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˙CONTROL OF ERGOSTEROL BIOSYNTHESIS IN YEAST

[EXISTENCE OF LIPID INHIBITORS]

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CONTROL OF ERGOSTEROL BIOSYNTHESIS IN YEAST[*]

[EXISTENCE OF LIPID INHIBITORS]

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Abbreviations: MVA, mevalonic acid; HMG, 3-hydroxy-3-

methylglutario acid; NSF, nonsaponifiable fraction; FAF, fatty

acids fraction.

It was demonstrated that the incorporation of radioactivity from $1-^{14}C$-acetate into the nonsaponifiable fraction in S. cerevisiae was depressed by the addition of ergosterol to the growing medium under strictly anaerobic conditions. These results suggested that ergosterol in the culture medium might control its synthesis. Unexpectedly with the cell-free extracts of this organism, ergosterol had no effect on the incorporation of radioactivity from $1-^{14}C$-acetate into the nonsaponifiable fraction. However, the acidic lipids extracted from this organism were found to inhibit ^{14}C-incorporation into the nonsaponifiable fraction from $1-^{14}C$-acetate as well as from $3-^{14}C$-3-hydroxy-3-methylglutaryl-CoA, but not from $2-^{14}C$-mevalonate. The evidence obtained suggested that the acidic lipids inhibited the reduction of 3-hydroxy-3-methylglutaryl-CoA to mevalonate. The inhibitors were purified by column chromatography followed by thin-layer chromatography. Four compounds which had inhibitory activities were found on the thin-layer plate. One of the inhibitors was purified 1,000 fold based on the inhibitory activity. Discussions are made on the possible role of these inhibitors in the control mechanism of ergosterol synthesis in yeast.

As early as 1953, it was pointed out by many workers that hepatic cholesterol biosynthesis in animals is depressed by

cholesterol feeding(2-4). Subsequent investigations demonstrated that cholesterol biosynthesis is depressed at a step prior to MVA (5-7). Siperstein et al. (8-9) obtained the results indicating that HMG-CoA reduction might be a step which is subjected to feed-back inhibition in cholesterol biosynthesis. Although these observations suggested that cholesterol apparently controlled its own rate of synthesis by a negative feed-back mechanism, the inhibition of cholesterol synthesis in vitro by the addition of cholesterol could not been demonstrated (10). Therefore, many workers investigated the inhibitory effects of some other substances related to cholesterol on its synthesis. Beher and Baker (11) reported the inhibition of cholesterol synthesis in rats by feeding a diet containing cholic acid. On the other hand, the removal of bile from enterohepatic circulation led to an increased rate of hepatic cholesterol synthesis (12, 13). With intestinal cholesterol synthesis, Dietschy and Siperstein (14) observed that it is inhibited by the infusion of bile into small intestine. In addition to these observations, Fimognari and Rodwell (15) demonstrated the inhibition of MVA synthesis from acetate in rat liver homogenates by cholic, deoxycholic, taurocholic, and taurodeoxycholic acids. These experiments suggested that MVA synthesis in animals is controlled by bile acids which are presumed to be the end products in cholesterol metabolism.

Fimognari and Rodwell (16) also reported that mevalonate:
NAD oxidoreductase [EC 1.1.1.x] from Mycobacterium and Pseudo-
monas is inhibited by deoxycholic acid. However since these
organisms are known not to produce any sterol, the physiological
significance of this inhibition remained unsolved.

Yeast is well known to produce ergosterol but nothing had
been reported about the control of the biosynthesis of this
sterol prior to a preliminary communication from this laboratory
(1). We reported in the communication that the activity of
HMG-CoA reduction in ergosterol synthesis in yeast undergoes
possibly a feed-back inhibition by acidic lipids which are
formed from ergosterol. In this paper, detailed studies on the
inhibition of ergosterol biosynthesis by the acidic lipids in
yeast and the purification of the inhibitors are described.

EXPERIMENTAL

Experimental Procedure with Growing Cells --- Saccharomyces
cerovisiae (ATCC 12341) was used throughout this study. Experiments
with growing cells were carried out under strictly anaerobic
conditions. The medium for the growth contained the following
components in a final volume of 25 ml: 2.5 g of glucose, 170 mg
of Difco yeast nitrogen base, 8.0 mg of potassium oleate, 25 mg
of Tween 80, 0.03 µmole of 1-^{14}C-acetate (1,400,000 cpm), 1.25

- 4 -

mmoles of succinate buffer (pH 5.0), and the indicated amounts

of ergosterol. Strictly anaerobic incubations were carried out

at $28^{\circ}C$ in a desiccator according to the method of Meyer and

Bloch (19). After incubation, the cells were harvested by

centrifugation, washed three times with distilled water, and

divided into two portions. One portion was used for the deter-

mination of growth; the other portion, for the determination

of the incorporation of radioactivity into NSF. Growth was

represented as mg of dry weight. Saponification was carried

out with 15 % KOH in methanol in a nitrogen atmosphere at $70^{\circ}C$

for 2.5 hr. NSF was extracted from the mixture with ether by

the conventional method.

Preparation of Cell-free Extracts --- For the preparation

of cell-free extracts, yeast was grown semi-anaerobically at

$28^{\circ}C$ for 48 hr in a medium containing 2 g of $(NH_4)_2SO_4$, 2 g of

KH_2PO_4, 0.5 g of $Na_2HPO_4 \cdot 12H_2O$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.025 g

of $MnSO_4 \cdot 4H_2O$, 20 g of glucose, and 1 g of yeast extract per

liter. The harvested cells were washed twice with 0.1 M

potassium-sodium phosphate buffer, pH 6.2, suspended in the

same buffer containing 10% glucose (20 mg wet weight/ml), and

shaken for 150 min at $28^{\circ}C$ according to the method of Klein (20).

The cells, harvested again by centrifugation, were washed twice

with 0.1 M potassium phosphate buffer, pH 7.0. The cells thus

obtained were ground with an equal weight of the mixture of

carborundum and Celite 535 (1:1), and the mixture was treated with· 0.1 M potassium phosphate buffer, pH 7.0 (1 ml/1 g of ,wet cells). The extracts were centrifuged at 5,000 x g for 1 hr and the cell debris sedimented was discarded. The supernatant obtained was centrifuged again at 80,000 x g for 1 hr. After the floating lipid layer was carefully removed, the supernatant was used as the cell-free extracts.

Reaction with the Cell-free Extracts —— Typical reaction mixture contained the following components in a final volume of 1.0 ml; ATP, 5 μmoles; GSH, 2 μmoles; CoA, 0.1 μmole; NADP, 1 μmole; glucose-6-phosphate, 10 μmoles; $MgSO_4 \cdot 7H_2O$, 2 μmoles; $MnSO_4 \cdot 4H_2O$, 1 μmole; Tween 80, 1 mg; potassium phosphate buffer 80 μmoles; 0.3 ml of the cell-free extracts (7 mg protein); and $1-^{14}C$-acetate, $2-^{14}C$-MVA, $3-^{14}C$-HMG or $3-^{14}C$-HMG-CoA as indicated. The amount of substrate used was indicated in each experiment. Reactions were carried out in test tubes with constant shaking at 37°C for 1 hr. The reaction mixture was saponified with 15 % KOH in 80 % methanol. NSF, and FAF where indicated, were isolated and their radioactivities were determined by a conventional method. Tween 80, which was proved not to interfere the reaction, was used for dispersing lipids in the reaction mixture.

Radioactivity was counted in a Nuclear Chicago liquid scintillation spectrometer with an efficiency of 81 % for ^{14}C. Samples were assayed in a scintillation fluid containing 0.01 %

1,4-bis-2-(5-phenyloxazolyl) benzene and 0.4 % 2,5-diphenyloxazole in toluene.

Materials ---- $1-{}^{14}C$-Acetate (25 μCi/μmole) and $2-{}^{14}C$-MVA (5.0 μCi/μmole) were obtained from Daiichi Chemicals Company; and $3-{}^{14}C$-HMG (2.0 μCi/μmole), from New England Nuclear Co. $3-{}^{14}C$-HMG-CoA was synthesized according to the method of Hiltz et al. (17) and used without separation from free $3-{}^{14}C$-HMG. ${}^{14}C$-Ergosterol was prepared by incubating yeast cells with $1-{}^{14}C$-acetate followed by purification on thin-layer plates (18). NADP, GSH, CoA and glucose-6-phosphate were purchased from Sigma Chemical Company; and ATP, from Schwartz BioResearch Inc.. The other organic and inorganic chemicals were supplied by Nakarai Chemicals Company.

RESULTS

Effect of Ergosterol on the Conversion of $1-{}^{14}C$-Acetate into NSF in Growing Cells ---- S. cerevisiae is known to be able to synthesize unsaturated fatty acids and ergosterol when grown under aerobic conditions. Under strictly anaerobic conditions, however, this organism requires an unsaturated fatty acid and ergosterol for growth (21). Of various unsaturated fatty acids tested, oleic acid was found most effective (22). In the presence of an adequate amount of oleic acid, growth of the organism is dependent on the amount of ergosterol added (1, 23). In order to investigate the effect of ergosterol on its own biosynthesis,

the conversion of $1-^{14}C$-acetate into NSF and FAF was examined

with the cells which were grown in the presence of various

concentrations of ergosterol under strictly anaerobic conditions.

As reported in the preliminary communication ($\underline{1}$), the conversion

into NSF remarkably decreased with increasing concentrations

of ergosterol in the medium, while the conversion into FAF did

not decrease. To investigate this problem further, the time

course experiment of ^{14}C-incorporation from $1-^{14}C$-acetate into

NSF was carried out with the growing cells in the presence and

absence of ergosterol (50 µg/ml). The results are shown in Fig. 1.

Fig. 1

In the presence of ergosterol, the growth of yeast increased

exponentially for the first 50 hr of incubation. Even in the

absence of ergosterol, a poor of growth was observed, which might

be due to a small amount of oxygen remained in the medium. In

the presence of ergosterol, the amount of ^{14}C-incorporation into

NSF decreased rapidly along with the incubation time. In the

absence of ergosterol, on the contrary, it showed a higher value,

remaining almost constant throughout the incubation time. More

than 90 % of the radioactivity in NSF was recovered as squalene,

an intermediate of ergosterol synthesis, when NSF was analyzed by

column chromatography on deactivated almina (24). 'From these

results, it was presumed that the conversion of 1-^{14}C-acetate

into NSF took place at an early stage of growth in which only

a limited amount of ergosterol was incorporated into the cells.

However, ^{14}C-incorporation into NSF was considered to be decreased

as the,incorporation òf ergosterol into the cells increased. It

might be possible to consider, that ergosterol or its metabolite

controls sterol synthesis from acetate in yeast.

The sterol requirement of S. cerevisiae is known not to be

confined to ergosterol (21). From these considerations, yeast

cells were grown with 1-^{14}C-acetate and various concentrations of

cholesterol and the incorporation of radioactivity from 1-^{14}C-

acetate into NSF was examined. The results were just the same as

those with ergosterol: cholesterol also promoted growth to almost

the same extent as ergosterol, and ^{14}C-incorporation decreased

with increasing concentrations of cholesterol. It might be

concluded that cholesterol or its metabolite also controls sterol

synthesis from acetate.

Conversion of 1-^{14}C-Acetate into NSF with the Cell-free

Extracts —— In order to elucidate the control mechanism, further

experiments were carried out with the cell-free extracts. In

the first place, the effect of ergosterol on the conversion of

1-^{14}C-acetate into NSF was examined. The results are summarized

in Table I. Unexpectedly, 1 mg of ergosterol showed no inhibitory

effect on the incorporation of radioactivity from 1-^{14}C-acetate

TABLE I

into NSF even if ergosterol and'the cell-free extracts were pre-

incubated at 37°C for 15 min. These results indicate that

ergosterol itself has no effect on the conversion of acetate

into NSF.

Therefore, it is conceivable that some substance(s) related

to ergosterol controls the sterol synthesis in yeast. To examinɔ

this possibility, the effect of the crude yeast lipids, which

were extracted from the disrupted cells with chloroform-methanol

(2:1), was investigated. As can be seen in Fig. 2, the addition

Fig. 2

of the crude yeast lipids reduced the ^{14}C-incorporation from

1-^{14}C-acetate into NSF but had no action on that from 2-^{14}C-MVA.

From these results, it appears that in the crude yeast lipids

there exists some substance(s),which inhibits the conversion of

acetate into MVA.

For the separation of the inhibitory substance(s), the crude yeast lipids were dissolved in ether and the solution was shaken with 1 $\underline{M}$ NaHCO$_3$ to separate the acidic lipids from the neutral ones. After separation, the NaHCO$_3$ solution was acidified with 6 $\underline{N}$ H$_2$SO$_4$ to pH 1.0 and was shaken with ether. The acidic- and neutral lipids were obtained by evaporation the other solutions. From 200 mg of crude yeast lipids, 64.0 mg of neutral lipids and 6.0 mg of' acidic lipids were obtained. The concentration of each lipid fraction which is necessary for 50 % inhibition was as follows: crude yeast lipids, 57 mg/ml; neutral lipids, >150 mg/ml; acidic lipids, 0.50 mg/ml. From these figures it is evident that most of the inhibitory activity is to be found in the acidic lipid fraction. The total inhibitory activity of the acidic lipids was higher than that of the original crude yeast lipids. This apparently low value of the crude yeast lipids was presumed to be due to the interfering action of other lipids contaminated in them.

Inhibition Step --- According to the present knowledge, the conversion of acetate into MVA involves four enzymatic reactions. An attempt was made to decide which step the acidic lipids inhibit. The results are shown in Table II. One mg of the acidic lipids showed a 85.6 % inhibition of the ^{14}C-incorporation into NSF from 1-^{14}C-acetate, and a 86.9 % inhibition of that from 3-^{14}C-HMG-CoA. On the contrary, the ^{14}C-incorporation from 2-^{14}C-MVA was not inhibited. These results strongly suggested that the enzyme

TABLE II

responsible for the reduction of HMG-CoA to MVA is inhibited by

acidic lipids. As described in EXPERIMENTAL, the preparation of

$3-^{14}C$-HMG-CoA used in this experiment contained free $3-^{14}C$-HMG.

Therefore, the conversion of $3-^{14}C$-HMG to NSF was examined. As

can be seen from the table, $3-^{14}C$-HMG-CoA was converted into NSF,

while $3-^{14}C$-HMG was not. In view of these findings, it is

concluded that HMG-CoA is an intermediate of ergosterol synthesis

and that HMG can not be utilized for sterol synthesis. This

conclusion was also supported by another experiment: the ^{14}C-

incorporation into NSF from $1-^{14}C$-acetate was found to be lowered

by the addition of non-labeled HMG-CoA (0.3 μmole) but not by the

addition of non-labeled HMG (1.0 μmole). This means that HMG-CoA

caused a dilution of radioactivity but HMG did not.

Purification of inhibitor(s) ---- It was found that in the

acidic lipids there is some substance(s) which inhibits the

reduction of HMG-CoA to MVA. Therefore, purification of the

inhibitor(s) was attempted. For estimation of inhibitory

activity at various purification steps, $I_{\frac{1}{2}}$ was employed, denoting

the concentration which exerts a 50 % inhibition on the conversion

of $1-^{14}C$-acetate into NSF. The acidic lipids obtained above were fractionated by column chromatography on silica·gel. The acidic lipids (156 mg) were dissolved in small volumes of hexane and applied on cilica gel (10 g). For elution, 100 ml portions of hexane-ether (50:50), ether-methanol (75:25), and methanol (100) were used successively. The dry weight and $I_{\frac{1}{2}}$ of each fraction obtained are summarized in Table III. Free fatty acids were found in the first

TABLE III

fraction. About 70 % of the total dry weight of the original lipids were in the first fraction, but in terms of the inhibitory activity, the lipids in the second fraction were most effective. For further purification, the second fraction was chromatographed on a thin-layer plate of Silica Gel G using a solvent system of benzene-dioxane-acetic acid (60:30:2). Anisaldehyde spray (25) revealed four spots as shown in Fig. 3. For reference cholic,

Fig. 3

deoxycholic, and lithocholic acids were run in parallel since these bile acids also specifically inhibited the reduction of HMG—CoA to MVA as will be discussed later. The four substances in Fig. 3 were eluted from silica gel with ether-methanol (50:50), respectively. The inhibitory activities of the four substances and their amounts are shown in Table IV. On rechromatography,

TABLE IV

Substance C, among them, gave a single spot (Rf 0.24) on a thin-layer plate in a solvent system of hexane-ethyl acetate-acetic acid (50:50:1). The other substances also showed inhibitory activities as shown in Table IV, but it was found that there were some impurities in them. Further purification is required for the determination of their exact inhibitory activities.

TABLE V

In Table V, is summarized the purification process of them. The yield of Substance C was 3.0 mg from 500 g of wet cells. The purification of Substance C was about 1000 fold in terms of the inhibitory activity.

Effect of Substance C on the Conversion of 1-^{14}C-Acetate

into NSF and FAF —— The effect of addition of increasing

amounts of Substance C on the incorporation of radioactivity from

1-^{14}C-acetate into NSF and FAF was examined. The results are

Fig. 4

shown in Fig. 4. Substance C inhibited the ^{14}C-incorporation from

1-^{14}C-acetate into NSF. On the other hand, it stimulated the

incorporation from 1-^{14}C-acetate into FAF, though the reason is

not clear. At least, it can be said that Substance C does not

cause an inhibition of fatty acid synthesis. The results

suggested a rather specific inhibition of sterol synthesis.

Conversion of Ergosterol to the Inhibitors —— The existence

of the inhibitors in yeast posed a question whether these

inhibitors from yeast are normal metabolites or not. For this

purpose, the conversion of ^{14}C-ergosterol into the inhibitors

was examined. ^{14}C-Ergosterol (10 μmoles, 10,000 cpm) was added

to 1 liter of growing culture of S. cerevisiae and the mixture

was incubated for 48 hr at 28°C with shaking. The inhibitors

were extracted from the cells according to the procedure

described above. The radioactivity of the ether-methanol (75:25)

fraction eluted from the silica gel column was 500 cpm. This

fraction was subjected to thin-layer chromatography using benzene-dioxane-acetic acid (60:30:2). After chromatography, radioactivity in the·1 cm segments of the developed plate was counted. Fig. 5 shows that the radioactivity on the plate was located at the spots

Fig. 5

which were revealed with anisaldehyde spray. About 86 % of the radioactivity was recovered in these four spots. These results strongly support the view that the inhibitors are normal metabolites which are derived from ergosterol.

DISCUSSION

During the course of studies on the control mechanism of ergosterol biosynthesis in yeast, it was found that the acidic lipids which are presumed to be derived from ergosterol inhibit the activity of HMG-CoA reduction as in the case of bile acids in the control of cholesterol synthesis in animal (15). These relationship is illustrated in Scheme 1. Because of the analogy

Scheme 1

of the acidic lipids to bile acids, the inhibitory activity of various bile acids was investigated with the cell-free extracts of yeast. The detailed results will be reported in a succeeding paper[*] but it was demonstrated that bile acids also inhibited specifically the reduction of HMG–CoA to MVA with various degrees of inhibitory activity. Comparing the inhibitory activity of Substance C from yeast with that of bile acids, Substance C was found stronger than cholic acid. Furthermore, in a separate experiment, the mechanism of the inhibition by these acidic lipids as well as bile acids was investigated. The inhibition was found not due to non-specific surface active action and it could be reversed by the addition of albumin (unpublished data).

These acidic lipids showed no effect on the conversion of MVA into squalene and sterols, or on the conversion of acetate into FAF. Therefore, this inhibition was considered specific to the HMG–CoA reduction step. These findings suggest that the acidic lipids may be responsible for the regulation of ergosterol synthesis in yeast.

Furthermore, with growing cells, it was demonstrated that ergosterol added to the medium depressed the synthesis of ergosterol from acetate. For this depression, the following two possible explanations may be possible: (1) the formation of inhibitors

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[*] Hatanaka, H., Kawaguohi, A., Kubota, F., Hayakawa, S., and Katsuki, H., Manuscript in preparation.

from ergosterol, and (2) the repression of the enzymes concerned. The results of ^{14}C-incorporation into the acidic lipids from ^{14}C-ergosterol suggested the validity of the first possibility, though the second possibility can not completely be excluded from the information available.

Although the structures of the acidic lipids have not been determined yet, some informations were obtained about their properties (unpublished data). After esterification with diazomethane, the mobility of the acidic lipids on a thin-layer plate was increased. Moreover, their mobility on a thin-layer plate was increased by the trifluoroacetylation of the methyl esters of the acidic lipids. From these observations, these acidic lipids are supposed to have both carboxyl- and hydroxyl groups in them. However, it is not clear that the acidic lipids are bile acid-like substances. Studies are now in progress on the structures and the properties of the acidic lipids.

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TABLE I

Effect of ergosterol on the conversion of 1-^{14}C-acetate

into NSF with the cell-free extracts

Reaction mixture contained the following components in a final volumn of

1.0 ml: ATP, 5 µmoles; GSH, 2 µmoles; NADP, 1 µmole; glucose 6-phosphate, 10

µmoles; CoA, 0.1 µmole; $MgSO_4 \cdot 7H_2O$, 2 µmoles; $MnSO_4 \cdot 4H_2O$, 1 µmole; Tween 80,

1 mg; potassium phosphate buffer (pH 7.0), 80 µmoles; 1-^{14}C-acetate, 0.012 µmole ·

(550,000 cpm); 0.3 ml of the cell-free extracts (7 mg protein). Reaction was

carried out at 37°C for 1 hr with constant shaking.

Ergosterol added (mg)	Preincubation[1]	Incorporation into NSF (cpm)
0	-	32,300
1	-	31,100
0	+	34,700
1	+	33,200

1) Preincubation was carried out with the cell-free extracts and ergosterol

at 37°C for 15 min.

TABLE II

Inhibition of the conversion into NSF by the acidic lipids

with the cell-free extracts

The experimental conditions were the same as those in Table I except for the use of $1-^{14}C$-acetate (0.012 μmole, 550,000 cpm), $3-^{14}C$-HMG (0.18 μmole, 690,000 cpm), $3-^{14}C$-HMG-CoA (2,100,000 cpm) [1], or $2-^{14}C$-MVA (0.022 μmole, 200,000 cpm) as indicated. Amount of the acidic lipids added was 1.0 mg.

Radioactive substrate	Acidic lipids	Incorporation into NSF (cpm)	Inhibition (%)
$1-^{14}C$-Acetate	-	52,900	
"	+	7,600	85.6
$3-^{14}C$-HMG	-	48	
$3-^{14}C$-HMG-CoA	-	79,400	
"	+	10,400	86.9
$2-^{14}C$-MVA	-	77,400	
"	+	70,100	9.4

1) $3-^{14}C$-HMG-CoA was used without separation from free $3-^{14}C$-HMG.

TABLE III

Fractionation of the acidic lipids on silica-gel column

Dry weight of the acidic lipids applied was 77.8 mg.

Elution solvent	Dry weight (mg)	$I_{\frac{1}{2}}$ (mg/ml)
Hexane-ether (50:50)	55.0	2.00
Ether-methanol (75:25)	15.6	0.40
Methanol (100)	1.4	1.80

TABLE IV

Inhibitory activity of the compounds purified on thin-layer plate

Ether-methanol fraction (15.6 mg) in Table III was applied to silica

gel G plate using the same solvent as that in Fig. 3.

Substance	Dry Weight (mg)	$I_{\frac{1}{2}}$ (mg/ml)
A	0.4	0.40
B	0.9	0.51
C	3.0	0.052
D	0.8	0.06

TABLE V

Purification of an inhibitor from yeast

Wet weight of yeast cells treated was 500 g.

Fraction	Dry weight (mg)	$I_{\frac{1}{2}}$ (mg/ml)
C-M (2:1) extracted lipids[1]	2,500	57.0
Acidic lipids	77.8	0.50
Silica-gel chromatography	15.6	0.40
Thin-layer chromatography		
(Substance C)	3.0	0.052

1) C-M shows the mixture of chloroform and methanol.

Fig. 1. Effect of ergosterol on the conversion of $1-^{14}C$-acetate into NSF with the growing cells.

The medium contained the following components in a final volume of 25 ml: glucose, 2.5 g; Difco yeast nitrogen base, 170 mg; potassium oleate, 8.0 mg; Tween 80, 25 mg; $1-^{14}C$-acetate, 0.03 μmole (1,400,000 cpm); succinate buffer (pH 5.0), 1.25 mmoles; and 750 μg of ergosterol when indicated. After yeast cells were inoculated in the above medium, incubations were carried out at $28^{\circ}C$ in an argon atmosphere during indicated period.
Growth:

- - -▨- - -   in the presence of ergosterol

- - -▢- - -   in the absence of ergosterol

Incorporation into NSF:

——●——   in the presence of ergosterol

——○——   in the absence of ergosterol

Fig. 2. Effect of yeast lipids on the conversion of $1-^{14}C$-acetate and $2-^{14}C$-MVA into NSF with the cell-free extracts.

The experimental conditions were the same as those in Table I except for the use of $1-^{14}C$-acetate (550,000 cpm) or $2-^{14}C$-MVA (200,000 cpm) as indicated.

Fig. 3. Thin-layer chromatogram of the inhibitors.

The solvent system was benzene-dioxane-acetic acid (60:30:2). The spots were visualized by spraying anisaldehyde reagent (25).

Fig. 4. Effect of Substance C on the conversion of $1-^{14}C-$ acetate into NSF and FAF.

The experimental conditions were the same as those in Table I.

Fig. 5. Thin-layer chromatogram of the inhibitors derived from ^{14}C-ergosterol.

Inhibitors were extracted from yeast cells as described in the text, and were chromatographed with benzene-dioxane-acetic acid (60:30:2). For assay of radioactivity, the 1 cm segments of the developed plate were scraped and put directly into counting vials containing scintillation fluid.
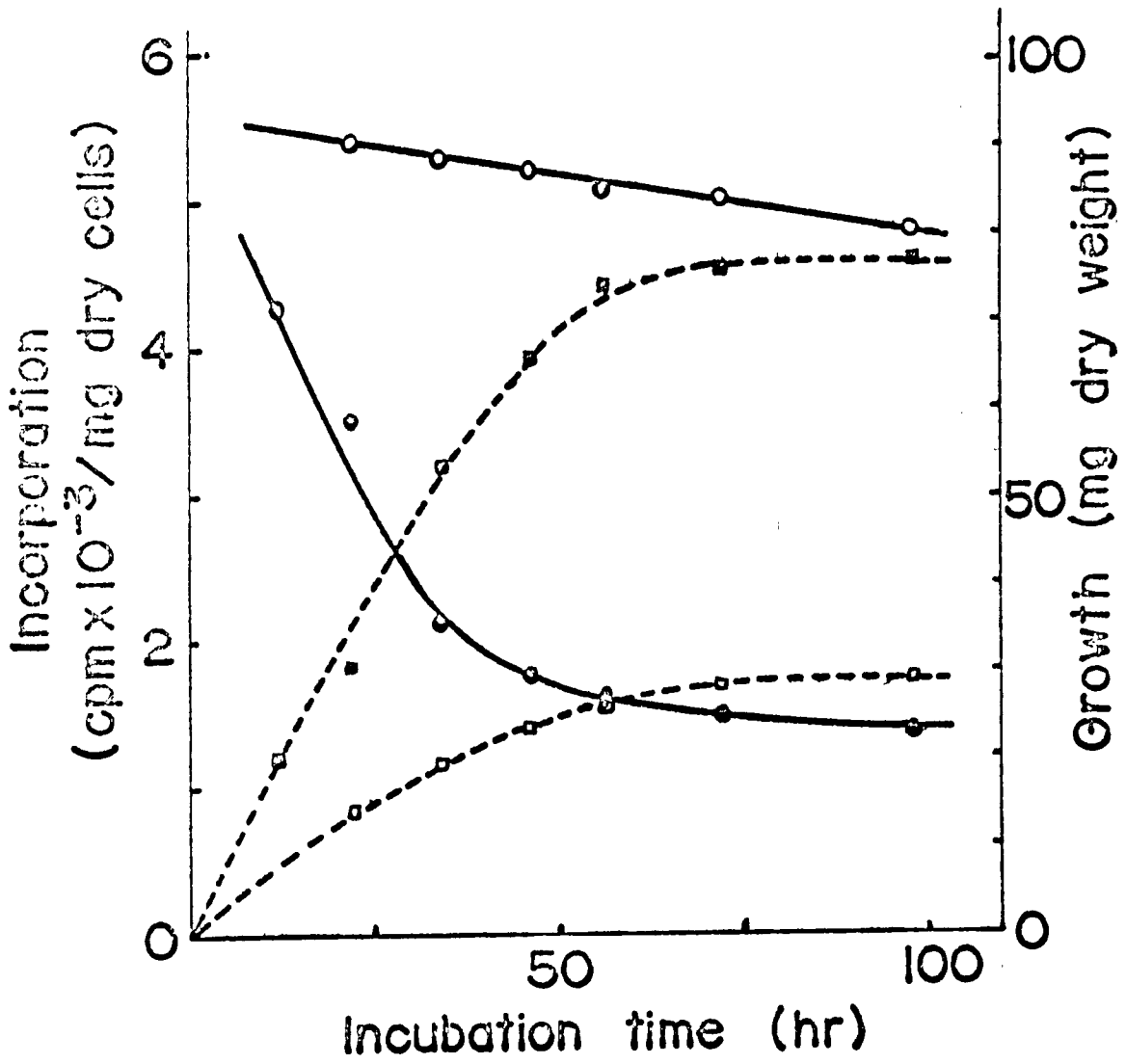
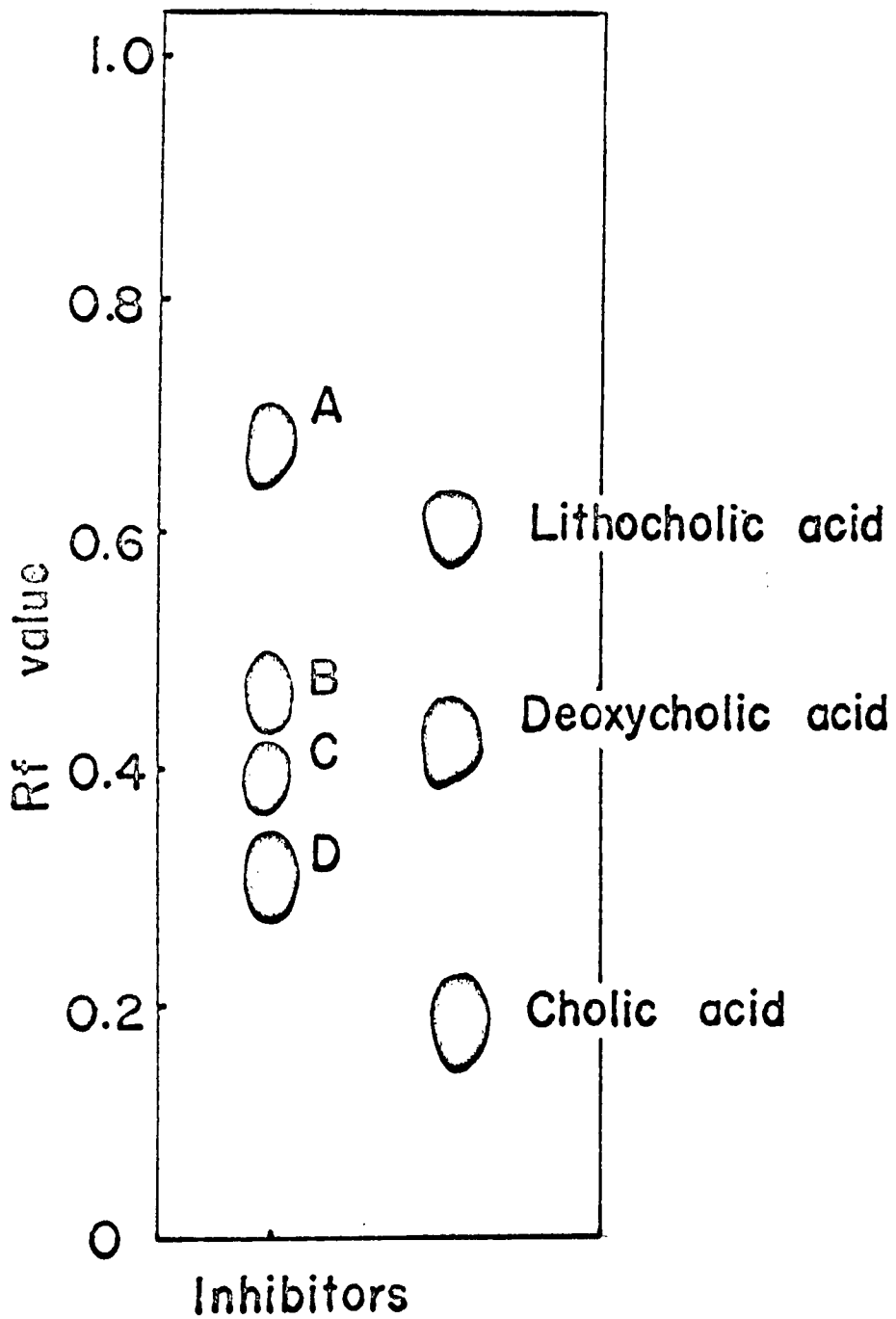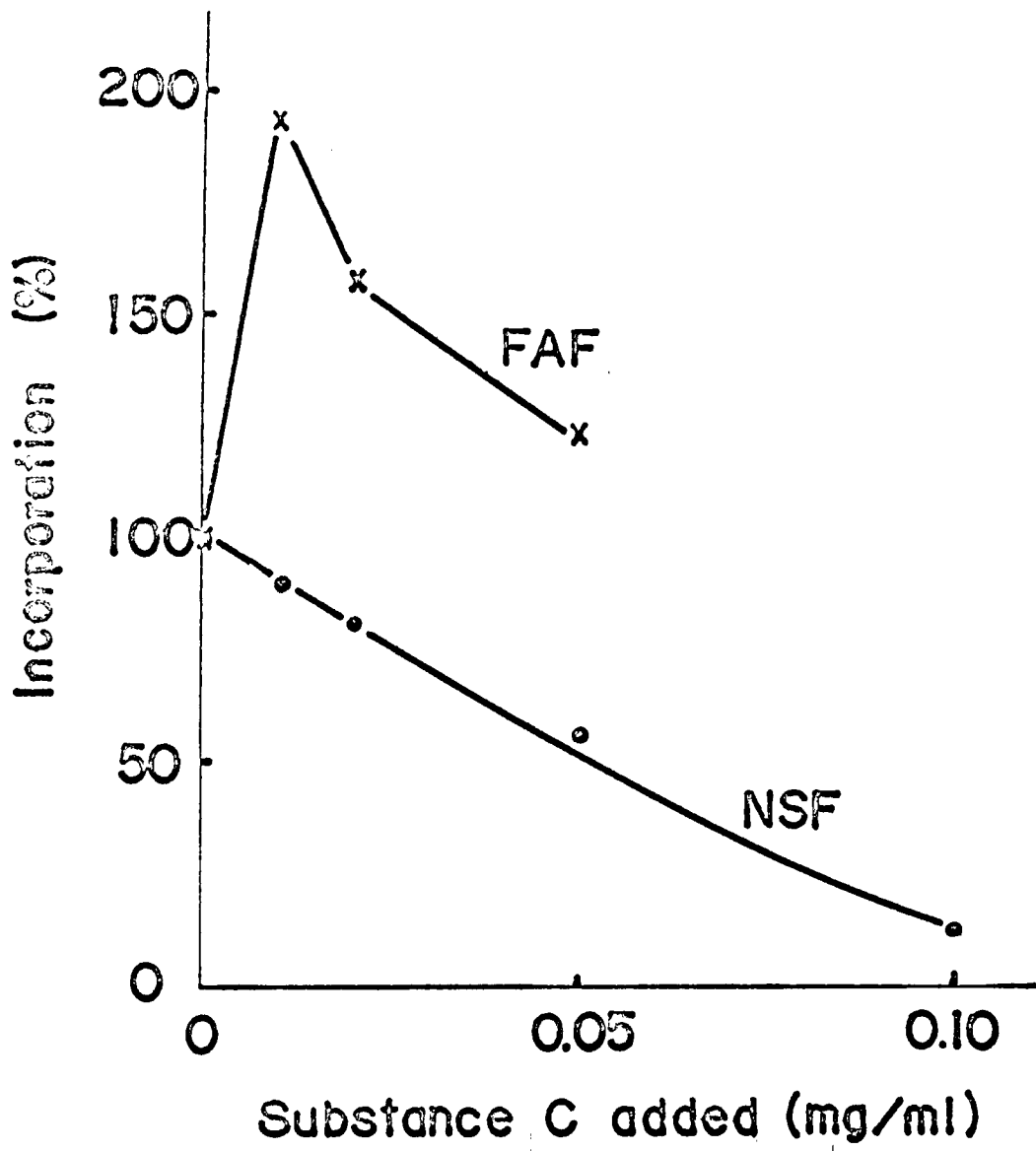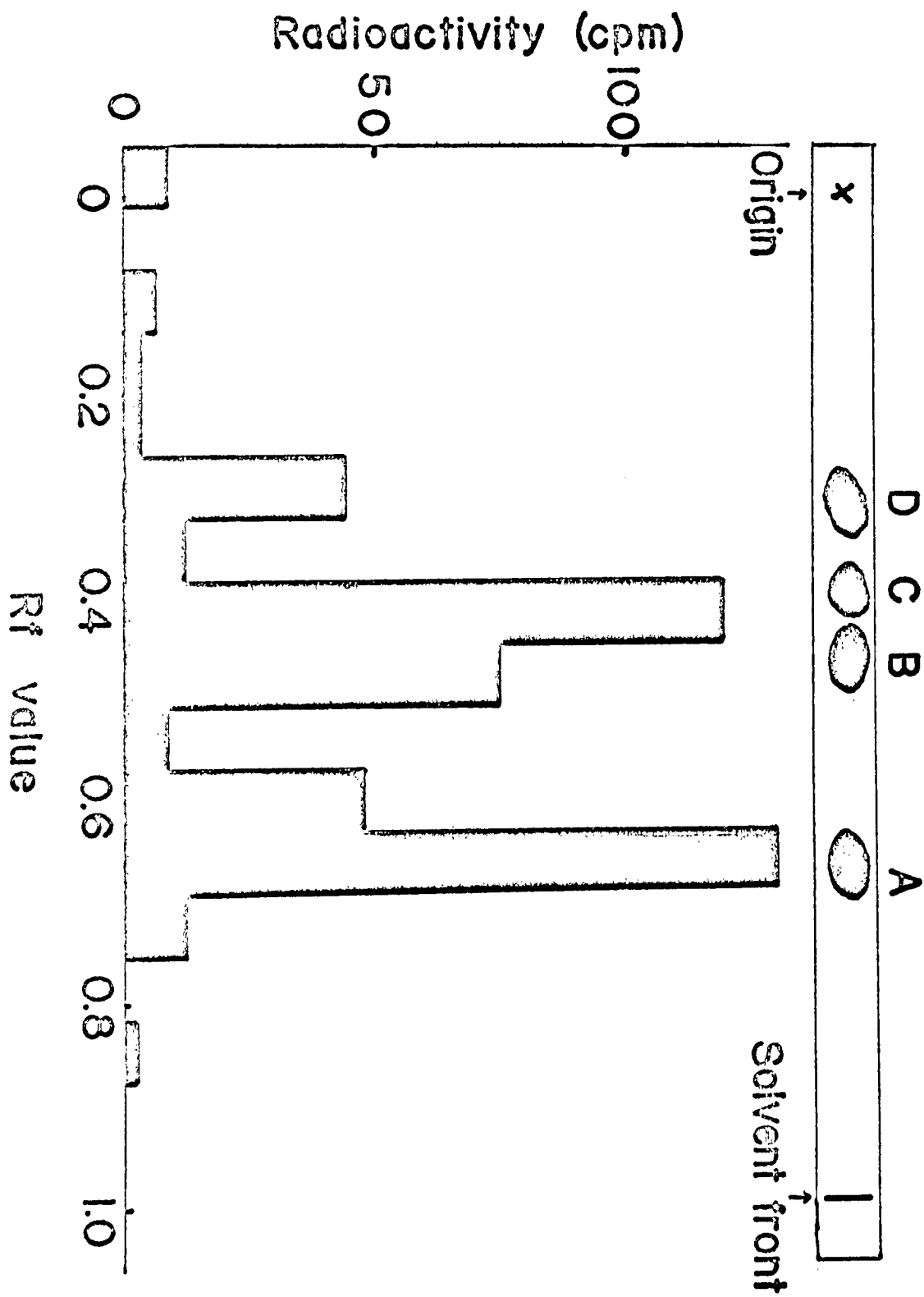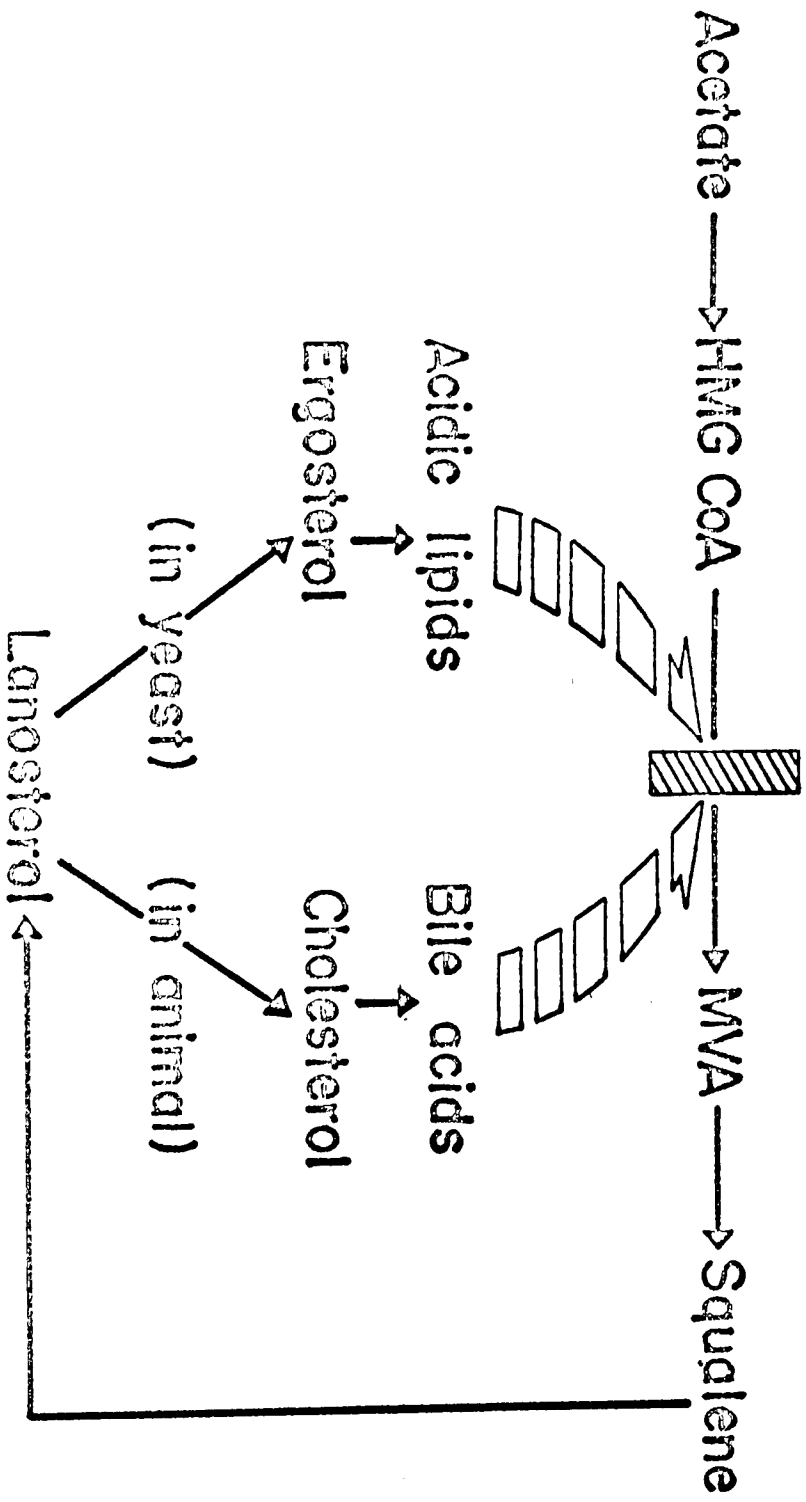Fig. 1

Fig. 2
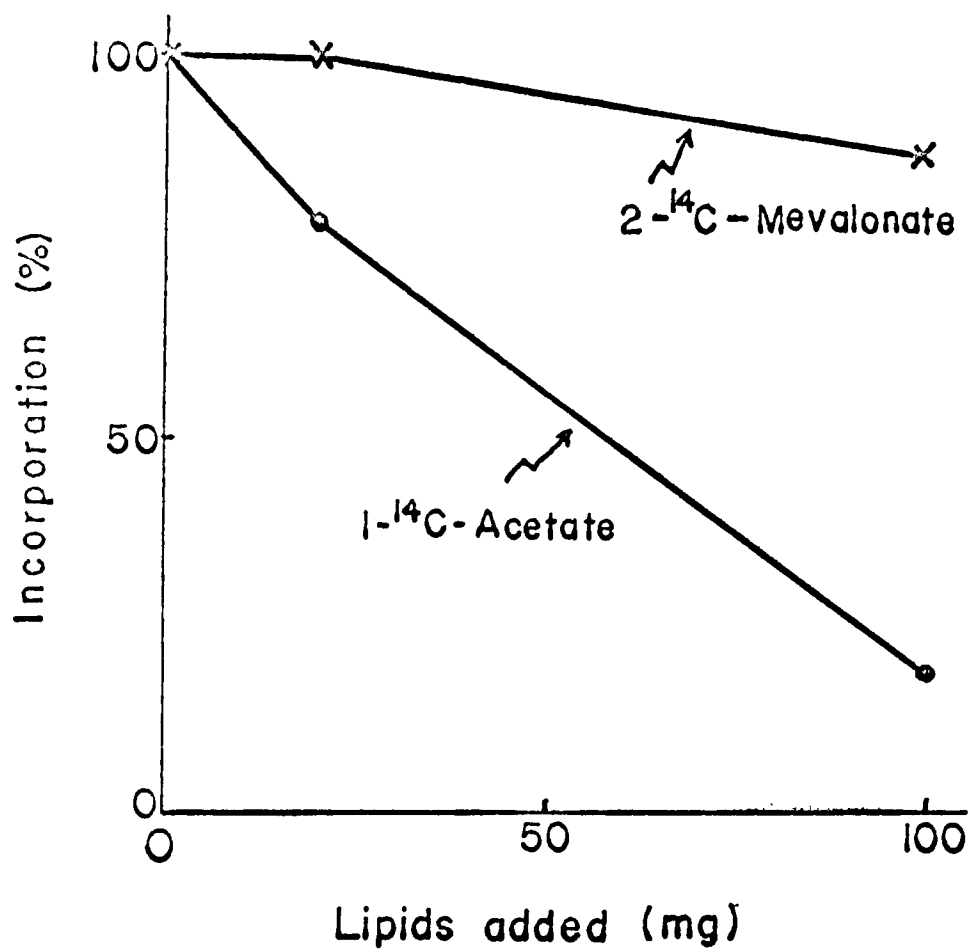
Fig. 2

Fig. 3

Fig. 3

Fig. 4

Fig. 4

Fig. 5

Fig. 5

Scheme 1