figure 19. The effect of 1.0 ml 1 mM 4-NPO on the incorporation of L-alanine-$^{14}C$ by *Lactobacillus casei*. 33µCi L-alanine-$^{14}C$.

TABLE 33

EFFECT OF 1.0 ml 1mM NDMA ON L-ALANINE-$^{14}C$
UPTAKE BY LACTOBACILLUS CASEI.
33$\mu$Ci L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 54,668 |
| 300 | 72,348 |
| 420 | 90,968 |
| 600 | 80,703 |

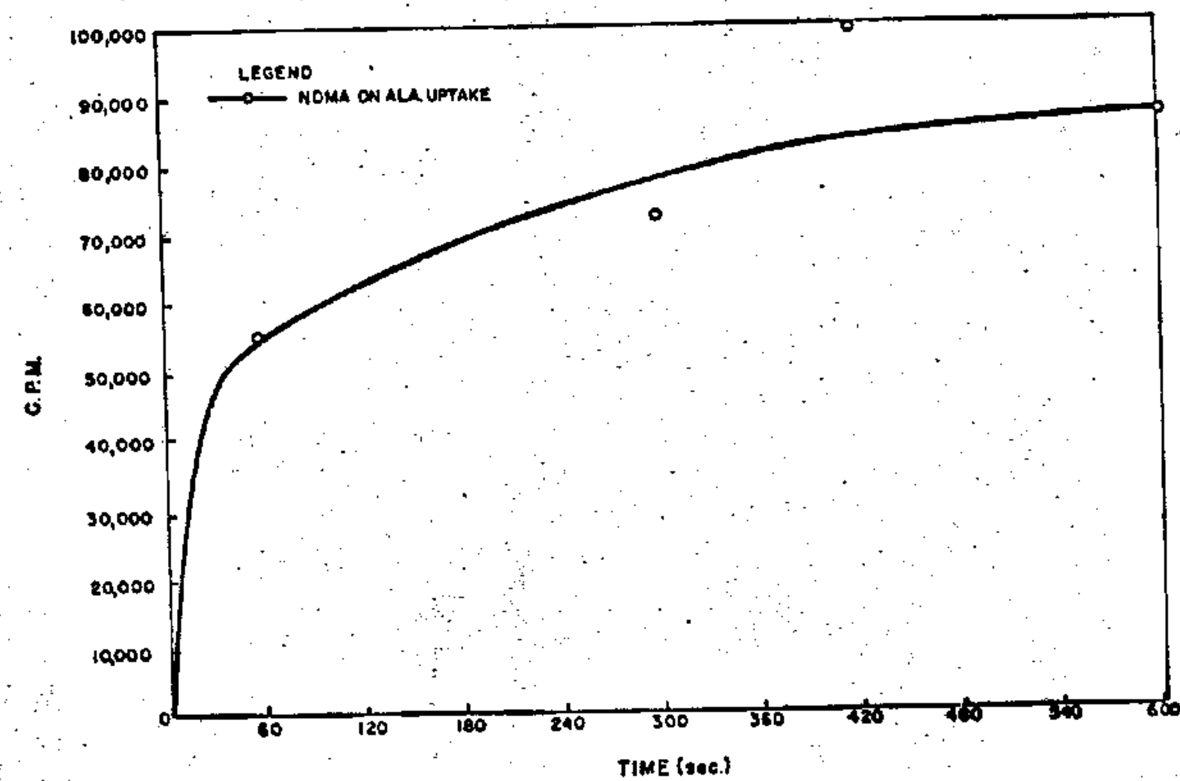



Figure 20. The effect of 1.0 ml 1 mM NDMA on the uptake of L-alanine-$^{14}C$ by *Lactobacillus casei*. 33µCi L-alanine-$^{14}C$.

TABLE 34

EFFECT OF 1.0 ml 1mM NDMA ON L-ALANINE-$^{14}C$ INCORPORATION BY LACTOBACILLUS CASEI.
33$\mu$Ci L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 17,122 |
| 180 | 55,906 |
| 420 | 85,376 |
| 660 | 70,018 |

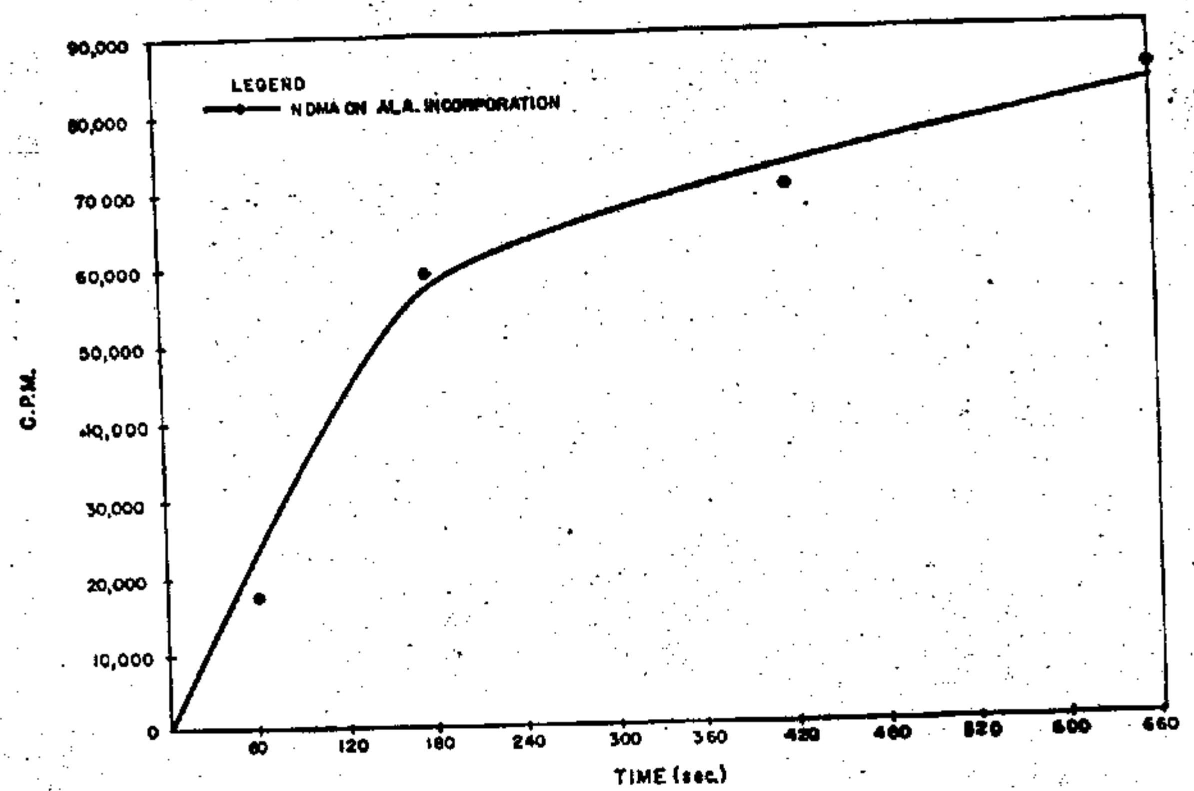



**Figure 21.** **The effect of** 1.0 ml 1 mM NDMA on the **incorporation of L-alanine-$^{14}C$** by <u>Lactobacillus</u> <u>casei</u>. 33μCi L-alanine-$^{14}C$.

TABLE 35

EFFECT OF 1.0 ml 1mM MNNG ON L-ALANINE-$^{14}C$
UPTAKE BY LACTOBACILLUS CASEI.
33μCi L-ALANINE-$^{14}C$

| Time (sec) | Counts per minute |
|---|---|
| 60 | 22,165 |
| 180 | 29,576 |
| 180 | 45,520 |
| 300 | 38,054 |
| 420 | 41,494 |
| 420 | 49,345 |
| 600 | 50,199 |

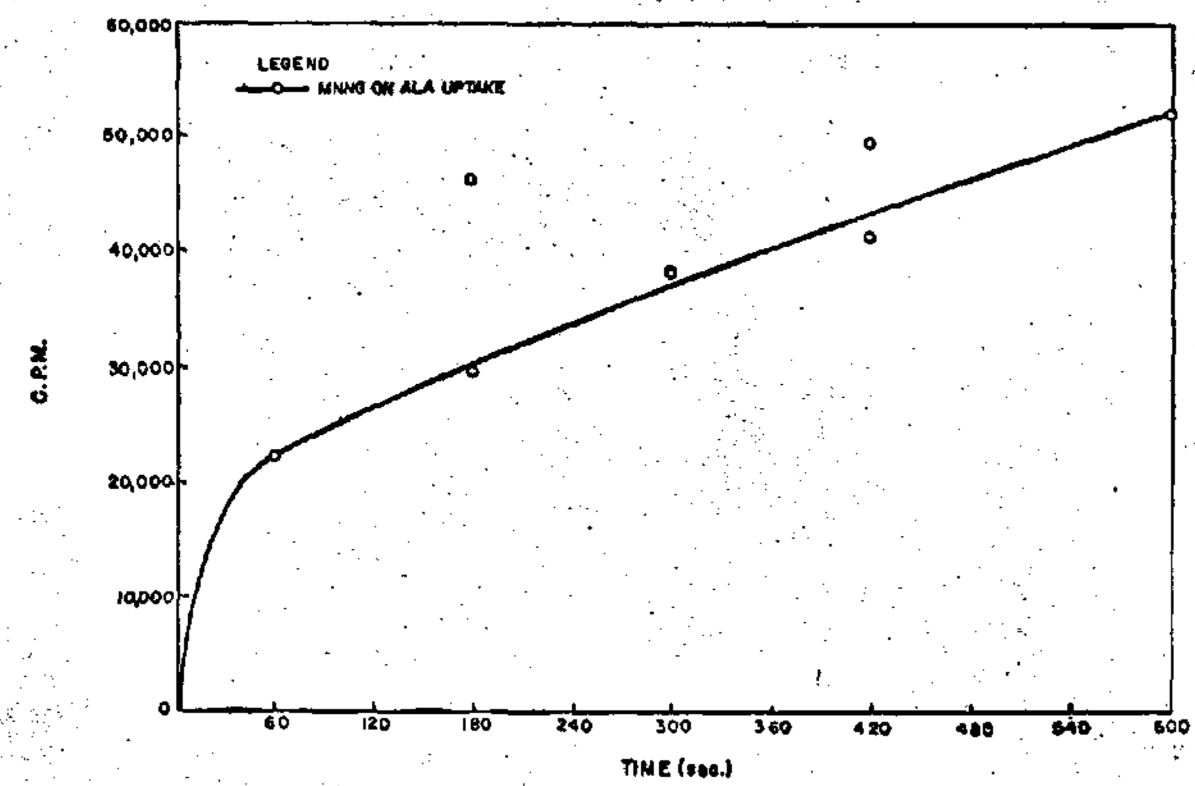



**Figure 22.** The effect of 1.0 ml 1 mM MNNG on the uptake of L-alanine-$^{14}C$ by <u>Lactobacillus</u> <u>casei</u>. 33$\mu$Ci L-alanine-$^{14}C$.

TABLE 36

EFFECT OF 1.0 ml 1mM MNNG ON L-ALANINE-$^{14}$C INCORPORATION BY <u>LACTOBACILLUS</u> <u>CASEI</u>. 33μCi L-ALANINE-$^{14}$C

| Time (sec) | Counts per minute |
|---|---|
| 60 | 18,851 |
| 180 | 21,745 |
| 300 | 14,602 |
| 420 | 24,118 |
| 600 | 25,872 |

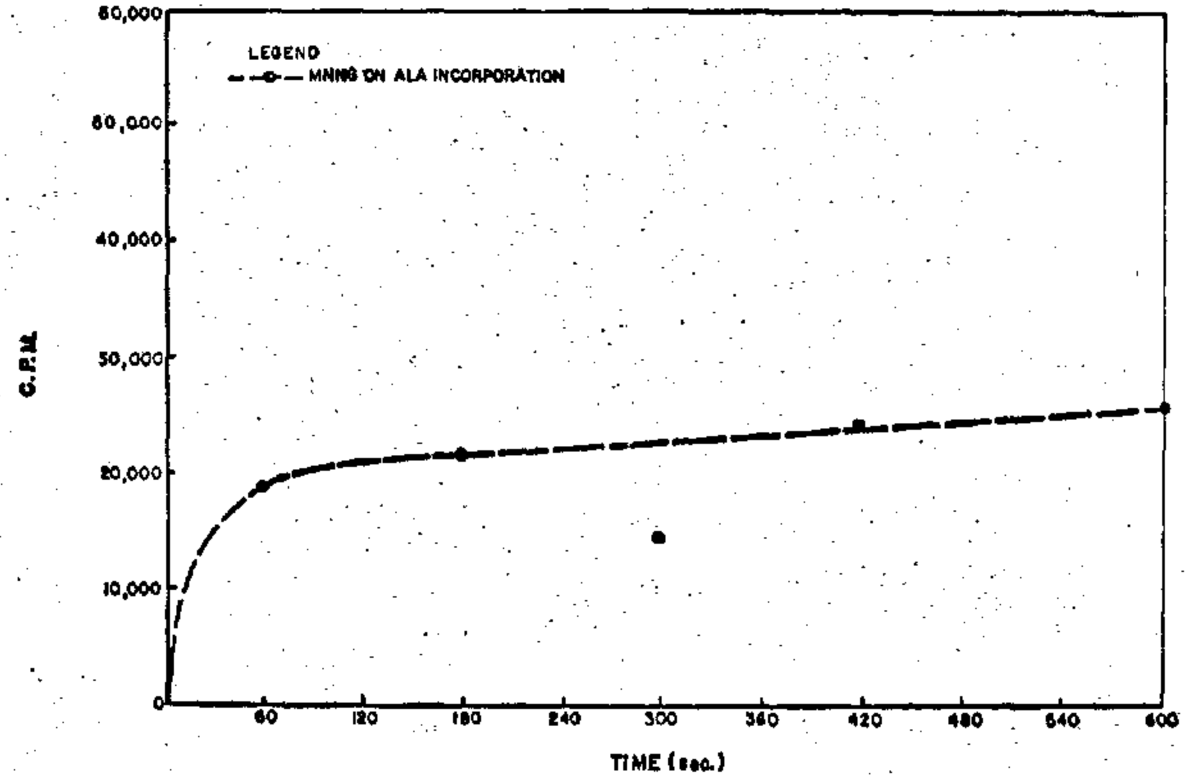



**Figure 23.** The effect of 1.0 ml 1 mM MNNG on the incorporation of L-alanine-$^{14}C$ by Lactobacillus casei. 33μCi L-alanine-$^{14}C$.

TABLE 37

UPTAKE OF 4-NITROQUINOLINE 1-OXIDE-$^3$H BY
LACTOBACILLUS CASEI IN THE PRESENCE OF ACTINOMYCIN D

| Time (sec) | Counts per minute |
|---|---|
| 60 | 128 |
| 120 | 111 |
| 240 | 102 |
| 300 | 103 |
| 360 | 90 |
| 420 | 91 |
| 540 | 74 |

## DISCUSSION

### A. Preface

It has now become apparent that a major portion of spontaneous oncogenic states in humans has been reproduced by chemical induction. It is therefore, not only desirable, but most necessary in our industrialized society to develop and utilize a simple, standard procedure to determine if a chemical has oncogenic properties. One of the most obvious physical characteristics of mammals suffering from cancer is an alteration of normal tissue. The relationship of tissue integrity to genetic function has stimulated an extensive search for alterations in genetic material by chemical oncogens.

Closely associated with deoxyribonucleic acids (DNA) and the various forms of ribonucleic acids (RNA) is protein synthesis and the amino acids. Most of the work that has been reported on the effect of known oncogens on protein synthesis has been its effect on gross synthesis, i.e. either the test organism grows slower or faster. It was therefore considered of interest to investigate the effect on the uptake and incorporation of amino acids by selected oncogens, with special emphasis on 4-nitroquinoline 1-oxide.

### B. Standard Uptake and Incorporation of Amino Acids

Because of the availability, ease of handling, nonpathogenicity and amount of previously published work, Lactobacillus casei was selected as the experimental organism for this in vivo work. Alanine is required in greater amounts for optimum growth of Lactobacillus casei than any other amino acid (See Table 6). Before the fate of alanine in resting Lactobacillus casei cells in the presence of oncogen could be determined, standard uptake and incorporation curves of L-alanine-$^{14}C$ were determined. These curves closely parallel those reported by Leach and Snell (139). Any discrepancies are most easily explained by the differences in methods. Characteristic rapid uptake levels were observed, with maximum levels being reached within ten minutes. The chief difficulty in determining the standard uptake of L-alanine-$^{14}C$ was in finding the appropriate ratio of bacteria cell concentration to labeled compound. Until saturation with $^{14}C$ label was accomplished a steady drift upward in alanine uptake was observed. It was determined that 33μCi was an appropriate level of 5.56 mg/ml Lactobacillus casei diluted to 6 ml. The addition of 50μCi L-alanine-$^{14}C$ did not alter the experimental results.

The incorporation of carbon-14 labeled alanine as expected was directly proportional to time. This was once again in agreement with reported data (139). Labeled glycine, methionine, and tyrosine were substituted for alanine with similar results. All exibited immediate rapid uptake of the amino acid with the incorporation being time dependent.

### C. *Uptake and Incorporation of 4-Nitroquinoline 1-oxide-$^3H$ by* Lactobacillus casei

In order to establish whether 4-nitroquinoline 1-oxide reacted with the macromolecules in the bacteria, tritium labeled 4-NQO was added to the experimental system. The Lactobacillus casei experienced an immediate and rapid uptake of tritium activity, presumably of the labeled oncogen. After approximately one minute the trend is sharply reversed by the bacteria and the amount of tritium found in the organism is rapidly lowered. The remaining label is then gradually lowered.

The incorporation of tritium activity into trichloroacetic acid insoluble protein also is initially rapid. The organism then begins an equally rapid removal of bound activity. After three minutes, most of the radioactivity present in the Lactobacillus casei is in bound form. This data lends further support to the hypothesis that the mechanism for 4-NQO-induced oncogenesis is a rapid process where the contact with the target tissue or organism need only be for a minimal time (141,142).

It is of interest to note at this time the speed with which an oncogenic state may be induced in this experimental system. This is of great merit when suggesting rapid test for possible oncogenic chemicals.

The addition of Actinomycin D (0.0042 mg) in addition to 1 ml 1 mM 4-NQO-$^3H$ prevented the uptake of the higher amounts reported above. The initial amounts were much lower than those given in the absence of Actinomycin D (see Tables 9 and 37).

Actinomycin D is known to prevent the synthesis of messenger RNA and in turn prevent new protein synthesis. However, 4-NQO was apparently not excluded from the organism because of its presence in the experimental system.

### D. Introduction of 4-Nitroquinoline 1-oxide into the Standard L-Alanine-$^{14}C$ Experimental System

The presence of 4-nitroquinoline 1-oxide in the experimental system had a distinct effect upon the uptake and incorporation of all the amino acids tested. L-alanine-$^{14}C$ was the most sensitive to the presence of 4-NQO. Quantities of 0.1 ml of 1 mM oncogen in saline diluted to six ml in the experimental system caused a decrease in the amount of L-alanine-$^{14}C$ taken up by the bacteria. These data were very much in line with previously reported work from this laboratory showing a decrease in growth of Lactobacillus casei in the presence of 4-NQO (110). When the level of oncogen is increased to 0.056 mM or 0.167 mM the amount of L-alanine-$^{14}C$ taken up is reduced to what is apparently the minimum uptake level in this test system of 10,000 to 12,000 CPM.

Far more startling than the lowering of amino acid uptake by the oncogen is the effect of 4-NQO on the amino acid incorporation. The incorporation of L-alanine-$^{14}C$ is initially increased above the levels of standard alanine incorporation. Upon examination of the curves and data it becomes apparent that incorporation of amino acid parallels amino acid uptake.

These data suggest that a potentially cancerous initiating state, as reflected by enhanced protein synthesis, has been induced in the test organism with only 0.1 ml of 1 mM 4-NQO. The

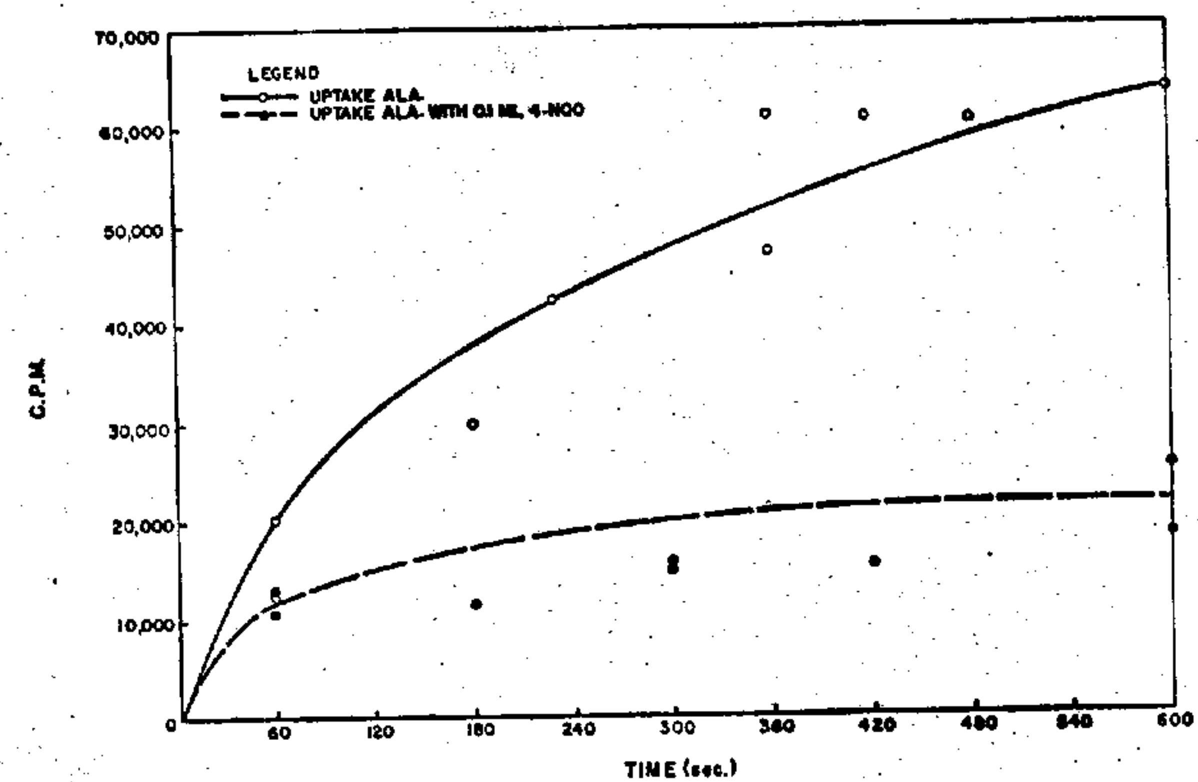



**Figure 24.** A comparison of the standard uptake of L-alanine-$^{14}C$ with L-alanine-$^{14}C$ uptake in the presence of 0.1 ml 1 mM 4-NQO. 33µCi L-alanine-$^{14}C$.

increase in amino acid incorporation, even though amino acid uptake is reduced, suggested that control mechanisms for protein synthesis in the bacteria have been impaired. In the remainder of the discussion there will be frequent reference to the shape of the amino acid incorporation curves. The standard curves demonstrate a direct relationship between time and uptake. The curves given by Lactobacillus casei in the presence of oncogen appear to be most dependent on the amino acid concentration in the bacteria cell.

One of the greatest difficulties in using in vivo systems for investigational purposes is that no two organism ever act exactly alike. Even though careful consideration was given to controlling experimental parameters some scatter was experienced. Some of these points have been included in the data section of this manuscript, not to demonstrate to lack of accuracy of the test system, but instead to show that shapes of various data curves are not altered appreciable even though they might be moved up or down the graphic scales.

### E. Reversal of the Effect of 4-Nitroquinoline 1-oxide on L-Alanine-$^{14}$C Uptake and Incorporation

As has been pointed out previously the addition of -SH group containing compounds reverse the biochemical effects of 4-NQO (110). Among other research groups, this laboratory has reported that cysteine, glutathione, and Cleland's Reagent (dithiothreitol) are examples of the variety of -SH compounds that will effect this reversal.

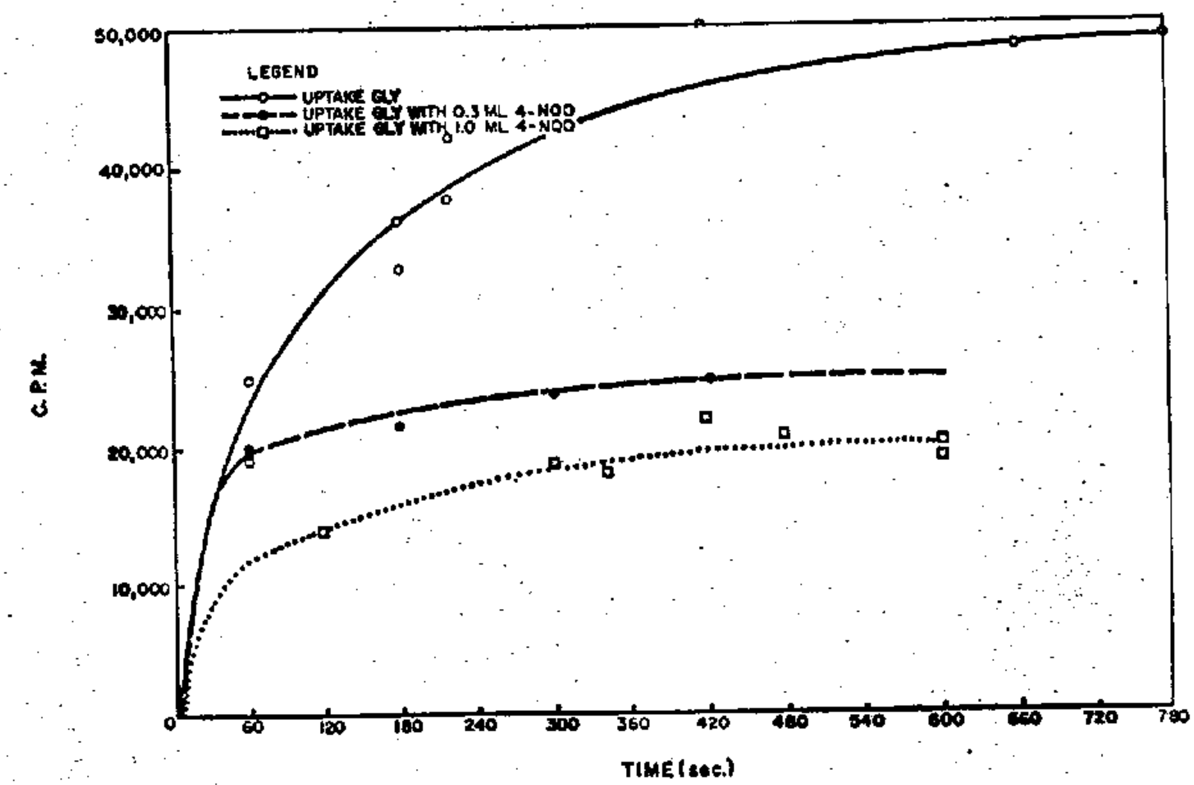



Figure 25. A comparison of the standard uptake of glycine-$^{14}C$ with glycine-$^{14}C$ in the presence of 0.3 ml 1 mM 4-NQO and 1.0 ml 1 mM 4-NQO. 33μCi glycine-$^{14}C$.

In the experimental system presently under discussion Cleland's Reagent had a reversing effect upon the biochemistry of 4-NQO. When used in a 1:1 molar:molar ratio the uptake of L-alanine-$^{14}$C was increased from the levels given in the presence of 0.0167 mM 4-NQO solution without Cleland's Reagent. The incorporation curves of L-alanine-$^{14}$C are also altered when Cleland's Reagent is added to the 4-NQO-bacterial cell suspension. The incorporation curve no longer follows the amino acid concentration dependent curve, demonstrating a biochemical effect that could be related to an initial carcinogenic state, but follows the time dependent curve of the standard incorporation work.

### F. Effect of 4-Nitroquinoline 1-oxide on Other Selected Amino Acids and Reagents

(1) Glycine

Standard uptake and incorporation curves of glycine-$^{14}$C were as expected. The uptake curve showed an initial rapid uptake with a saturation level less than that observed for alanine. The effect of 4-NQO on glycine uptake was less pronounced than that for alanine. The addition of 0.1 ml 1 mM 4-NQO solution had no obvious effect on the uptake curves. By increasing the amount of 1 mM 4-NQO solution to 0.3 ml and 1.0 ml an oncogen concentration-glycine uptake dependency was established. The greater the 4-NQO concentration the less total glycine was taken up by the organism (see Figure 25). These data suggest that 4-NQO may induce an alteration in the membrane of the bacteria reducing the amino acid uptake.

Initially, the 0.3 ml of 4-NQO (1 mM) enhanced the glycine incorporation even though the uptake was lowered by the addition of the same level of oncogen. A comparison of standard glycine-$^{14}C$ incorporation and incorporation in the presence of 0.3 ml 4-NQO is illustrated in Figure 26. A possible interpretation of these results is that two mechanisms are involved in the organisms response to the presence of 4-NQO; one response being a depression of the ability of the bacteria to efficiently transport amino acids across its membrane and the other may be either the loss of a specific protein coding-sequence mechanism or a general growth enhancement caused by a loss of inhibition control over protein synthesis.

(2) Methionine

The uptake of L-methionine-$^{14}C$ had a higher maximum limit than the other amino acids tested. The curve shape remained the same as other amino acids i.e. an initial rapid uptake rate followed by a leveling off of the curve. The uptake followed the characteristic time dependent pattern (Figure 11).

The addition of 1.0 ml 1 mM 4-NQO failed to lower the uptake of L-methionine-$^{14}C$ as much as had been expected. This indicates that the methionine uptake mechanism is less sensitive to the presence of the oncogen. The methionine, much like the glycine has its incorporation initially enhanced by the presence of oncogen. It has been reported (138, unpublished information) that methionine reacts with 4-NQO. Assuming this to be correct, methionine may have a reversing effect on 4-NQO-induced biological alteration. This reversing might be accomplished by simple

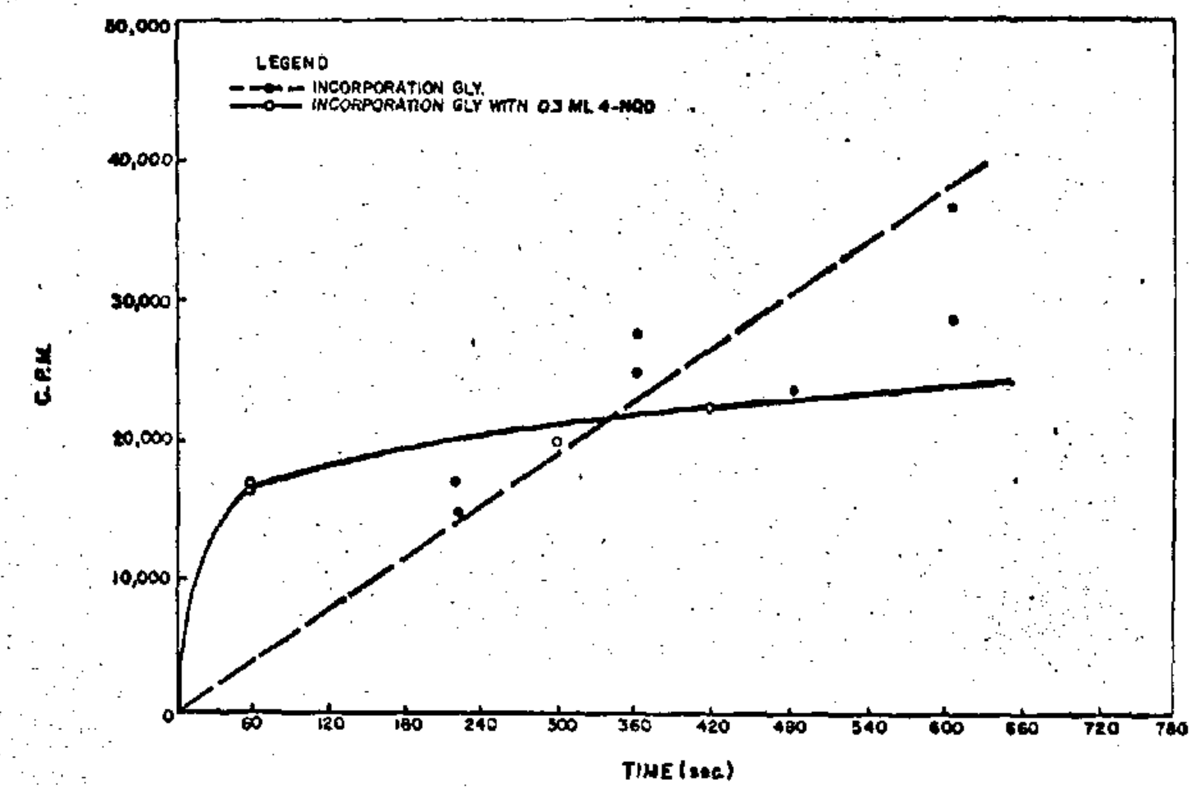



**Figure 26.** A comparison of the standard incorporation of glycine-$^{14}C$ with glycine-$^{14}C$ incorporation in the presence of 0.3 ml 1 mM 4-NQO. 33μCi glycine-$^{14}C$.

extracellular chemical reaction with the soluble 4-NQO or through a competetive replacement reaction with the 4-NQO-macromolecule complexes.

(3) Tyrosine

The uptake and incorporation of L-tyrosine-$^{14}C$ showed the expected results of quick initial uptake and a time dependent incorporation curve (Figure 12).

In the same discussion that alludes to 4-NQO reacting with methionine it was also pointed out that tyrosine reacts with 4-NQO to form a ninhydrin positive compound that was not tyrosine. Data on the uptake of L-tyrosine-$^{14}C$ by Lactobacillus casei indicates that, much like methionine, tyrosine is not greatly inhibited. The incorporation which is apparently stimulated poses some problems in interpretation. The data would lead to an interpretation of a greater incorporation of $^{14}C$ than of uptake. This being impossible, we propose that the 4-NQO alters or reacts with the tyrosine in such a way that it no longer can be washed through the millipore filter. Either the size of the complex is such that it is too large or it is precipitated with the other TCA insoluble protein.

(4) N-methyl-N'-nitro-N-nitrosoguanidine

Since alanine had proven to be the most sensitive amino acid to the presence of 4-NQO it was chosen for experimental work with another water soluble oncogen N-methyl-N'-nitro-N-nitroso guanidine (MNNG). One ml of 1 mM MNNG solution was introduced into the test system with only modest loss of L-alanine-$^{14}C$ uptake levels. The incorporation curve was altered in appearance very much like those that were amino acid concentration dependent. It is interesting to note that MNNG after an initial stimulation of alanine incorporation, reached an early apparent saturation of incorporation.

(5) N-nitrosodimethyl amine

The third known oncogen used in the experimental system was N-nitrosodimethyl amine (NDMA). When 1.0 ml 1 mM solution was used alanine uptake was stimulated modestly. The incorporation under the same experimental conditions showed a marked stimulation of $^{14}C$ alanine incorporation similar to that shown by alanine in the presence of 0.1 ml 1 mM 4-NQO.

(6) 4-Nitropyridine N-oxide

The presence of 1.0 ml 1 mM 4-nitropyridine N-oxide in the experimental system produced a small inhibition from the standard uptake of $^{14}C$ alanine curves. However, the inhibition was not of the severity the 0.1 ml 1 mM 4-NQO produced. The incorporation curve is a time dependent curve very similar to the standard $^{14}C$ alanine curve.

### G. A Proposed Mechanism

Before drawing final conclusions as to the possible routes through which 4-nitroquinoline 1-oxide may alter normal biological processes and thus initiate oncogenesis, it would be beneficial to survey briefly the currently accepted route of protein synthesis. The specific coding for the order of amino acids in proteins is found in the deoxyribonucleic acid (DNA). This coding is transcribed from the DNA to the messenger ribonucleic acid RNA (mRNA). The actual protein synthesis occurs at the ribosomal RNA (rRNA) level where the mRNA is held in place so that the codons of mRNA and the anticodons of the amino acid-charged transfer RNA (tRNA) can be paired. As is usual for metabolic processes, these steps are enzymatically catalyzed.

*We shall now consider each one of these steps in light* of the data given above.

Whereas it is quite possible, even probable, that there is some 4-NQO-DNA interaction the rate of amino acid incorporation in the organism is not totally inhibited at the levels used. Therefore the mRNA must continue to be transcribed from the DNA at much the same rate as before since it is apparently available or incorporation would not continue at the same rate as before, not to mention a possibility of increased rate of incorporation that was demonstrated in the data presented (see Table 22, 24, 26, 28, and 30).

In searching the data on 4-NQO-$^{3}$H we should be reminded that the amount of 4-NQO-$^{3}$H in the organism is considerably less when the labeled amino acid is added than when 4-NQO-$^{3}$H is initially added (see Figure 5). We would therefore suspect an oncogenesis reaction that would require a minimum amount of 4-NQO binding. When the components of DNA and RNA are examined we observe that both contain ribose sugar, phosphate residues and the aromatic nitrogen-containing bases guanine (G), adenine (A), and cytosine (C). DNA also contains thymine (T) and RNA contains uracil (U). It has been reported that 4-NQO reacts with guanine (133,143). It could therefore react with either DNA or RNA. 4-NQO does not appear to have a pronounced effect on tRNA (144,145). The terminal end of tRNA that attaches to specific amino acids contains A,C,C. There is no guanine present to react with 4-NQO and thus hamper the charging of the tRNA with an amino acid. The data in this paper also suggest that sufficient tRNA-amino acid complexes are available to continue protein synthesis at the normal or even increased rate (Tables 22, 24, 25, 28 and 30).

There is more rRNA in the cell than any other form of RNA. It does not appear to be completely logical to assume that the small amount of 4-NQO bound in the cell could alter all of the guanine in rRNA or even a sufficient amount to alter its biological properties.

This line of reasoning now leaves only the mRNA. A default judgement that mRNA was the point of 4-NQO attack would not be appropriate. In our examination of the role mRNA plays in protein synthesis we noted the codons of mRNA and the anti codons of tRNA. A listing of the amino acids involved in this research and their mRNA codon sequence is given in Table 38. Recalling that 4-NQO reacts with G, Table 38 lends itself to the following explanation: (1) Alanine is the most sensitive amino acid tested in the presence of 4-NQO and each one of its codons has at least one G. (2) Glycine, which appears to be less sensitive to 4-NQO, has at least 2G per codon. This descrepancy is best explained that by reacting with the 4-NQO fewer total codons are affected by 4-NQO interaction. (3) Methionine, even with one G per codon may react with the 4-NQO and thus prevent it from reacting with its codon. (4) Tyrosine which is affected least by the presence of 4-NQO has no C in its codon to react with the 4-NQO. (5) At any point where 4-NQO has attached itself to the codon the anticodon of tRNA may be sufficiently held to allow the formation of nonsense proteins.

In conclusion, we note that there are multiple possible 4-NQO-macromolecular interactions. We feel, however, from the above data and reasoning, that the 4-NQO-mRNA interaction may prove to be an initiating point for 4-NQO-induced oncogensis. It would also be appropriate to note that the other oncogens tested all changed their L-alanine amino acid incorporation curves from time dependent to concentration dependent. Even though no obvious chemical similarities can be drawn from structures their alteration of these curves may prove to be significant as a method of screening potential oncogens.

# SUMMARY

The test system developed for this work utilizing <u>Lactobacillus</u> <u>casei</u> gave the following results:

1. 4-Nitroquinoline 1-oxide-$^3$H is taken up rapidly by the bacteria in the experimental system. There is an equally rapid reduction in tritium label retained by the <u>Lactobacillus</u> <u>casei</u>.
2. 4-Nitroquinoline 1-oxide-$^3$H is incorporated into the bacteria initially and shows a reduction of incorporated tritium label with time.
3. Actinomycin D was added to interrupt the synthesis of messenger ribonucleic acid. This did not prevent the uptake of tritium labeled 4-nitroquinoline 1-oxide.
4. Standard glycine-$^{14}$C, L-alanine-$^{14}$C, L-methionine-$^{14}$C, L-tyrosine-$^{14}$C, uptake data show the characteristic initial rapid uptake, followed by a leveling off of the $^{14}$C amino acid concentrations in the cells.
5. Standard glycine-$^{14}$C, L-alanine-$^{14}$C, L-methionine-$^{14}$C, L-tyrosine-$^{14}$C incorporation data shows a time dependent series of values.
6. The L-alanine-$^{14}$C is the most sensitive to levels of 4-nitroquinoline 1-oxide of the amino acids tested.

7. The glycine-$^{14}C$ uptake levels were shown to be inversely dependent upon 4-nitroquinoline 1-oxide levels.
8. All amino acids tested experienced alteration of their standard incorporation curves in the presence of 4-nitroquinoline 1-oxide. L-Alanine-$^{14}C$ was altered when the level in the test system reached 0.0167 mM. Glycine-$^{14}C$ incorporation was changed when the oncogen level reached 0.05 mM. L-Methionine-$^{14}C$ and L-tyrosine-$^{14}C$ were altered from their standard incorporation values when the 4-nitroquinoline 1-oxide level was raised to 0.167 mM.
9. Other known oncogens tested in this experimental system were N-nitrosodimethyl amine and N-methyl-N'-nitro-N-nitrosoquanidine. They gave an alteration much like that given by 4-nitroquinoline 1-oxide.
10. The nononcogen 4-nitropyridine 1-oxide was tested and gave no alteration of the L-alanine-$^{14}C$ incorporation curve.

From the results presented a mechanism for the 4-nitroquinoline 1-oxide alteration of biological metabolism was suggested. Specifically, this was the interaction of the quanine residue in the messenger ribonucleic acid codon. It was also noted that the experimental method developed for this work may prove to be useful as a test system for chemicals suspected of oncogenic character.

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VITA

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