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(54) **Titre :** METHODE POUR RENFORCER L'ACTION ANTIBACTERIENNE DES ANTIBIOTIQUES
(54) **Title:** METHOD OF STRENGTHENING ANTIBACTERIAL ACTION OF ANTIBIOTICS

(57) **Abrégé/Abstract:**

A method of increasing the activity of an antibiotic against methicillin resistant Staphylococcus aureus in a patient by administering to the patient the antibiotic in combination with a catechin of the formula (I) and/or a theaflavin of the formula (II).



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ABSTRACT OF THE DISCLOSURE

A method of increasing the activity of an antibiotic against methicillin resistant Staphylococcus aureus in a patient by administering to the patient the antibiotic in combination with a catechin of the formula (I) and/or a theaflavin of the formula (II).

METHOD OF STRENGTHENING ANTIBACTERIAL ACTION OF ANTIBIOTICS

FIELD OF THE INVENTION

The present invention relates to a method of strengthening antibacterial action of antibiotics. More specifically, the present invention relates to a method of strengthening antibacterial action of antibiotics against methicillin resistant Staphylococcus aureus (abbreviated as MRSA from hereon) by a combined use of the antibiotics with tea catechin and/or theaflavin.

BACKGROUND INFORMATION

With the popularization and rapid development of medical treatments, various kinds of antibiotics are being used in the medical world. However, bacteria resistant to these antibiotics have emerged, and MRSA is representative of such.

MRSA shows resistancy to not only methicillin but to most medications and since up until now there has been no conclusively effective treatment, arbekacin or vancomycin is at present being used in combination with other medications. However, this kind of combination treatment requires the intake of many kinds of medications in large amounts, and thus presents problems such as the increased possibility of detrimental side-effects. So with the increase in "compromised hosts" a method of controlling MRSA is urgently required.

The present inventors have shown and reported that tea extractions and tea catechins in clinical use show an antibacterial

action against MRSA isolate (Japanese J. Bacteriol., 46, 839-845, 1991). For example, administration of 250 mg/ml of epigallocatechin gallate showed an antibacterial action against MRSA, and within 24 hours 10^4 cells/ml of MRSA were destroyed. We have also reported that tea catechins combined with exotoxin, for example α -toxin of Staphylococcus aureus, and rendered them inactive (Japanese J. Bacteriol., 45, 561-566, 1990; Japanese J. Bacteriol., 45, 913-919, 1990).

Further, a method of preventing the transmission of infection caused by MRSA with the use of tea polyphenols is disclosed in U. S. Patent No. 5,358,713.

SUMMARY OF THE INVENTION

We now found that the activity of an antibiotic against methicillin resistant Staphylococcus aureus is increased, when the antibiotic is combined with a synergistic amount of at least one polyphenol selected from the group consisting of a catechin and a theaflavin.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain H-5. Figure 1B shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain H-8. Figure 1C shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain H-13. Figure 1D shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain H-18. Figure 1E shows synergistic

effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain 21. Figure 1F shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain H-28.

Figure 2A shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain F-8. Figure 2B shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain F-10. Figure 2C shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain F-49. Figure 2D shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain F-68. Figure 2E shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain F-74. Figure 2F shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain F-96.

Figure 3 shows synergistic effect of tea catechin (Polyphenon 100) with oxacillin against MRSA strain F-98.

Figure 4A shows synergistic effect of tea catechins (Polyphenon 100) with oxacillin against MRSA strain H-5. Figure 4B shows synergistic effect of tea catechins (Polyphenon 100) with oxacillin against MRSA strain H-9. Figure 4C shows synergistic effect of tea catechins (Polyphenon 100) with oxacillin against MRSA strain F-41. Figure 4D shows synergistic effect of tea catechins (Polyphenon 100) with oxacillin against MRSA strain F-84.

Figure 5A shows the synergistic effect of tea catechins (Polyphenon 100) with oxacillin against MRSA F-51. Figure 5B shows the synergistic effect of tea catechins (Polyphenon 100) with methicillin against MRSA F-51. Figure 5C shows the synergistic effect of tea catechins (Polyphenon 100) with aminobenzyl penicillin against MRSA F-51.

Figure 5D shows the synergistic effect of tea catechins (Polyphenon 100) with cephalexin against MRSA F-51. Figure 5E shows the synergistic effect of tea catechins (Polyphenon 100) with penicillin G against MRSA F-51. Figure 5F shows the synergistic effect of tea catechins (Polyphenon 100) with amikacin against MRSA F-51.

Figure 6A shows the synergistic effect of tea catechins (Polyphenon 100) with tetracycline against MRSA F-51. Figure 6B shows the synergistic effect of tea catechins (Polyphenon 100) with chloramphenicol against MRSA F-51. Figure 6C shows the synergistic effect of tea catechins (Polyphenon 100) with gentamicin against MRSA F-51.

Figure 7 shows the synergistic effect of epicatechin gallate with oxacillin against MRSA F-51.

Figure 8 shows the synergistic effect of epigallocatechin gallate with oxacillin against MRSA F-51.

Figure 9 shows the synergistic effect of theaflavin digallates with oxacillin against MRSA F-51.

Figure 10A shows the ineffectiveness of tea catechins ((-)-epigallocatechin gallate) with kanamycin against MRSA F-51. Figure 10B shows the ineffectiveness of tea catechins ((-)-epigallocatechin gallate) with erythromycin against MRSA F-51. Figure 10C shows the ineffectiveness of tea catechins ((-)-epigallocatechin gallate) with clindamycin against MRSA F-51. Figure 10D shows the ineffectiveness of tea catechins ((-)-epigallocatechin gallate) with colistin against MRSA F-51.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present inventors conducted the oxacillin antibacterial test using the orthodox cup method on clinical MRSA isolate and found that when less than the minimum inhibitory concentration (MIC) of tea catechins (25-100 μ g/ml) was added to the agar plate, oxacillin showed an antibacterial action on all of the MRSA isolates and the MIC was 5-12.5 μ g/ml. However, without the addition of tea catechins, even a concentration of 40 μ g/ml oxacillin had no antibacterial action.

Next, by counting the number of viable cells at intervals we observed the antibacterial action against MRSA when tea catechins and oxacillin were added simultaneously. When oxacillin was added independently (5 μ g/ml), no antibacterial effect was confirmed and the same growth curve as the control was observed; whereas when tea catechins (100 μ g/ml) were added with oxacillin, a synergistic effect was apparent and after 24 hours the viable cells had decreased to 1/100-1/10000.

These results demonstrate that the combination of tea catechins with oxacillin has a synergistic effect and it was confirmed that tea catechins stimulate the antibacterial action of oxacillin against MRSA. The same effect was observed when theaflavins were used instead of tea catechins.

Further, the influence of tea catechins on the antibacterial action of each kind of antibiotic to which MRSA showed resistancy was measured using the cup method as described above. Results showed that when 100 μ g/ml of tea catechin were combined with specific antibiotics, i.e. methicillin (12.5 μ g/ml), aminobenzyl penicillin (32 μ g/ml), tetracycline (2.5 μ g/ml) or chloramphenicol (12.5 μ g/ml), these antibiotics had an antibacterial action on MRSA, and thus the

effectiveness of tea catechins when used in combination with antibiotics other than oxacillin was confirmed.

According to these investigations, it was found that when antibiotics which showed no antibacterial action on MRSA when used independently, were used in conjunction with tea catechins and/or theaflavins in a concentration less than the MIC, there was an apparent antibacterial action on MRSA.

On the basis of these discoveries, the present inventors developed and completed a method of strengthening
10 antibacterial action of antibiotics against MRSA.

Thus the present invention provides a pharmaceutical composition which comprises (a) an effective antibiotic amount of an antibiotic in combination with (b) at least one polyphenol selected from the group consisting of a catechin and a theaflavin in an amount effective to synergistically increase the antibiotic activity of the antibiotic against methicillin resistant Staphylococcus aureus.

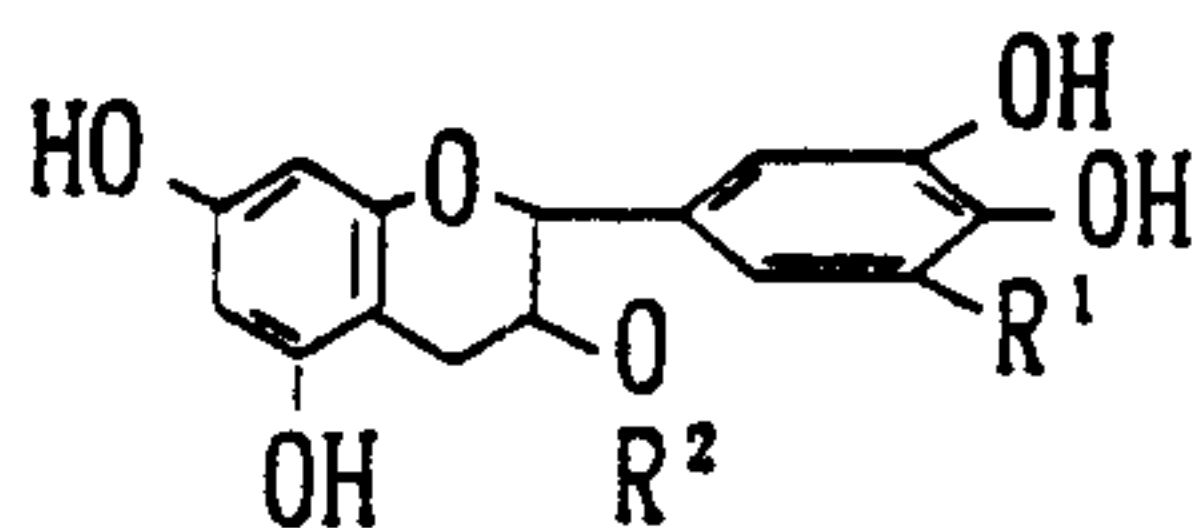
The antibiotics used in the present invention may show no antibacterial action against MRSA when used independ-
20 ently at a certain concentration. Such antibiotics include β -lactams, aminoglycosides, etc. Specific examples of such antibiotics include oxacillin, methicillin, aminobenzyl penicillin, cephalixin, penicillin G, amikacin, tetracycline, chloramphenicol, gentamicin and the like.

Out of these antibiotics, for example, oxacillin had a MIC of more than 1,000 $\mu\text{g/ml}$ against MRSA.

The pharmaceutical composition is particularly advantageous when used for treating or preventing infection of methicillin resistant Staphylococcus aureus, although it may also be used for treating infections of other bacteria.

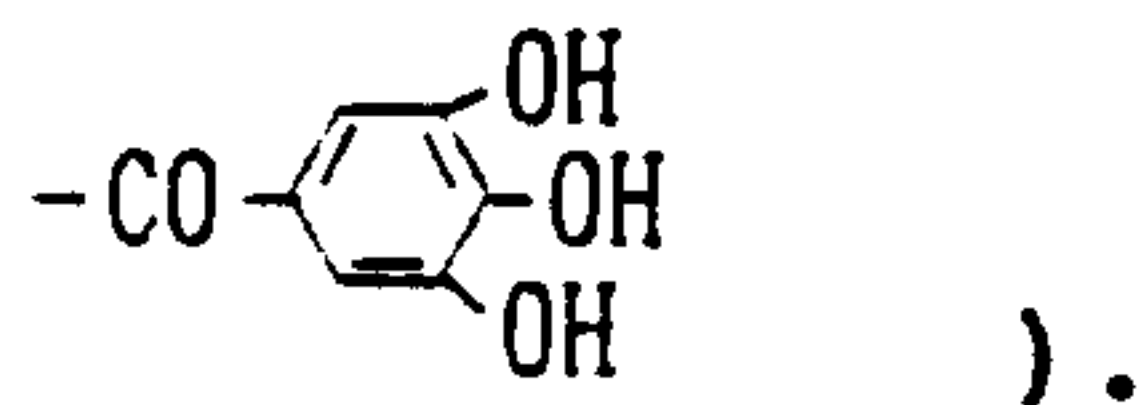
According to the present invention, the antibiotics may be used even in such a small amount that they would not be effective against MRSA if used independently. The amount of the catechin or theaflavin may vary widely as far as the activity of the antibiotics is synergistically increased against MRSA. In certain embodiments, the amount of the catechin or theaflavin is preferably from about 0.5 to about 500 parts by weight, more preferably from about 2 to about 100 parts by weight per part by weight of the antibiotics. When the antibiotic is oxacillin, a preferred amount of the catechin or theaflavin appears to be from about 10 to 100 parts by weight per part of oxacillin. When the antibiotic is methicillin, a preferred amount of the catechin or the theaflavin appears to be from about 5 to 50 parts by weight per part of methicillin.

The tea catechin used in the present invention is preferably one represented by the formula (I):



(I)

(wherein R^1 represents H or OH and R^2 represents H or



Tea catechins may be for example, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-gallocatechin, etc. (inclusive of isomers thereof) and some of these catechins can be used singly or a combination of two or more compounds may be used.

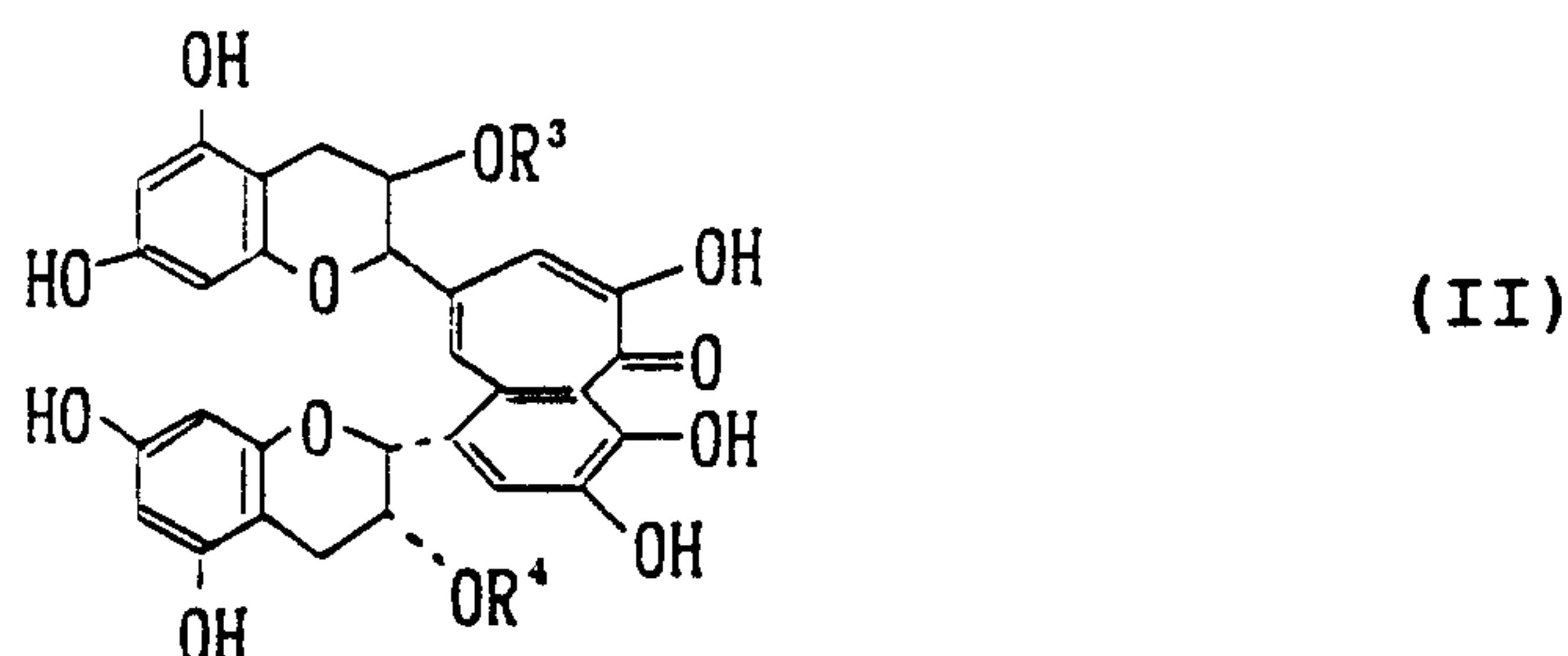
Among these catechins, compounds containing (-)-epicatechin gallate and/or (-)-epigallocatechin gallate are preferable.

10 In particular, tea catechin which contains (-)-epigallocatechin gallate as its main component is desirable, for example, "Polyphenon 100"TM (product of Mitsui Norin Co. Ltd., composition: (+)-gallocatechin 1.44%, (-)-epicatechin 5.81%, (-)-epigallocatechin 17.57%, (-)-epicatechin gallate 12.51%, (-)-epigallocatechin gallate 53.9%) or "Polyphenon E"TM (product of Mitsui Norin Co. Ltd., composition: (-)-epicatechin 10.8%, (-)-epigallocatechin 9.2%, (-)-epicatechin gallate 6.5%, (-)-epigallocatechin gallate 54.8%, (-)-gallocatechin gallate 4.0%) are preferable.

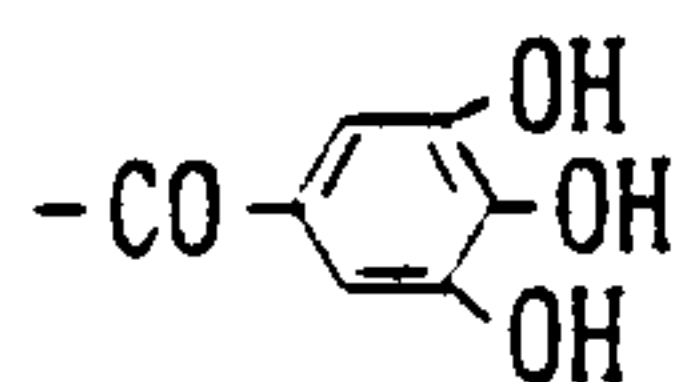
20 The MIC of tea catechin against MRSA is 125-250 µg/ml, and when tea catechin was tested at a concentration less than the MIC, such as about 5 to 120 µg/ml, it was found still effective to synergistically increase the activity of antibiotics.

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The theaflavin used in the present invention is preferably one represented by the formula (II):



(wherein R^3 and R^4 represent H or



and R^3 and R^4 may be the same or different from each other).

Theaflavins may be for example, free theaflavins, theaflavin monogallate A, theaflavin monogallate B, theaflavin digallate, etc. (inclusive of isomers thereof) and these
 10 theaflavins may be used singly or a combination of two or more compounds may be used. Among these theaflavins, compounds containing theaflavin digallate are preferable. In particular, the compound containing theaflavin digallate as its main component is desirable, for example "Polyphenon TF"TM (product of Mitsui Norin Co. Ltd., composition: theaflavin 16.8%, theaflavin monogallate A 19.5%, theaflavin monogallate B 16.1%, theaflavin digallate 31.4%).

The MIC of theaflavin against MRSA is 200 μ g/ml, and when the theaflavin was tested at a concentration less than
 20 the MIC, such as about 5 to 100 μ g/ml, it was found still effective to synergistically increase the activity of antibiotics. According to the present invention, a combination of more than two of the above-mentioned tea catechins and/or theaflavins may be used.

In order to determine the proportional concentrations of tea catechins and/or theaflavins to the antibiotics, the antibacterial action of each antibiotic in the presence of 100 μg of tea catechins was measured using the cup method, and it was found that in the case of 5 $\mu\text{g}/\text{ml}$ of oxacillin, 12.5 $\mu\text{g}/\text{ml}$ of methicillin, 32 $\mu\text{g}/\text{ml}$ of aminobenzyl pencillin, 2.5 $\mu\text{g}/\text{ml}$ of tetracycline and 12.5 $\mu\text{g}/\text{ml}$ of chloramphenicol, there was an apparent antibacterial action against MRSA. Thus by using these amounts of antibiotics as a guide, it can be determined in what proportions to mix these two kinds of compounds.

In order to make use of the antibacterial action against MRSA according to the present invention, antibiotics and tea catechins and/or theaflavins are mixed in the proportions stated above to formulate a pharmaceutical composition. The pharmaceutical composition may take various forms. For example, it may be mixed with a pharmaceutically acceptable excipient and used for an oral administration and/or non-oral administration. It may further be mixed in suitable proportions with supplementary substances such as lubricating agents, emulsifying agents, dispersing agents or the like.

As well known in the art, for practical use of the pharmaceutical composition, usually it is put in a commercial package. Such a commercial package normally includes a written matter associated with the pharmaceutical composition.

The written matter states for what purpose the pharmaceutical composition can or should be used.

For an oral administration, the pharmaceutical composition may be in the form of a liquid, powder, tablet, capsule, granules, etc. and in such cases, the excipients used are apart from water, sugar, starch, dextran, calcium phosphate, calcium carbonate, magnesium oxide, magnesium stearate, aluminium silicate, aluminium hydroxide, sodium bicarbonate, glycerin, etc.

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As a non-oral administration, the pharmaceutical composition may be in the form of an

injection, a drip, an ointment etc. and it may be mixed with a common substance such as distilled water, physiological saline, vegetable oils such as olive oil etc., alcohol such as ethanol etc., polyethylene glycol and so on.

The present invention will be explained in detail with reference to the following examples.

Example 1

10 strains of MRSA isolated at Showa University Hospital and 10 strains MRSA isolated at Showa University Fujigaoka Hospital were used for the following experiments. Firstly, the MIC of these bacteria was determined by the agar culture dilution method.

As a control, methicillin sensitive Staphylococcus aureus 209P (referred to as MSSA from hereon) was used.

A treated agar plate (10ml) was prepared by adding oxacillin (product of Sigma) in a final concentration of 0.25 to 1000 $\mu\text{g/ml}$ and tea catechins in a final concentration of 8 to 500 $\mu\text{g/ml}$ to Mueller-Hinton agar (Difco Lab. U.S.A.).

The tea catechin used was "Polyphenon 100"™ (product of Mitsui Norin Co. Ltd.).

The bacteria were cultured on the Mueller-Hinton agar plate for 20 hours and then a $1-5 \times 10^6/\text{ml}$ bacterial solution in physiological saline was prepared. 10 μl of this was spotted onto the treated agar plates of respective concentrations and cultured at 35°C for 24 hours to determine the MIC.

Results are shown in Table 1. As is clear from the table, the MIC of tea catechin was 125 $\mu\text{g/ml}$ with 6 strains, 250 $\mu\text{g/ml}$ with 14 strains. On the other hand, the MIC of oxacillin was 32 $\mu\text{g/ml}$ with one

strain, 63 $\mu\text{g/ml}$ with one strain, 125 $\mu\text{g/ml}$ with 4 strains, 250 $\mu\text{g/ml}$ with one strain, 500 $\mu\text{g/ml}$ with 8 strains and 1000 $\mu\text{g/ml}$ with 5 strains.

Table 1

Strains	MIC($\mu\text{g/ml}$)	
	Tea catechin	Oxacillin
MSSA 209P	250	0.5
MRSA H-5	125	500
H-6	250	500
H-7	125	1000
H-8	250	500
H-9	250	500
H-13	250	125
H-18	125	500
H-21	125	250
H-28	250	125
H-29	250	63
F-8	250	125
F-10	125	500
F-41	250	125
F-49	250	500
F-51	250	1000
F-68	125	1000
F-74	250	1000
F-84	250	1000
F-96	250	500
F-98	250	32

Example 2

The influence of tea catechins on the antibacterial action of oxacillin against MRSA was determined using the cup method. As in Example 1 the tea catechin used was "Polyphenon 100"TM (product of Mitsui Norin Co. Ltd.). Tea catechin in a concentration less than the MIC was dissolved in sterilized water and added in final concentrations of 25, 50, 100 $\mu\text{g/ml}$ to a melting Mueller-Hinton agar by keeping at a temperature of 60°C. 2ml of the Mueller-Hinton broth (Difco Lab.

U.S.A.) in which test bacteria had been cultivated for 18 hours was added to 98ml of the agar plate containing the tea catechin, and mixed well. Immediately thereafter, 10ml samples were poured into petri dishes.

After cooling, a stainless cup (ϕ 8mm) for the examination of the antibiotics was put on the agar plate in the dish, and into the cup was poured 0.1ml of each of the various dilutions of oxacillin (5, 10, 20, 40 μ g/ml), and after refrigerating for an hour, they were cultured overnight in a 36°C incubator and the following day the differences in inhibition (size of growth inhibition zone) were measured by slide calipers. Results are shown in Figure 1A to 1F, Figure 2A to 2F and Figure 3. Sterilized water only was added into the cup as a control.

In the Figures, ○ indicates "Polyphenon 100" at a concentration of 100 μ g/ml, ● indicates "Polyphenon 100" at a concentration of 50 μ g/ml, □ indicates "Polyphenon 100" at a concentration of 25 μ g/ml, ■ indicates control, i.e. no addition of "Polyphenon 100". The tested bacterial strains were resistant to oxacillin, and oxacillin up to a concentration of 40 μ g/ml showed no antibacterial action as measured by the cup method.

As is evident from Figures 1 to 3, due to the addition of tea catechins, the antibacterial activity of oxacillin against MRSA was apparent with all the bacteria tested. In particular, with the addition of 25 μ g/ml of tea catechins, only 5 μ g/ml of oxacillin were required to show an antibacterial action against MRSA strains H-5, H-8, F-49, F-68, F-74.

Moreover, with the addition of 10, 20, 40 μ g/ml of oxacillin, the size of the growth inhibition zone increased according to the

oxacillin concentration. Also when the amount of tea catechins was increased to $50\mu\text{g/ml}$ or $100\mu\text{g/ml}$, there was an even greater enlargement of the growth inhibition zone, proving the dose-dependency.

Example 3

The influence of tea catechins on the antibacterial action of oxacillin against MRSA was determined by counting the viable cells at intervals as described above. In this case, also the tea catechin used was "Polyphenon 100" (product of Mitsui Norin Co. Ltd.).

The test bacteria which had been incubated for 18 hours in Mueller-Hinton agar was scraped with a platinum needle and a bacterial solution 10^6 cfu/ml was prepared with physiological saline. A denary dilution of the above solution was made using a two-fold Mueller-Hinton broth (Difco Lab. USA) to which Ca^{2+} 25mg/liter, Mg^{2+} 12.5mg/liter were added in order to increase the sensitivity of the assay and the stability of the antibiotics.

To this bacterial solution, tea catechins were added to make the final concentration $100\mu\text{g/ml}$ and oxacillin was added to make a final concentration of $5\mu\text{g/ml}$. After mixing well, the number of viable cells were counted at intervals of 1, 3, 6 and 24 hours.

Results are shown in Figure 4A to 4D. Results of the control group without the addition of the antibiotics and the groups where two compounds were used independently are also shown in Figure 4A to 4D. In the Figure, ○ represents the control group, ● represents the group to which $5\mu\text{g/ml}$ of oxacillin only was added, □ represents the group to which $100\mu\text{g/ml}$ of "Polyphenon 100" only was added, and ■ represents the group to which $5\mu\text{g/ml}$ of oxacillin and $100\mu\text{g/ml}$ of "Polyphenon 100" were added.

The group to which oxacillin only was added, showed a growth curve similar to that of the control. In the group to which tea catechin only was added, there were some bacterial strains which at one point showed a decrease in the number of viable cells, but after that there was an increase. In the group where tea catechin was combined with oxacillin at a concentration which independently showed no antibacterial action, oxacillin showed an antibacterial action on all of the bacteria. When the viable cells were counted after 24 hours, compared with the control, H-5 was about 1/1000 and H-9, F-41, F-84 strains were about 1/1000-1/10000.

Example 4

The influence of tea catechins on the antibacterial effect of oxacillin on MRSA was investigated using the cup method as described in Example 2 and the results are shown in Figure 5A-5F and Figure 6A-6C.

F-51, a strain of bacteria with a high resistancy against oxacillin was used and the tea polyphenols used were in the form of Polyphenon 100 (Mitsui Norin Co. Ltd.). The antibiotics used were oxacillin (Sigma Chemical Company)(Fig. 5A), methillicin (Sigma Chemical Company)(Fig. 5B), aminobenzyl penicillin(Ban-yu Pharmaceutical Co. Ltd.)(Fig. 5C), cephalixin (Wako Pure Chemical Industries)(Fig. 5D), penicillin G (Meiji Pharmaceutical Co. Ltd.)(Fig. 5E), amikacin (Ban-yu Seiyaku)(Fig. 5F), tetracycline (Wako Pure Chemical Industries)(Fig. 6A), chloramphenicol (Wako Pure Chemical Industries)(Fig. 6B), gentamicin (Wako Pure Chemical Industries)(Fig. 6C).

In the figure, ○ indicates Polyphenon 100 at a concentration of 100 μ g/ml, ● indicates Polyphenon 100 at a concentration of 50 μ g/ml, □ indicates Polyphenon 100 at a concentration of 25 μ g/ml, ■

indicates the control containing no Polyphenon 100.

As is evident from this figure, under the cup method F-51 strain showed resistancy with up to 2000 $\mu\text{g/ml}$ oxacillin, but when 25 $\mu\text{g/ml}$ of Polyphenon 100 was added to the culture, oxacillin showed an antibacterial action at 10 $\mu\text{g/ml}$, and the size of the growth inhibition zone depended on the concentration.

In the same way, the synergistic effect of methicillin, aminobenzyl penicillin, cephalixin, penicillin G i.e. β -lactams, was confirmed. Amikacin, tetracycline, chloramphenicol also showed a synergistic effect but the antibacterial effect of gentamicin with the addition of tea catechins was low. F-51 was susceptible to vancomycin but the antibacterial activity was not influenced by the addition of tea catechins.

Example 5

The influence of individual pure catechins and a pure theaflavin on the antibacterial effect of oxacillin on MRSA was determined by the cup method as in Example 2, and the results are shown in Figures 7-9.

The strain of bacteria F-51, which has high resistancy to oxacillin was used, and tea catechins and theaflavins used were (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate and theaflavin digallates. Oxacillin (Sigma Chemical Co.) was used as antibiotic.

In Fig. 7, ○ indicates (-)-epicatechin gallate at a concentration of 25 $\mu\text{g/ml}$, ● indicates a concentration of 12.5 $\mu\text{g/ml}$, □ indicates a concentration of 6.3 $\mu\text{g/ml}$, and ■ indicates no addition of (-)-epicatechin gallate.

In Fig. 8, ○ indicates (-)-epigallocatechin gallate at a

concentration of $50\mu\text{g/ml}$. ● indicates a concentration of $25\mu\text{g/ml}$. □ indicates a concentration of $12.5\mu\text{g/ml}$. ■ indicates a concentration of $6.3\mu\text{g/ml}$, and △ indicates no addition of (-)-epigallocatechin gallate.

In Fig. 9, ○ indicates theaflavin gallate at a concentration of $100\mu\text{g/ml}$. ● indicates a concentration of $50\mu\text{g/ml}$. □ indicates a concentration of $25\mu\text{g/ml}$. ■ indicates a concentration of $12.5\mu\text{g/ml}$. △ indicates a concentration of $6.3\mu\text{g/ml}$, and ▲ indicates no addition of theaflavin gallate.

The addition of (+)-gallo-catechins up to a concentration of $800\mu\text{g/ml}$ to $40\mu\text{g/ml}$ oxacillin showed no antibacterial effect. In the same way the addition of (-)-epicatechin up to a concentration of $100\mu\text{g/ml}$, or (-)-epigallocatechin up to a concentration of $50\mu\text{g/ml}$ showed no antibacterial effect.

On the other hand, as is shown in Figures 7-9, with (-)-epicatechin gallate, (-)-epigallocatechin gallate and theaflavin digallates an antibacterial effect was apparant. These results show that the galloyl moiety of catechins and theaflavins is crucial to their synergistic effects with antibiotics.

Comparative Example

This experiment was carried out in the same way as in Example 4 except that the following antibiotics were used: kanamycin (Wako Pure Chemicals), erythromycin (Wako Pure Chemicals), clindamycin (Sigma Chemical Company), and colistin (Wako Pure Chemicals) and the effect of each catechin on the antibacterial action of each antibiotic was investigated by the cup method as in Example 2.

Results obtained are shown in Figure 10A-D. F-51 strain which

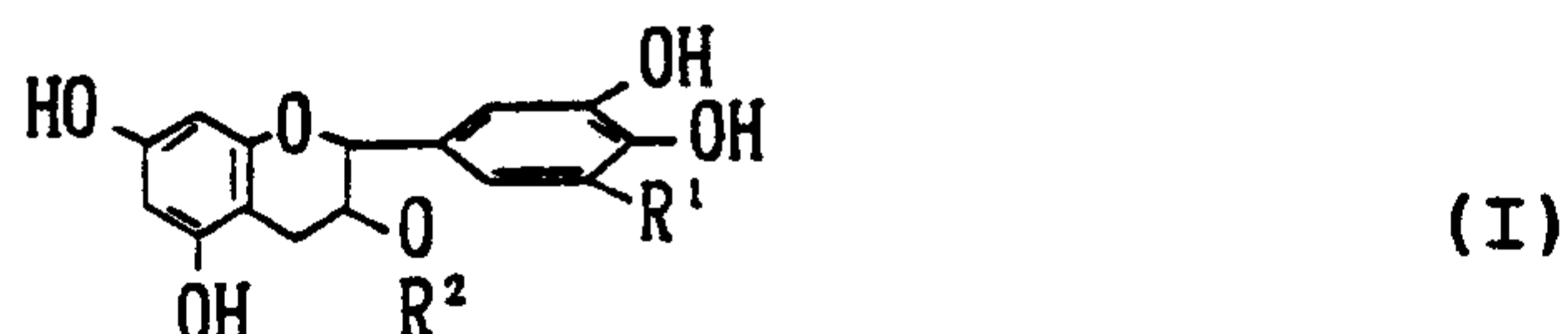
showed a high resistancy to oxacillin was used as the test bacteria and (-)-epigallocatechin gallate was used as the tea catechin. In the figure, ○ indicates (-)-epigallocatechin gallate at a concentration of 50 μ g/ml, ● indicates (-)-epigallocatechin gallate at a concentration of 25 μ g/ml, □ indicates (-)-epigallocatechin gallate at a concentration of 12.5 μ g/ml, ■ indicates (-)-epigallocatechin gallate at a concentration of 6.3 μ g/ml, and △ indicates no addition of (-)-epigallocatechin gallate.

As is evident from the figure, under the cup method F-51 strain showed its resistancy with up to 400 μ g/ml of each of the antibiotics, and no improved synergistic antibacterial effects could be observed even though (-)-epigallocatechin was added at any concentration and combined with the antibiotics, and there was no change in the size of the inhibition zone.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A pharmaceutical composition which comprises (a) an effective antibiotic amount of an antibiotic in combination with (b) at least one polyphenol selected from the group consisting of a catechin and a theaflavin in an amount effective to synergistically increase the antibiotic activity of the antibiotic against methicillin resistance Staphylococcus aureus.

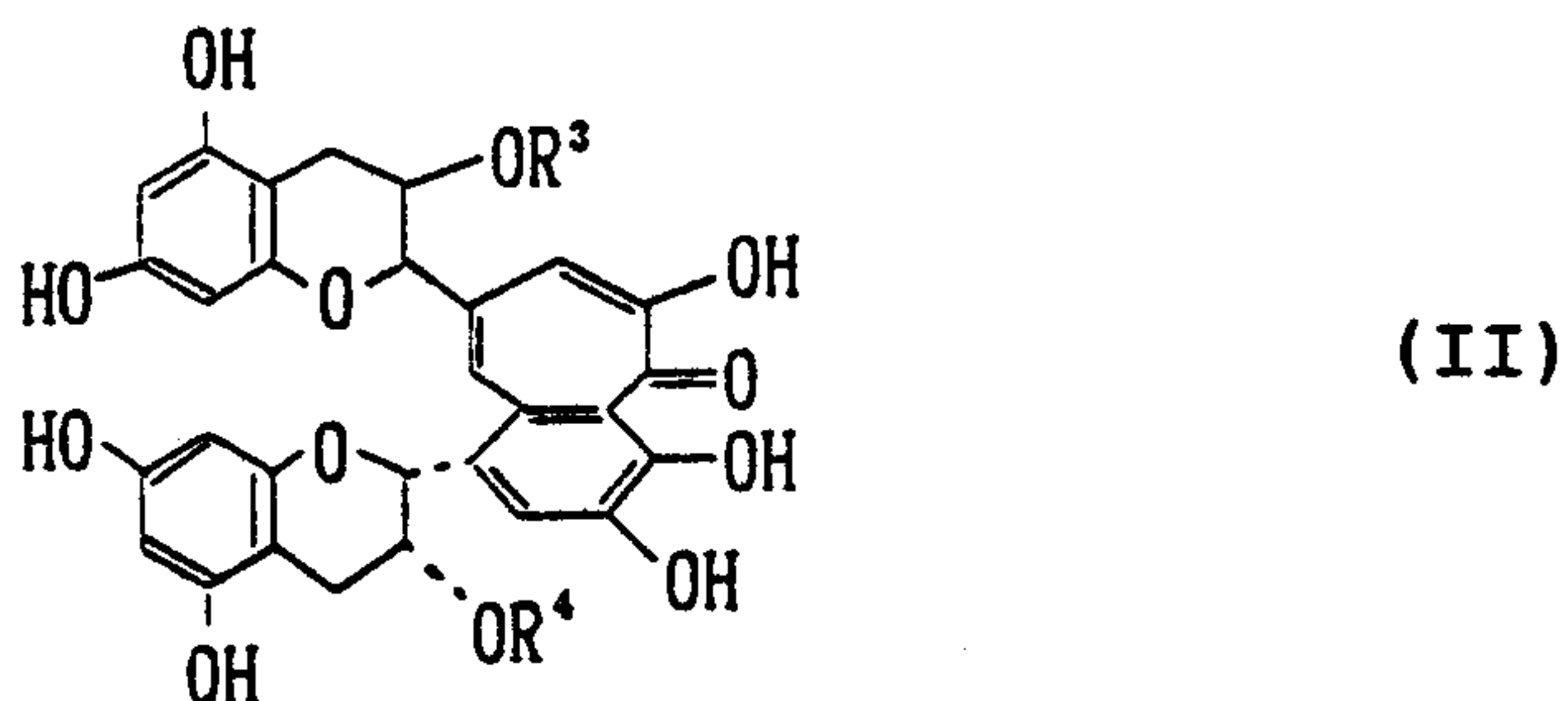
2. The composition according to claim 1, wherein the catechin has the formula (I):



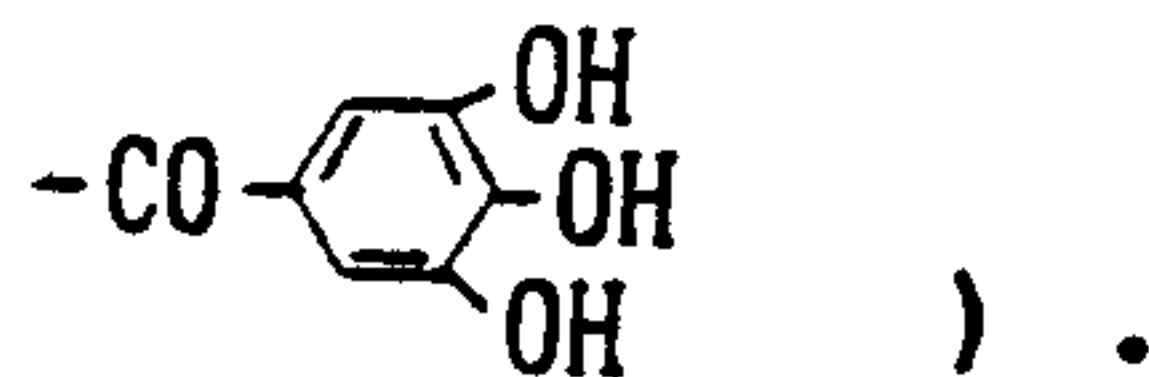
(wherein R¹ represents H or OH and R² represents H or



the theaflavin has the formula (II):



(wherein R^3 and R^4 each represent H or



3. The composition according to claim 2, wherein R^2 in

the formula (I) is $-\text{CO}-\text{C}_6\text{H}_2(\text{OH})_3$ and at least one of R^3 and R^4

represents $-\text{CO}-\text{C}_6\text{H}_2(\text{OH})_3$.

4. The composition according to claim 1, 2 or 3, wherein the antibiotic is ineffective against methicillin resistant Staphylococcus aureus.

5. The composition according to claim 4, wherein the antibiotic is β -lactam or aminoglycoside.

6. The composition according to claim 4, wherein the antibiotic is at least one member selected from the group consisting of oxacillin, methicillin, aminobenzyl penicillin, cephalixin, penicillin G, amikacin, tetracycline and chloramphenicol.

7. The composition according to claim 4, which contains as the catechin, epicatechin gallate or epigallocatechin gallate.

8. The composition according to claim 4, which contains the catechin having as its main component epigallocatechin gallate.

9. The composition according to claim 4, which contains the theaflavin having as its main component theaflavin digallate.

10. The composition according to any one of claims 1 to 9, which further contains a pharmaceutically acceptable excipient.

11. The composition according to any one of claims 1 to 10, which contains from about 0.5 to about 500 parts by weight of the polyphenol per part by weight of the antibiotic.

12. The composition according to claim 11, which contains from about 2 to about 100 parts by weight of the polyphenol per part by weight of the antibiotic.

13. The composition according to any one of claims 1 to 12, which is for treating or preventing infection of methicillin resistant Staphylococcus aureus.

14. A commercial package comprising the composition according to claim 13 and a written matter associated therewith, the written matter stating that the composition should or can be used for treating or preventing infection of methicillin resistant Staphylococcus aureus.

15. A method of increasing the activity of an antibiotic against methicillin resistant Staphylococcus aureus, which comprises admixing an effective antibiotic amount of the antibiotic with at least one polyphenol selected from the

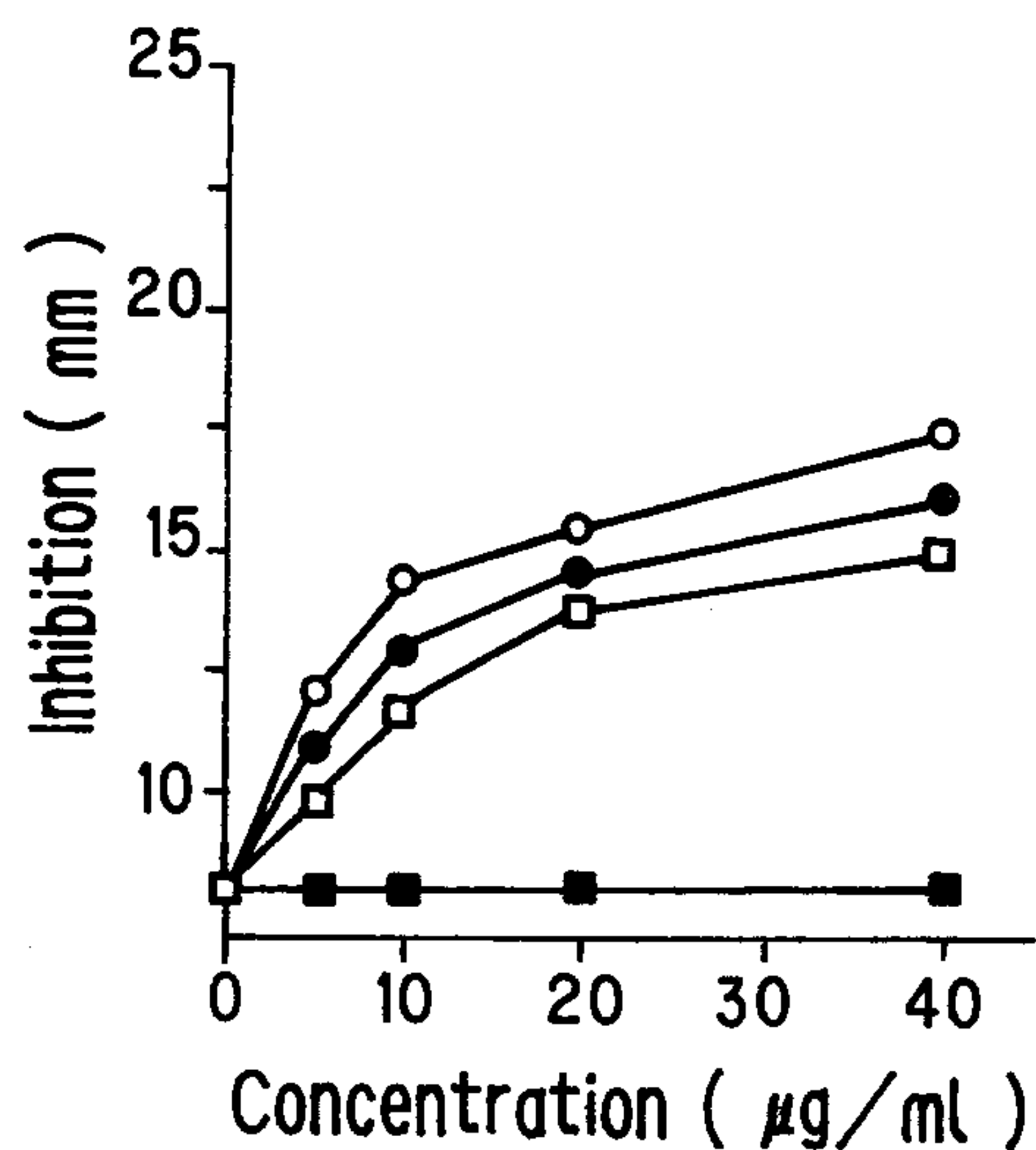
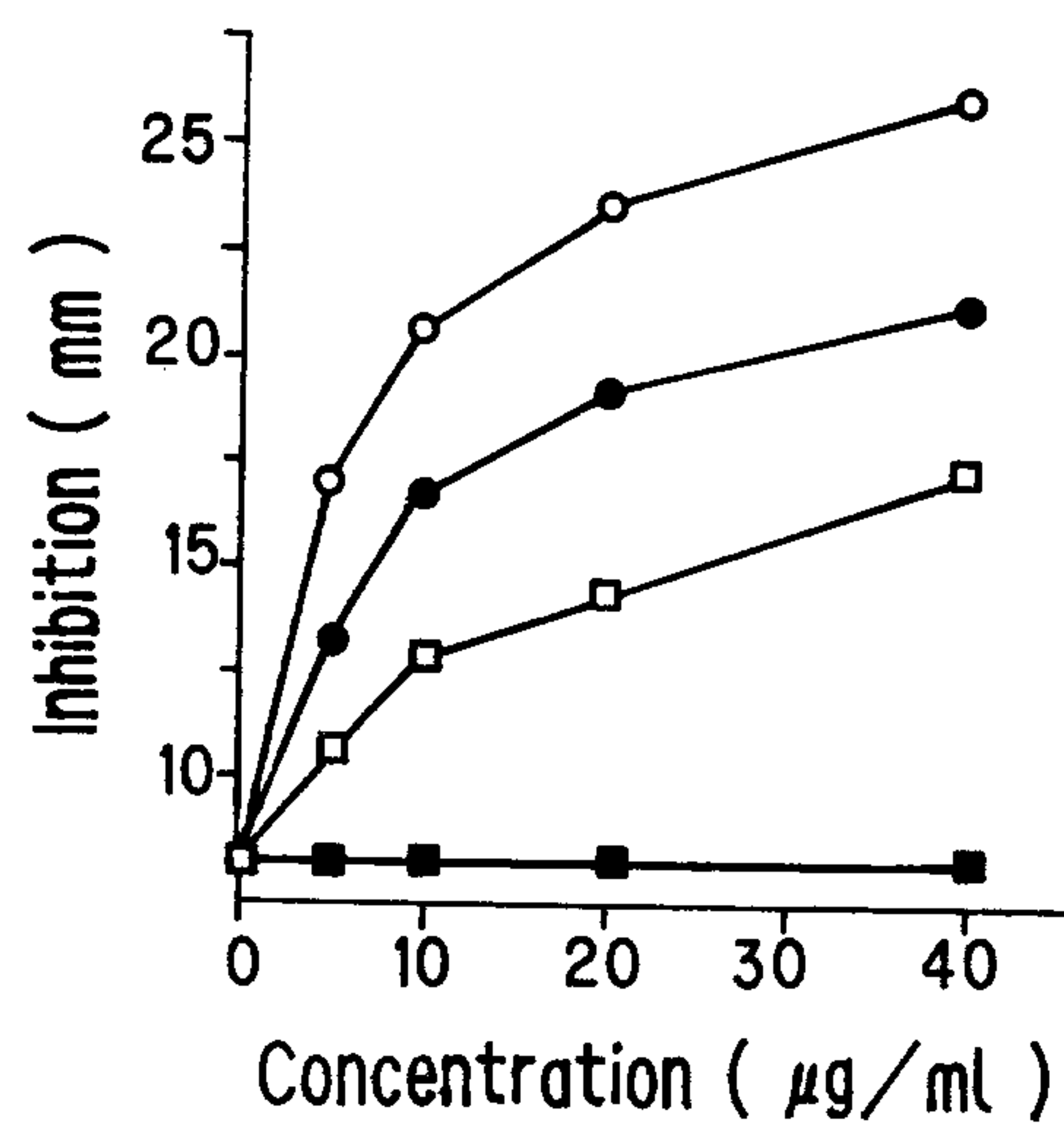
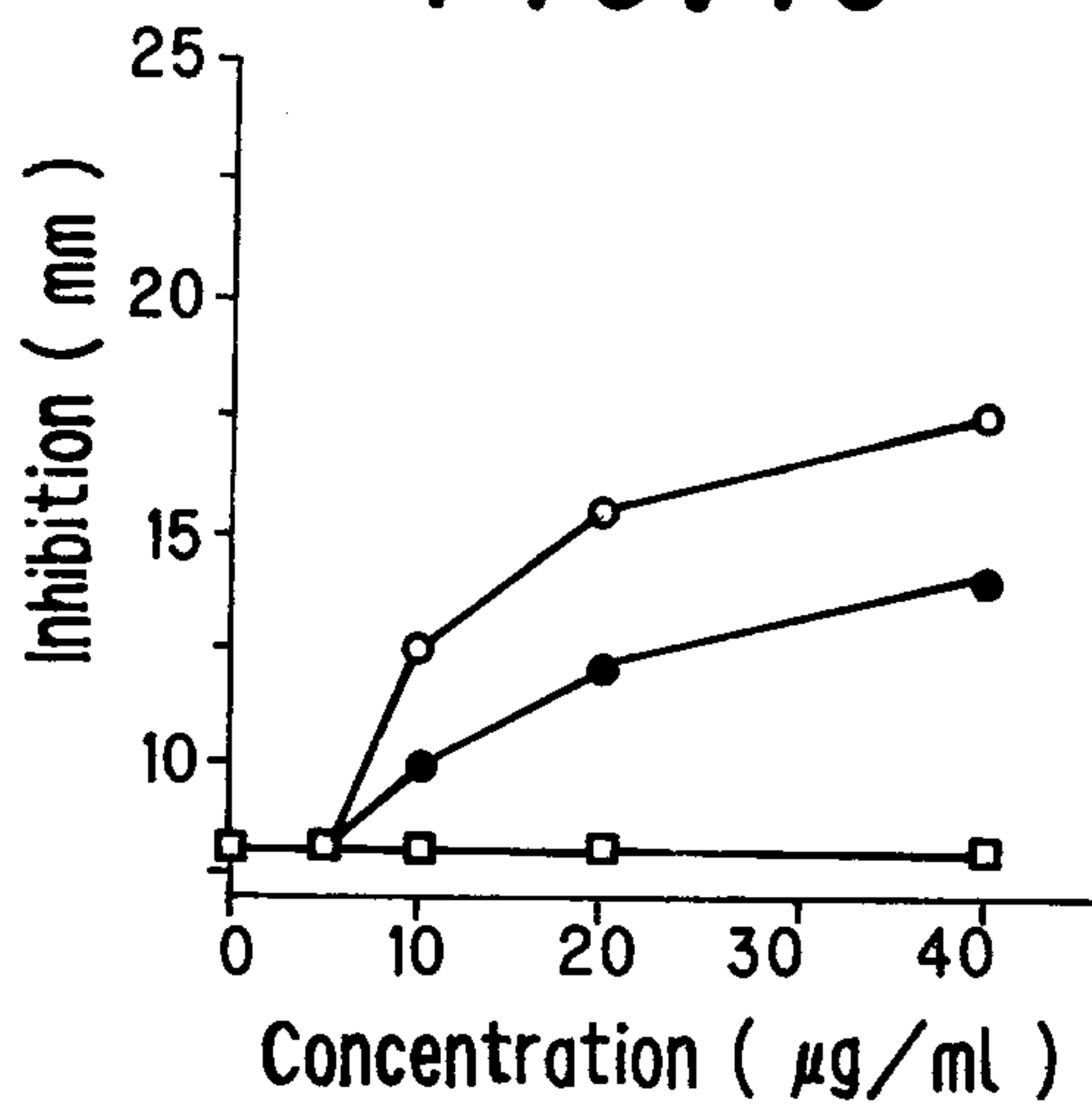
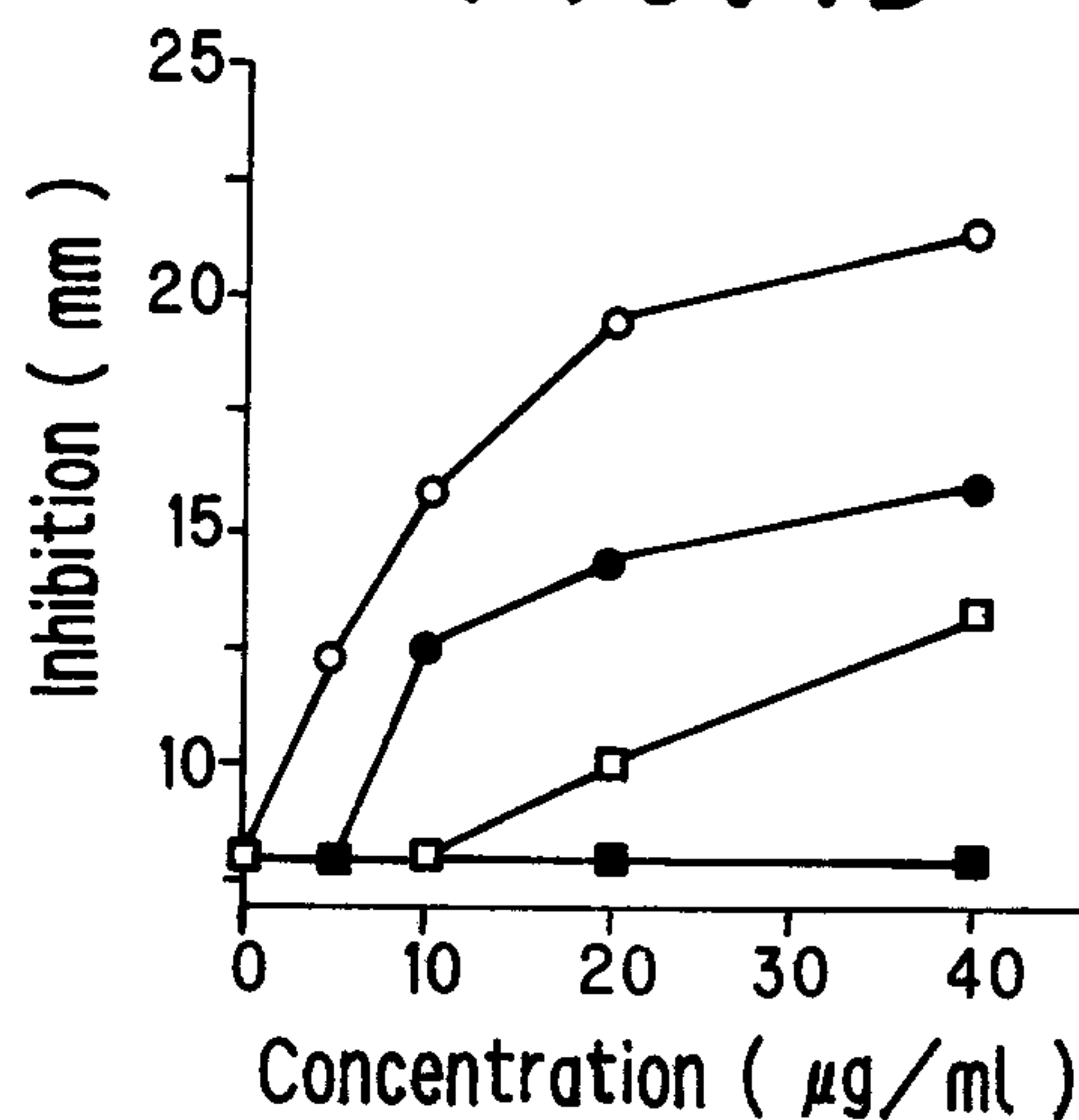
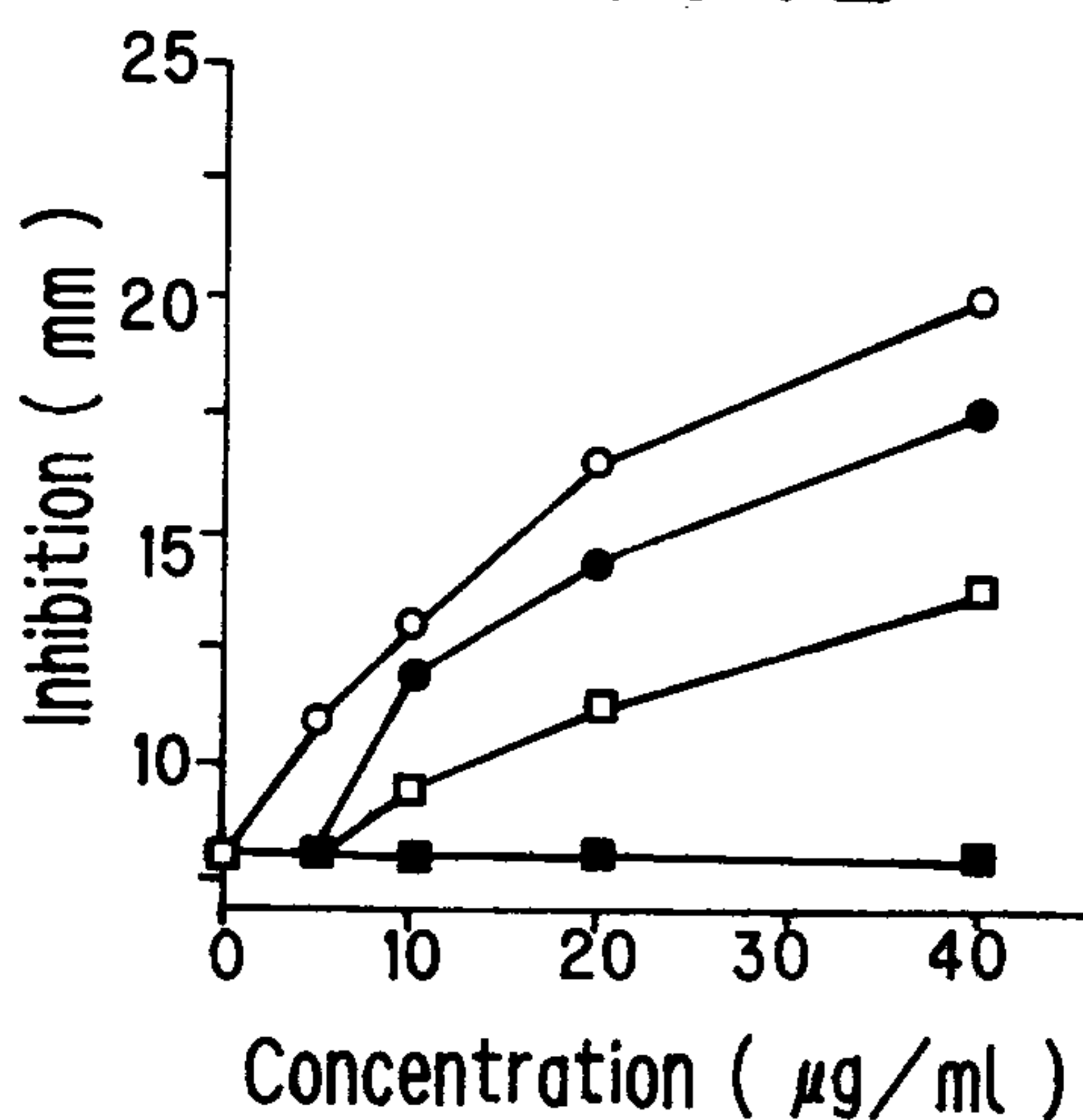
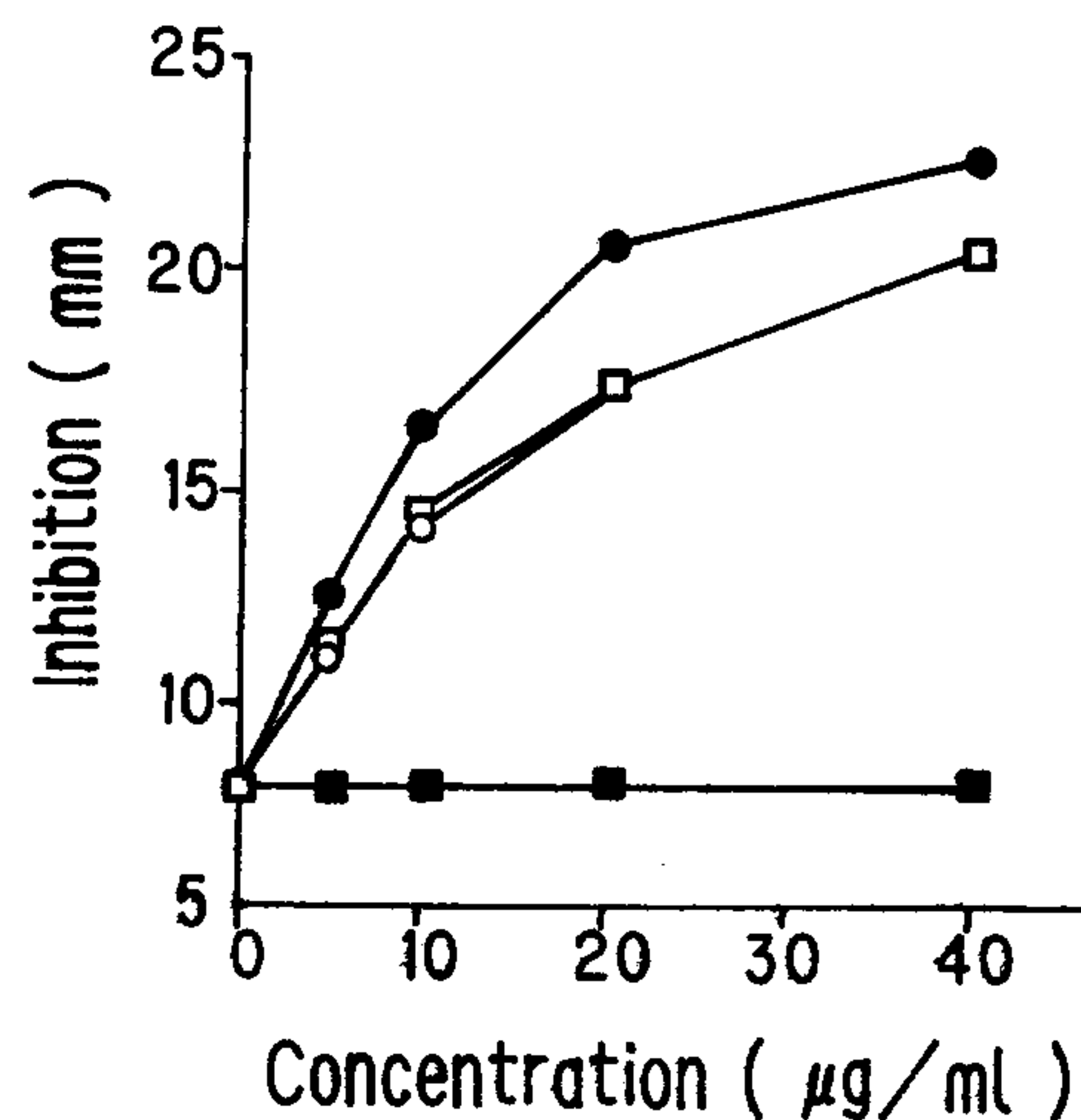
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group consisting of a catechin and a theaflavin in an amount effective to synergistically increase the antibiotic activity of the antibiotic.

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FIG. 1A**FIG. 1B****FIG. 1C****FIG. 1D****FIG. 1E****FIG. 1F**

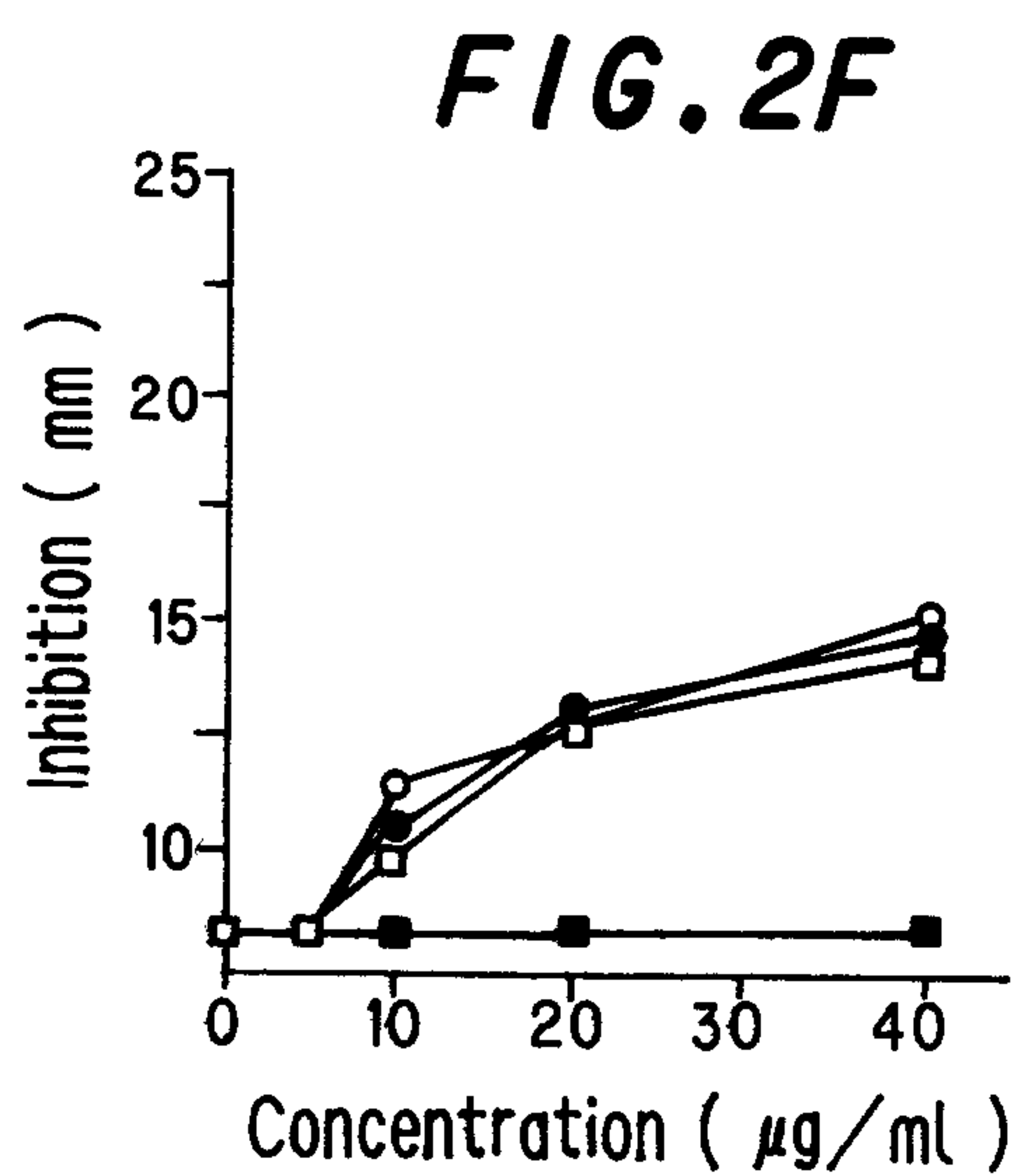
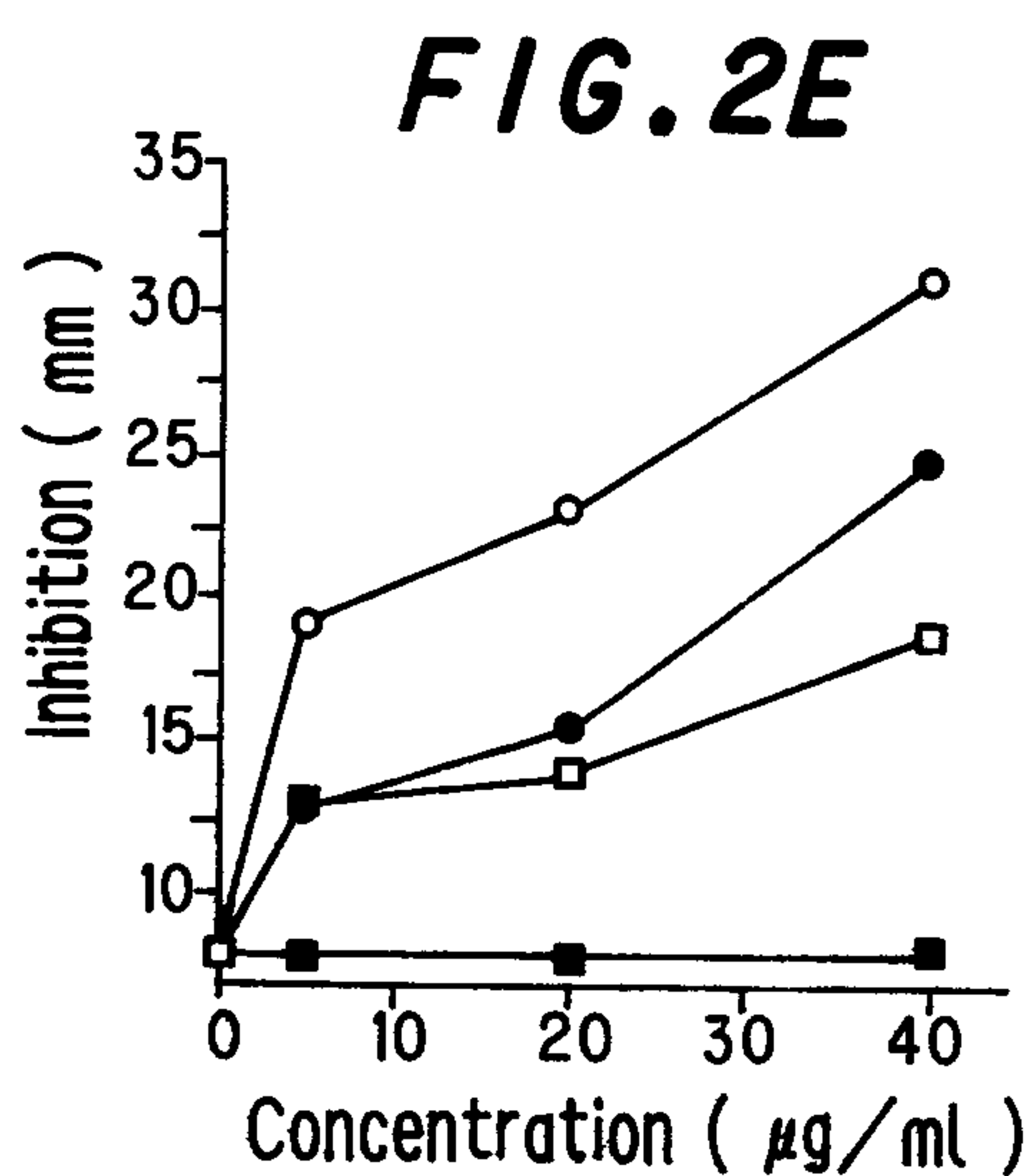
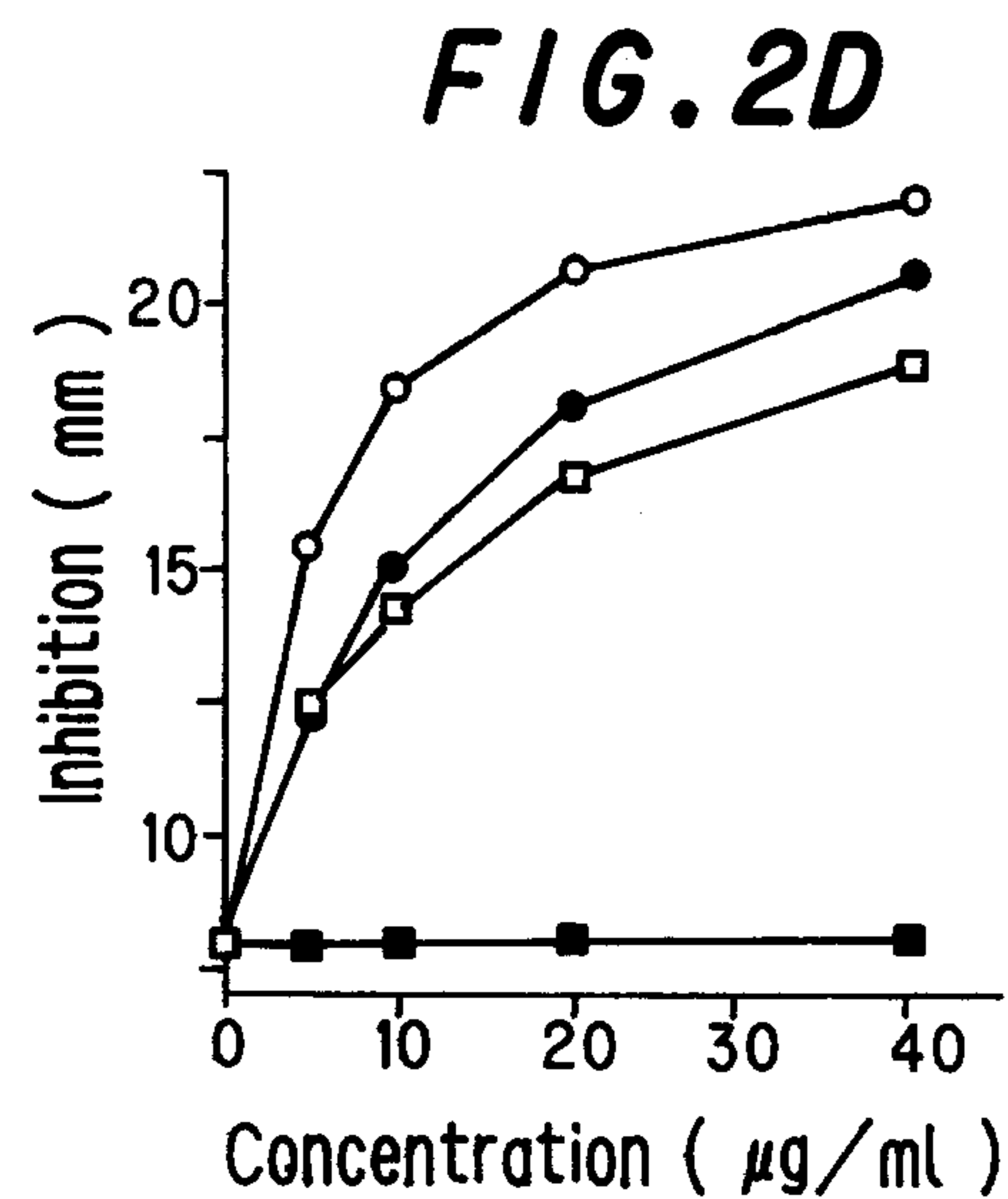
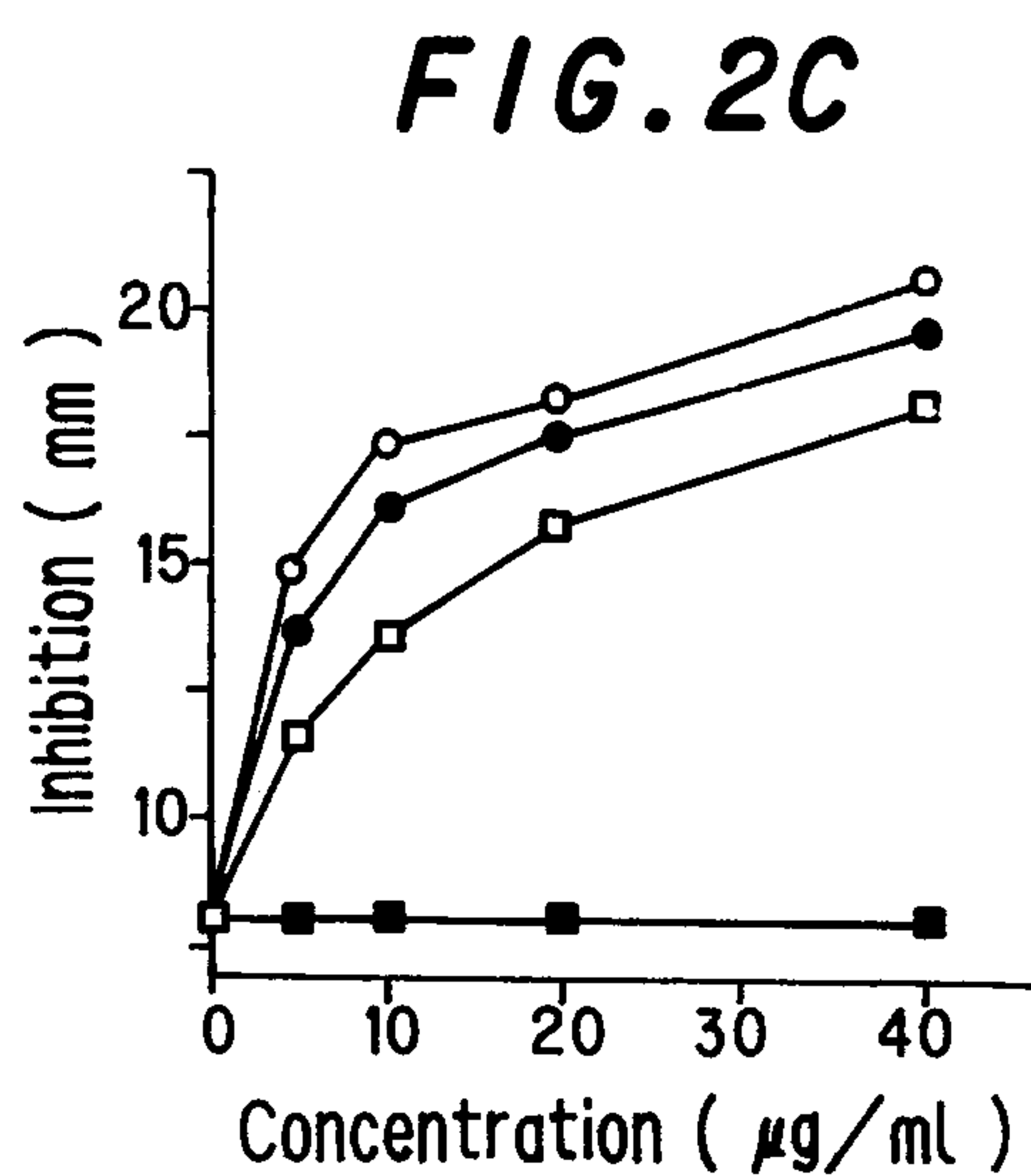
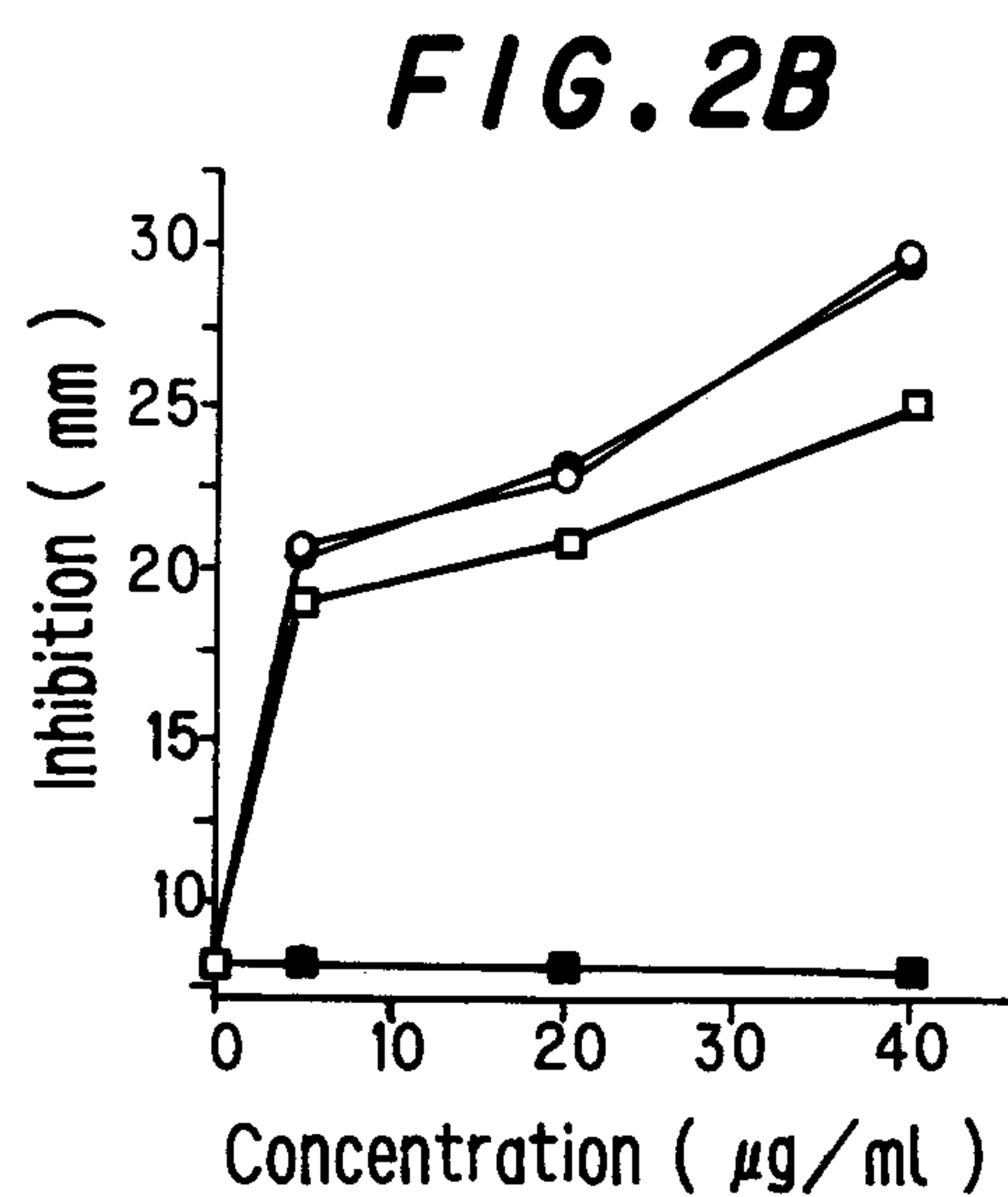
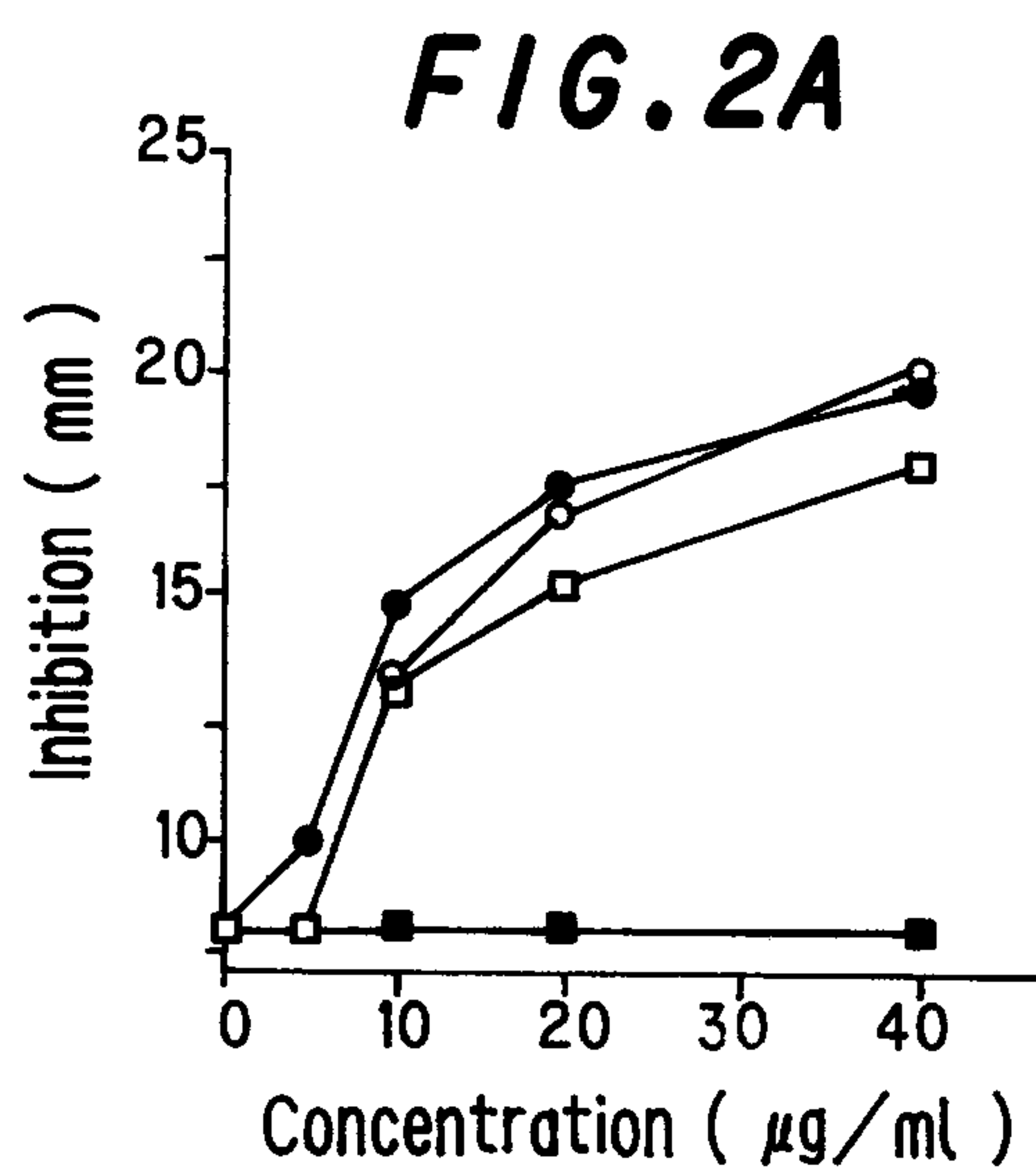


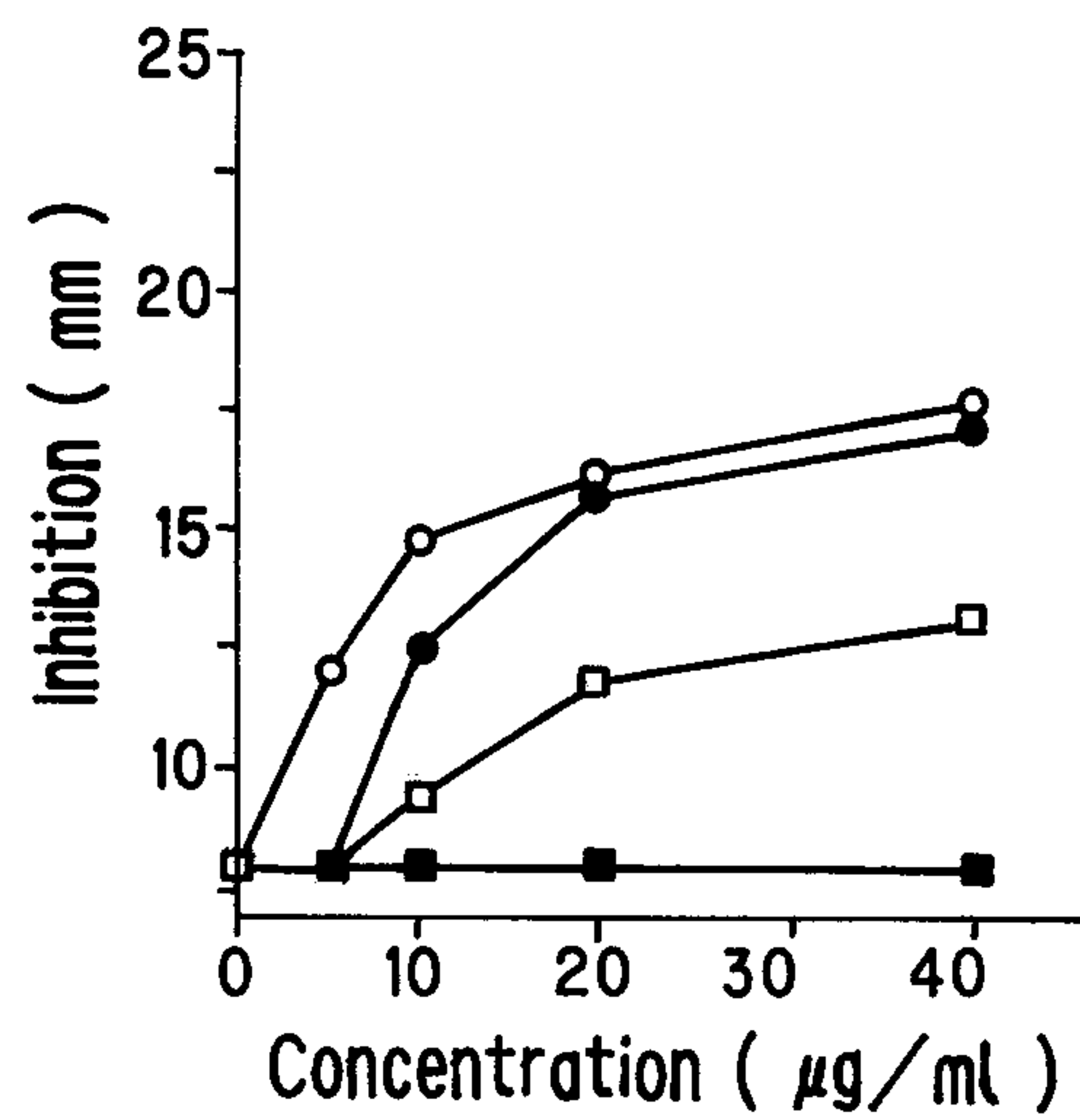
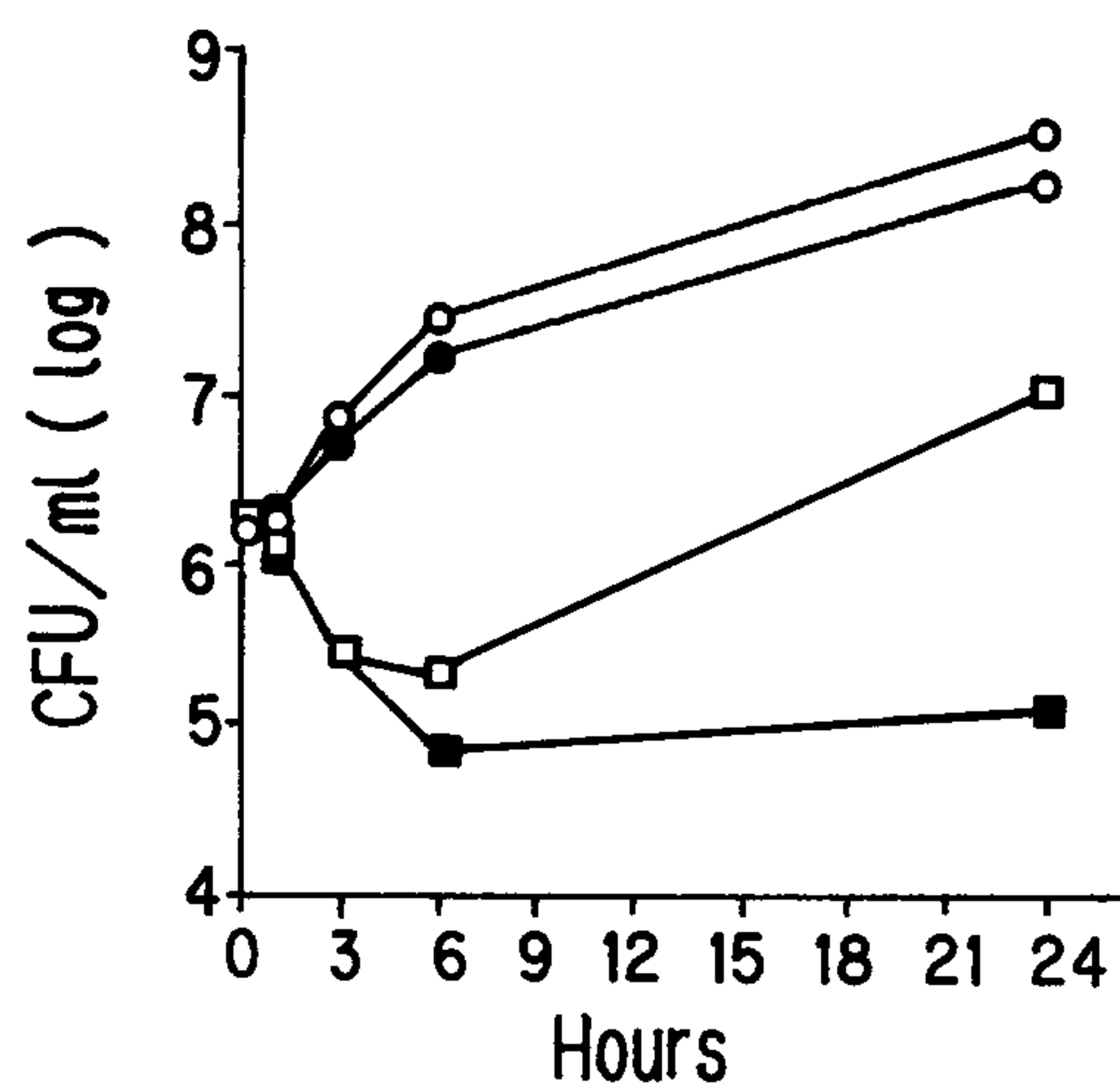
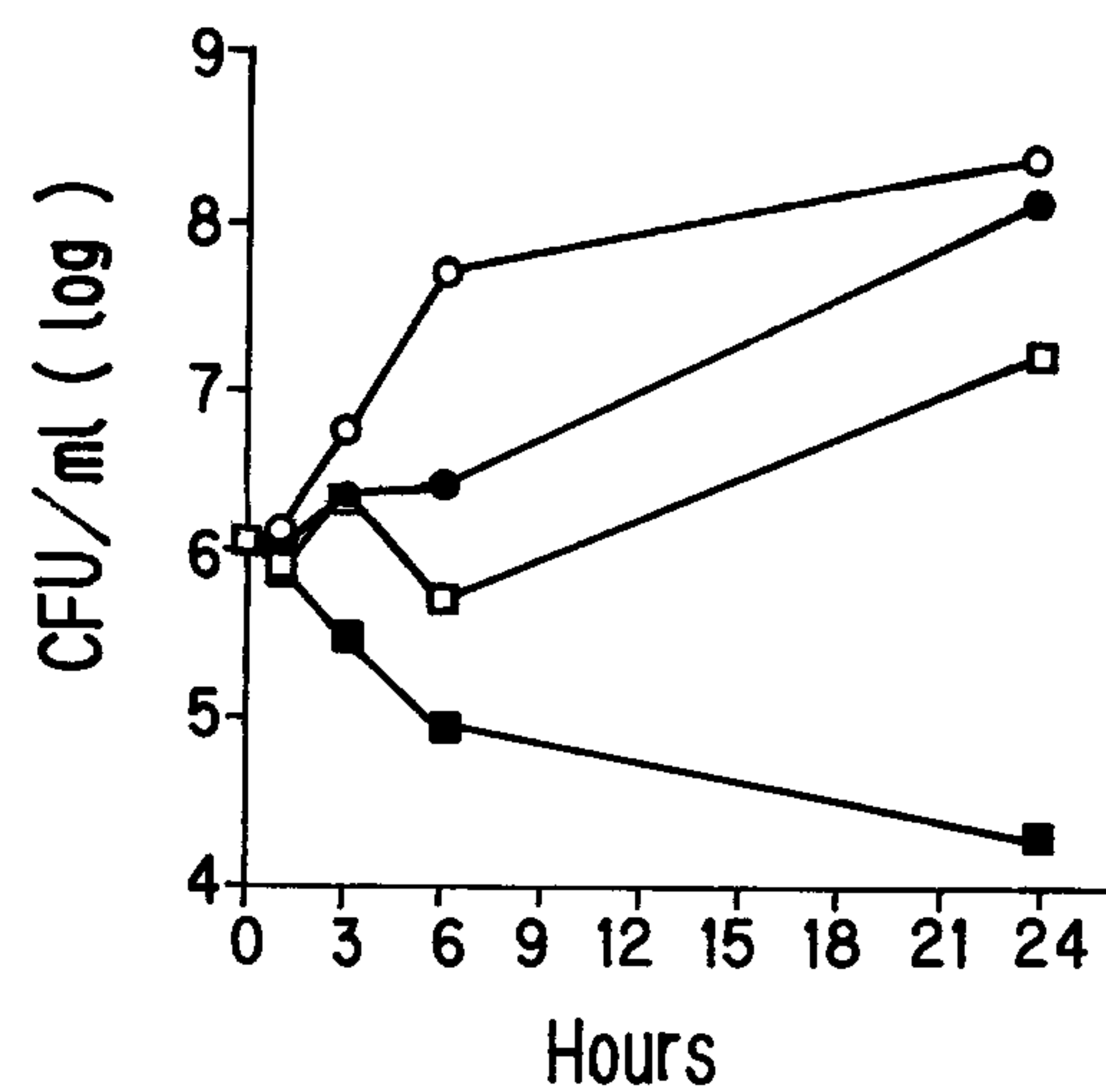
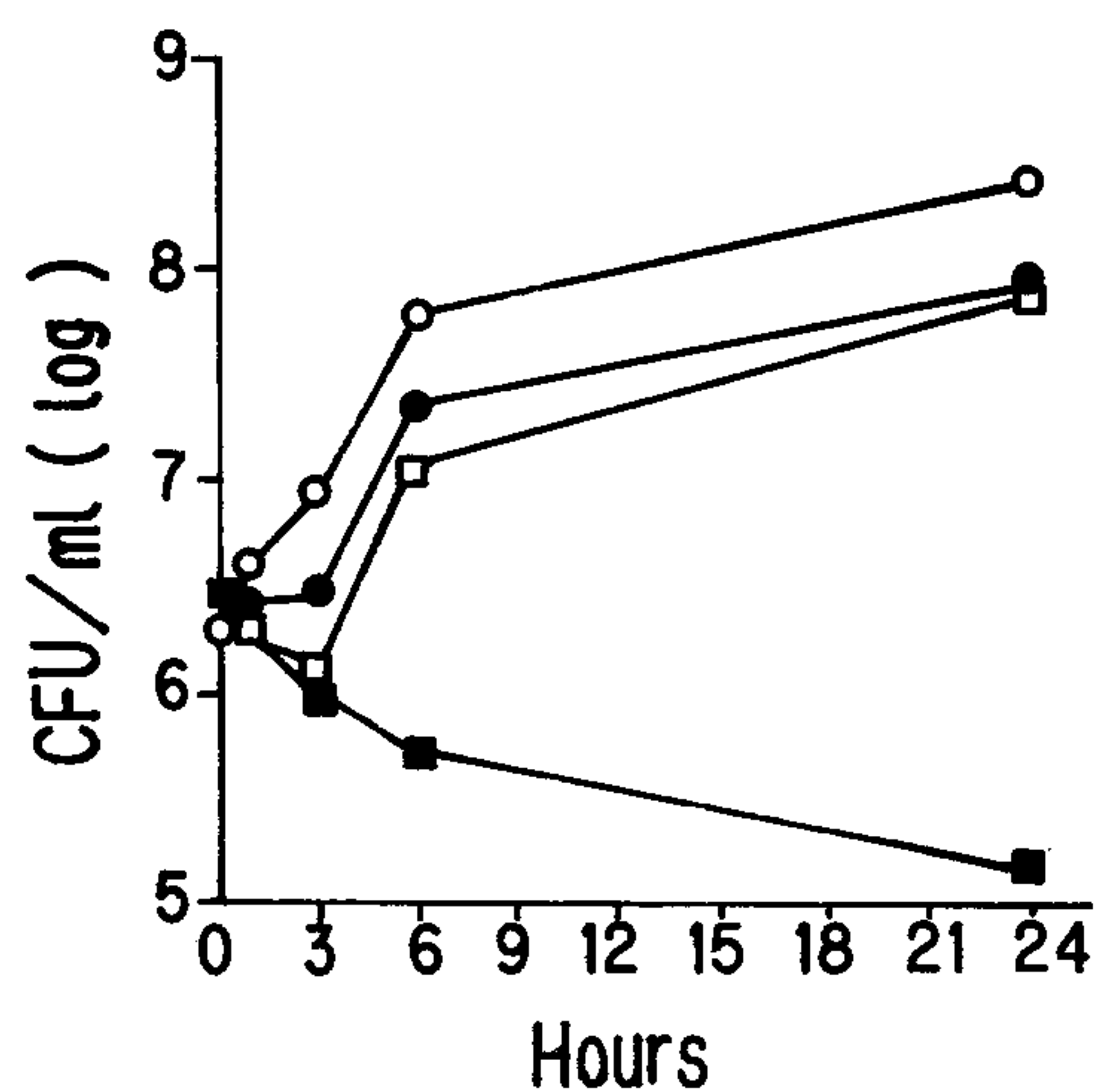
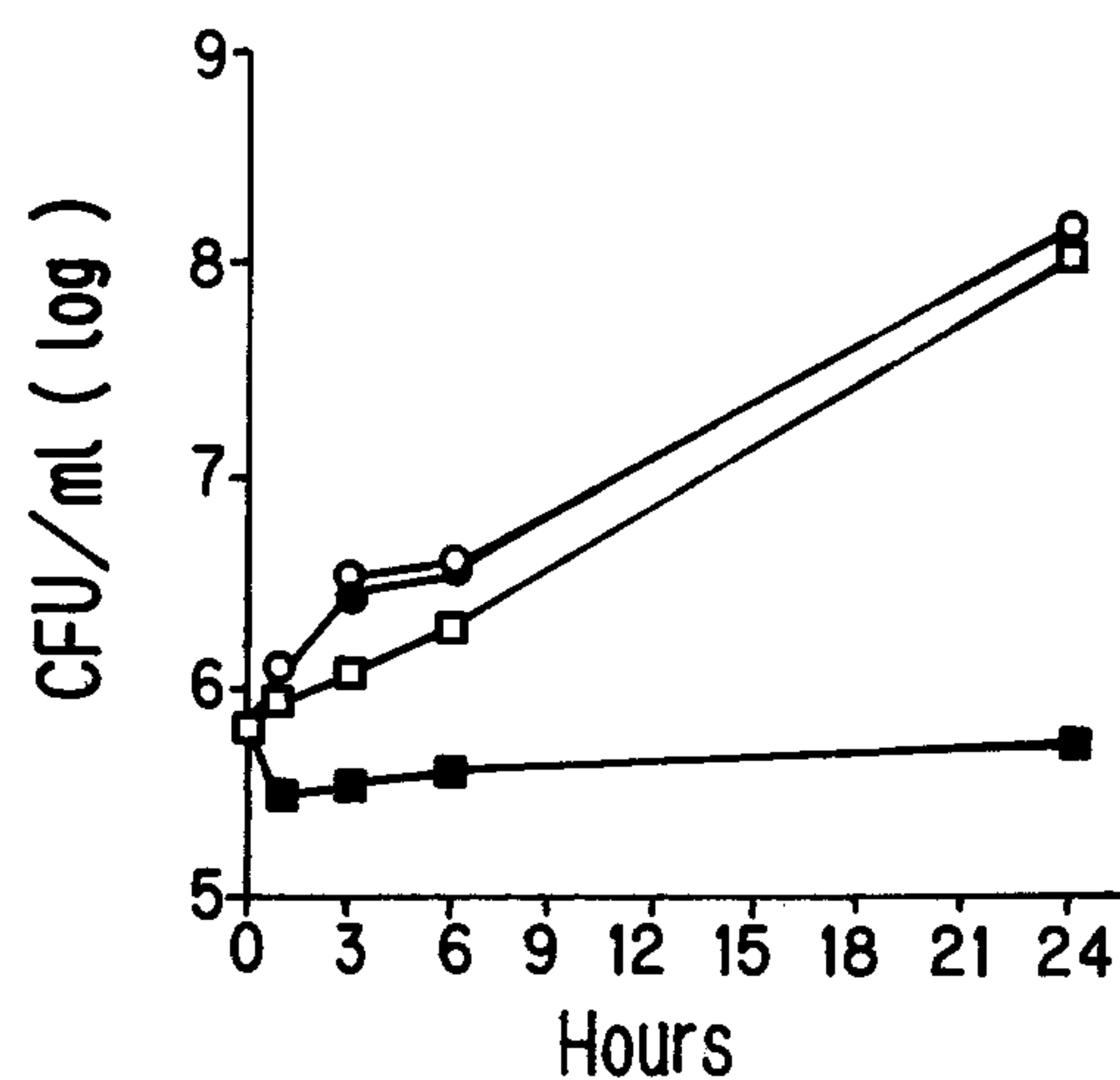
FIG. 3**FIG. 4A****FIG. 4B****FIG. 4C****FIG. 4D**

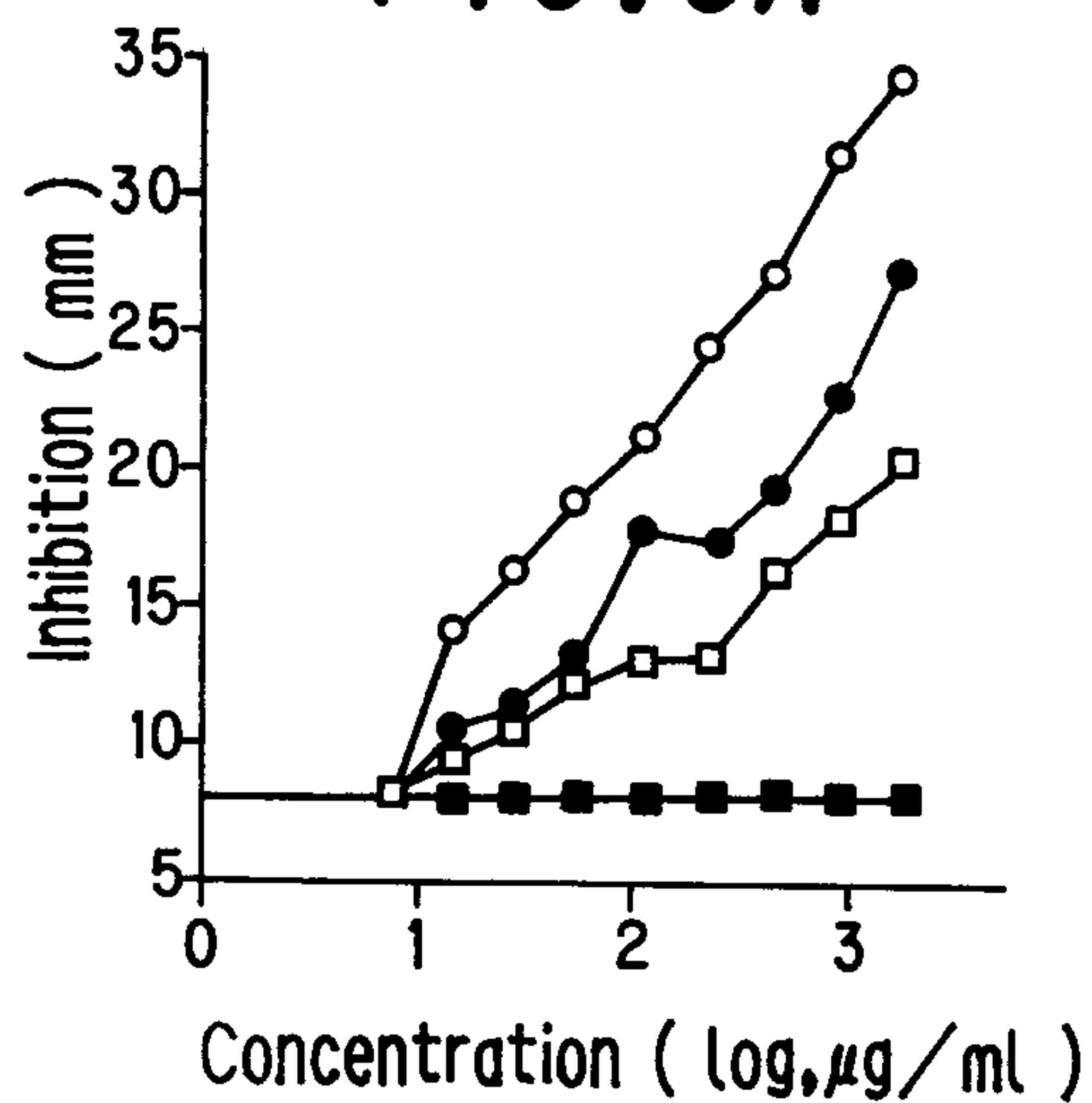
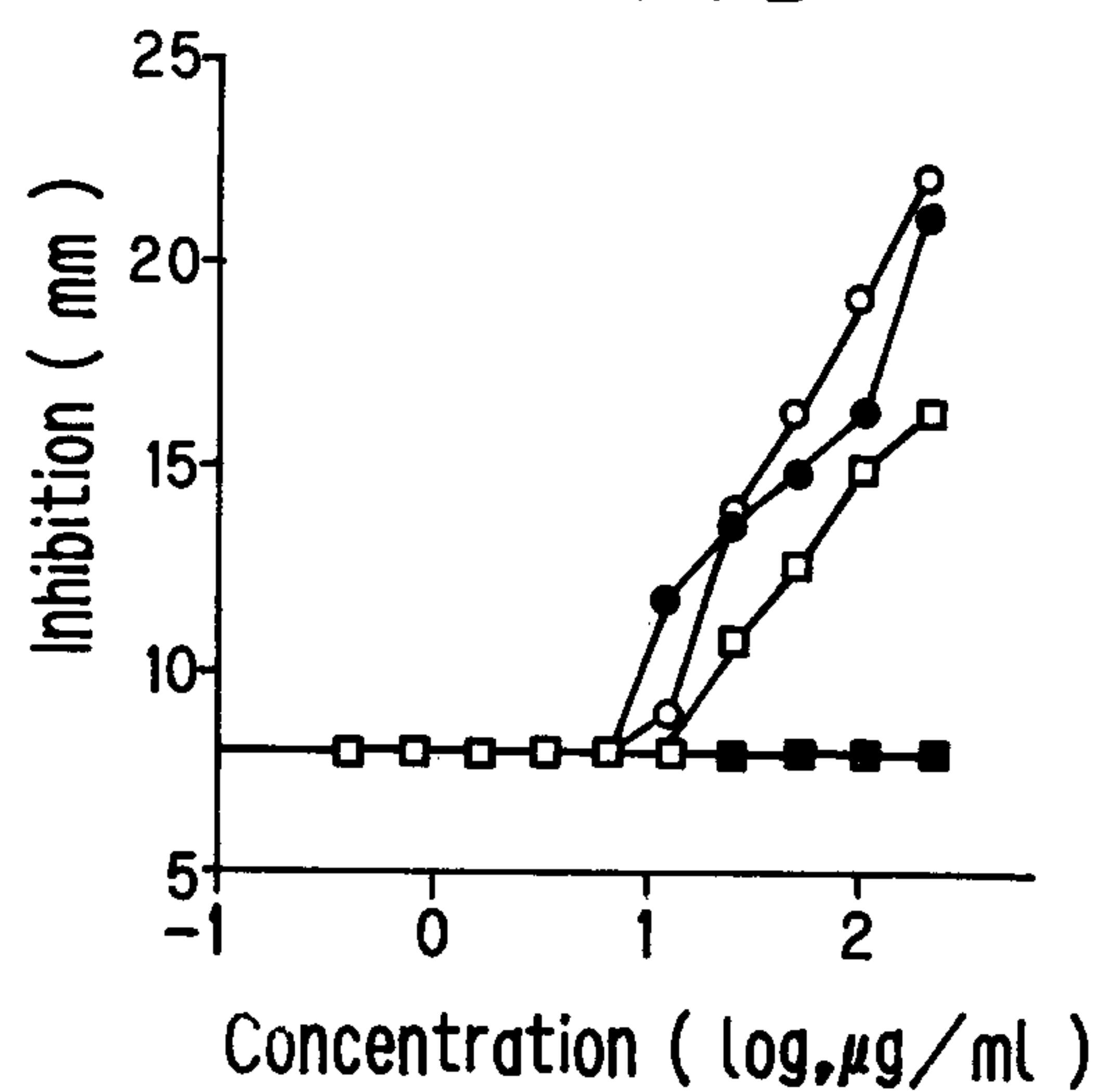
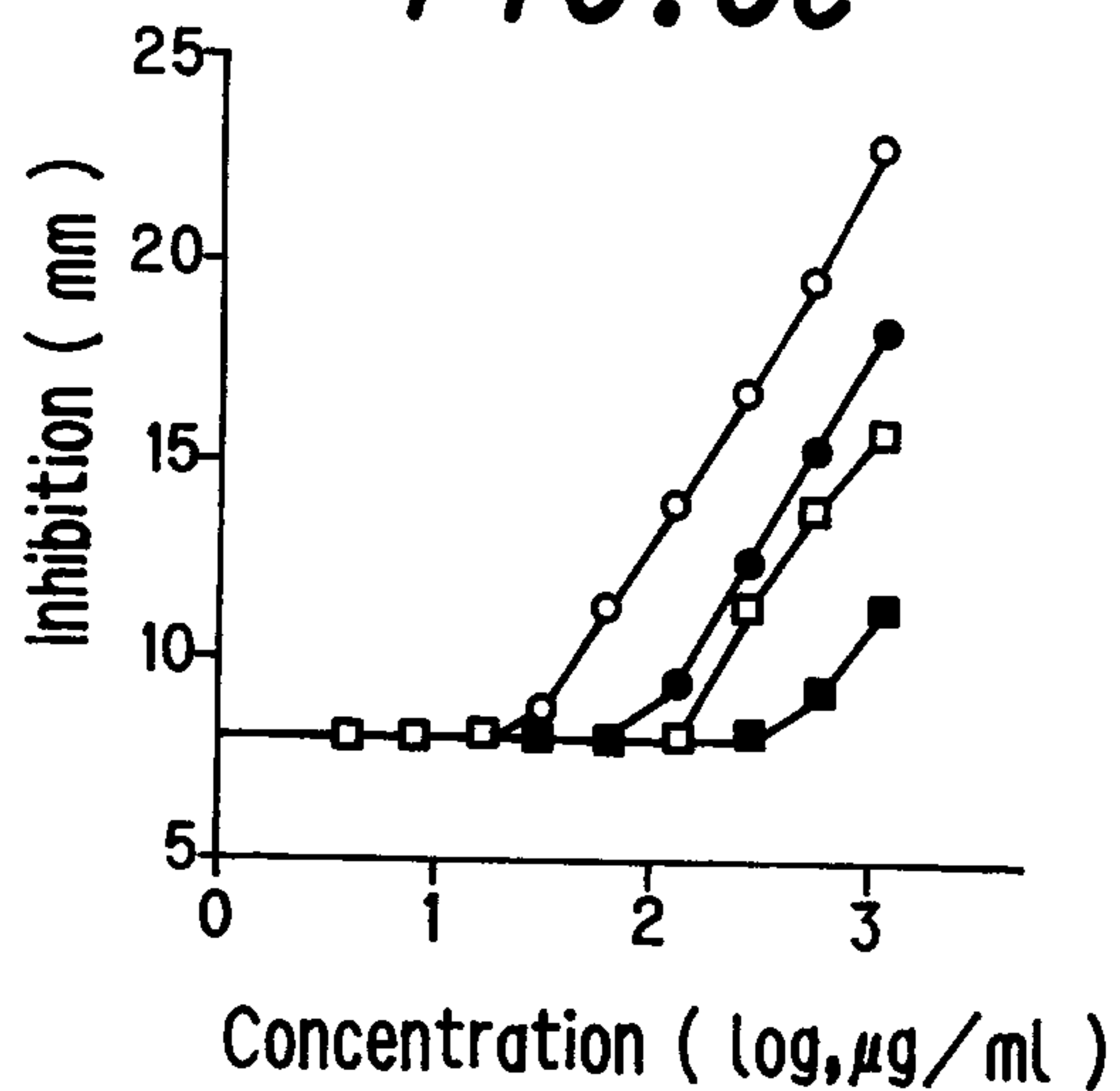
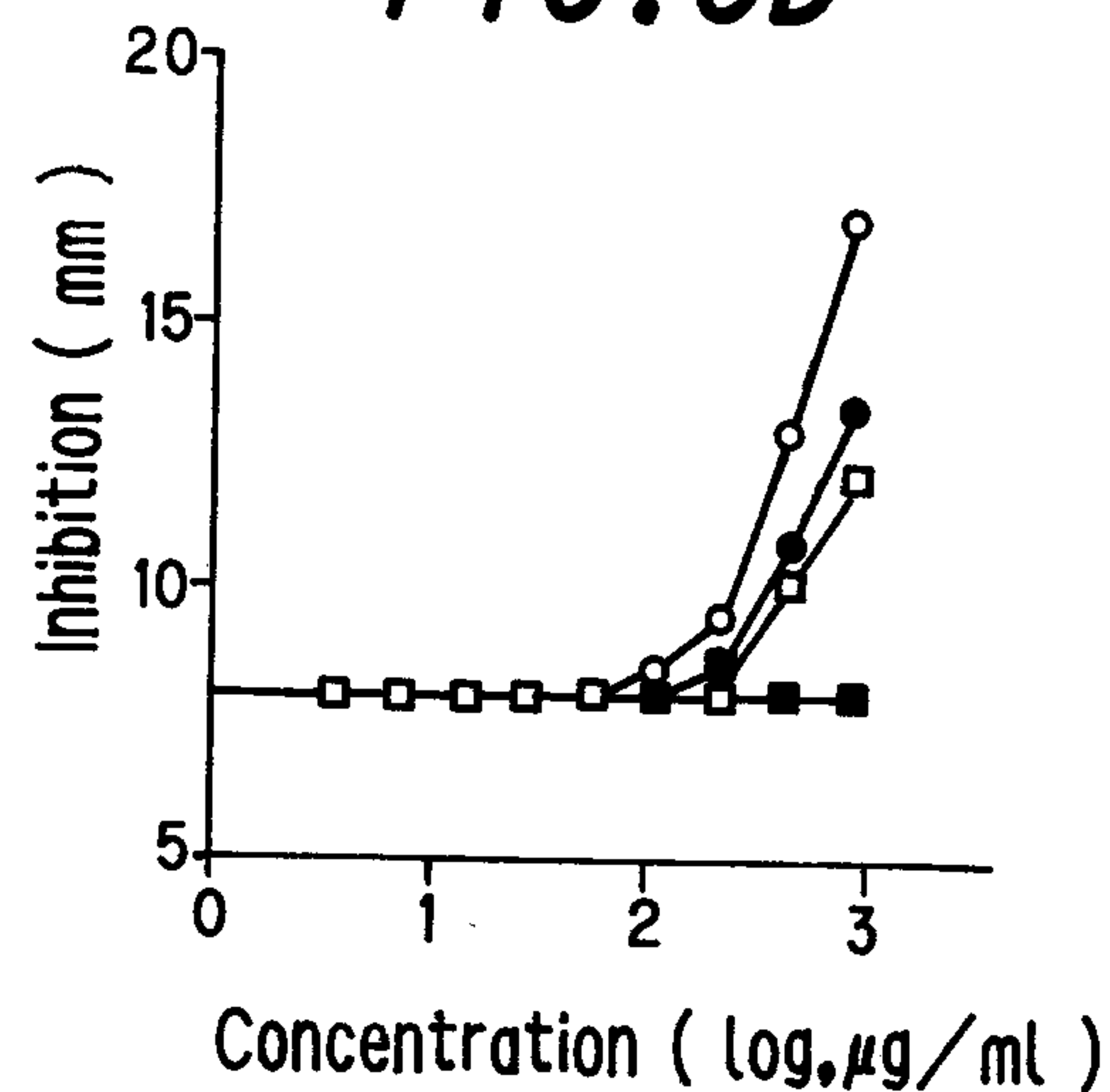
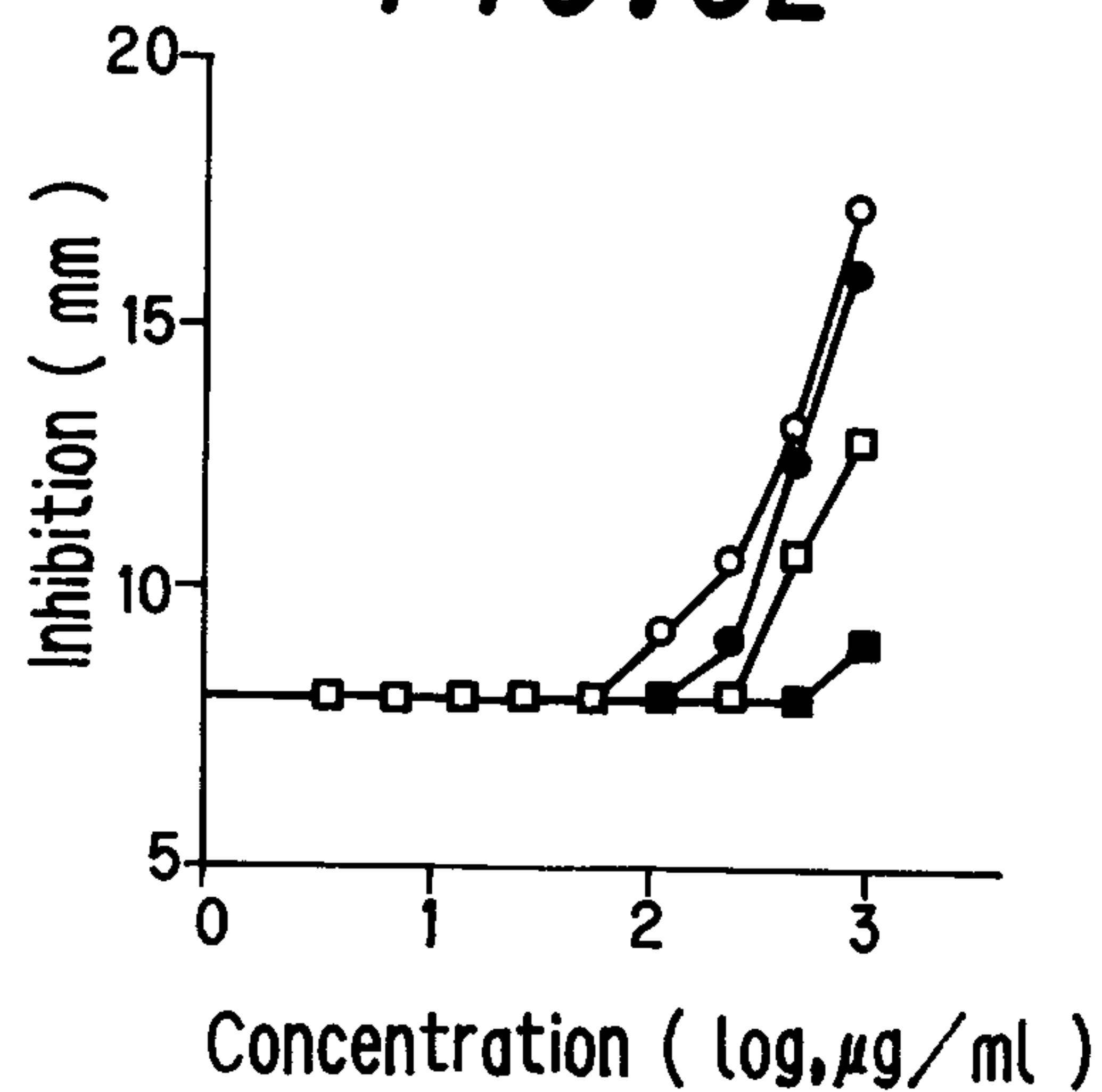
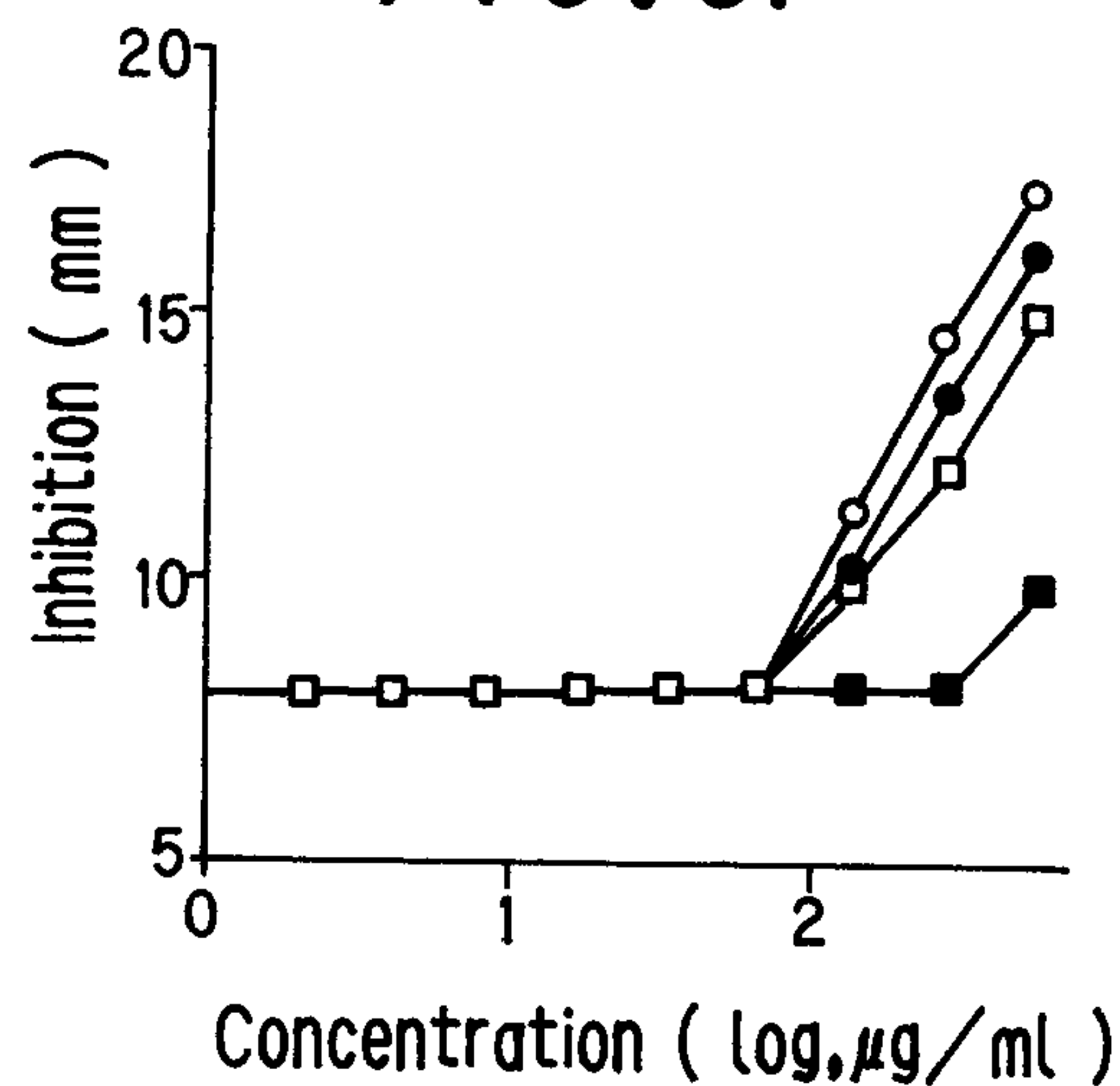
FIG. 5A**FIG. 5B****FIG. 5C****FIG. 5D****FIG. 5E****FIG. 5F**

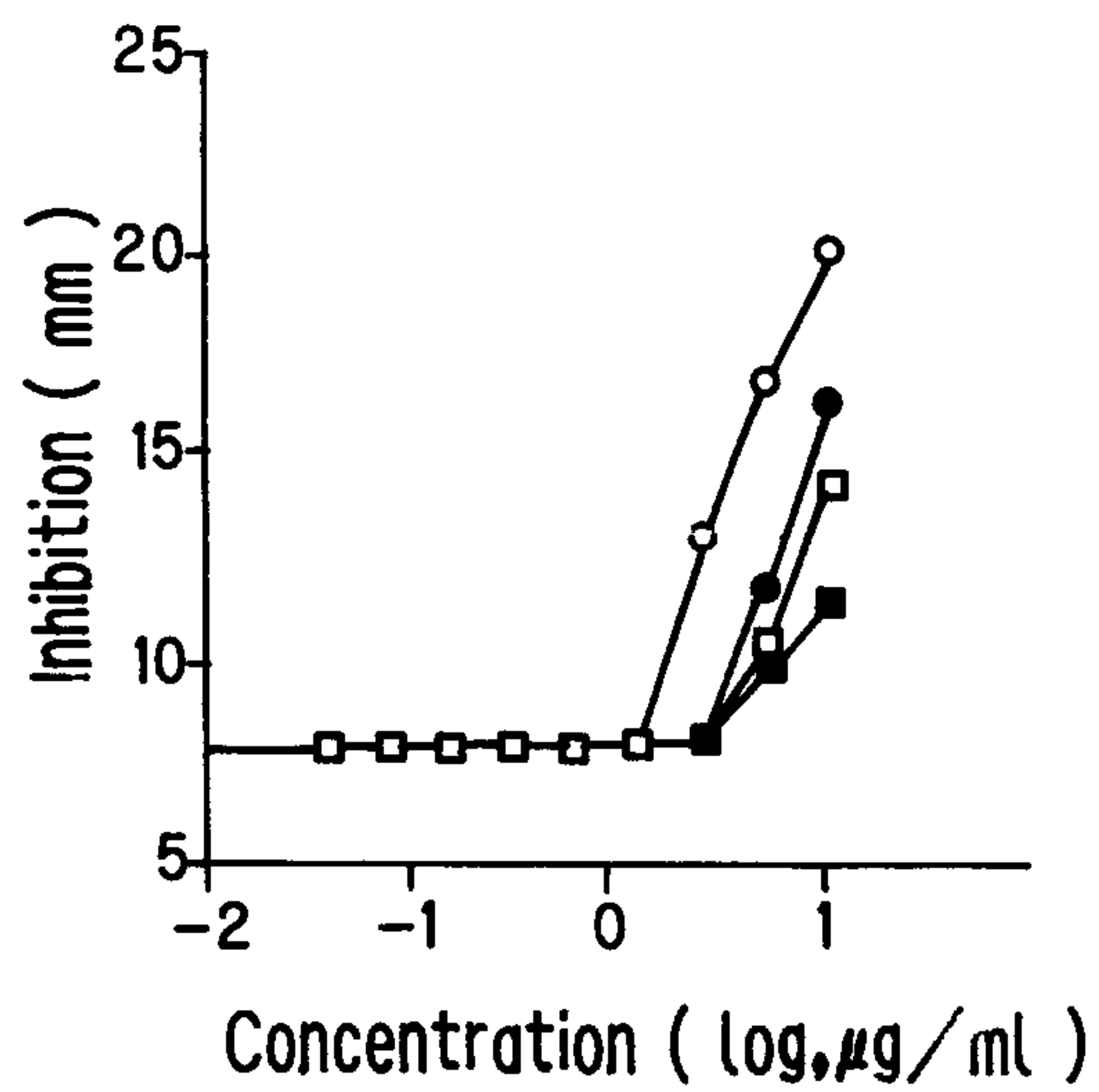
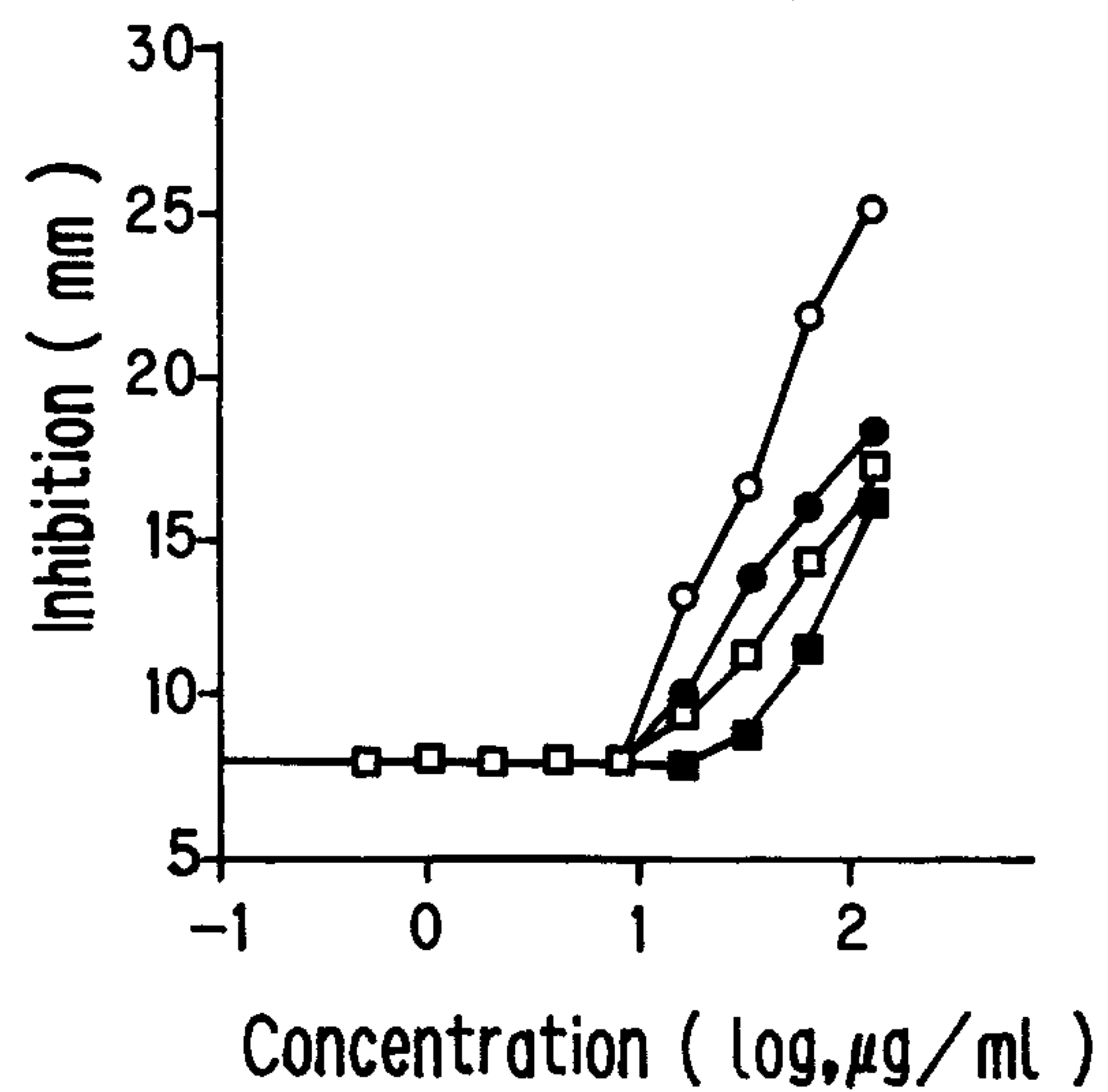
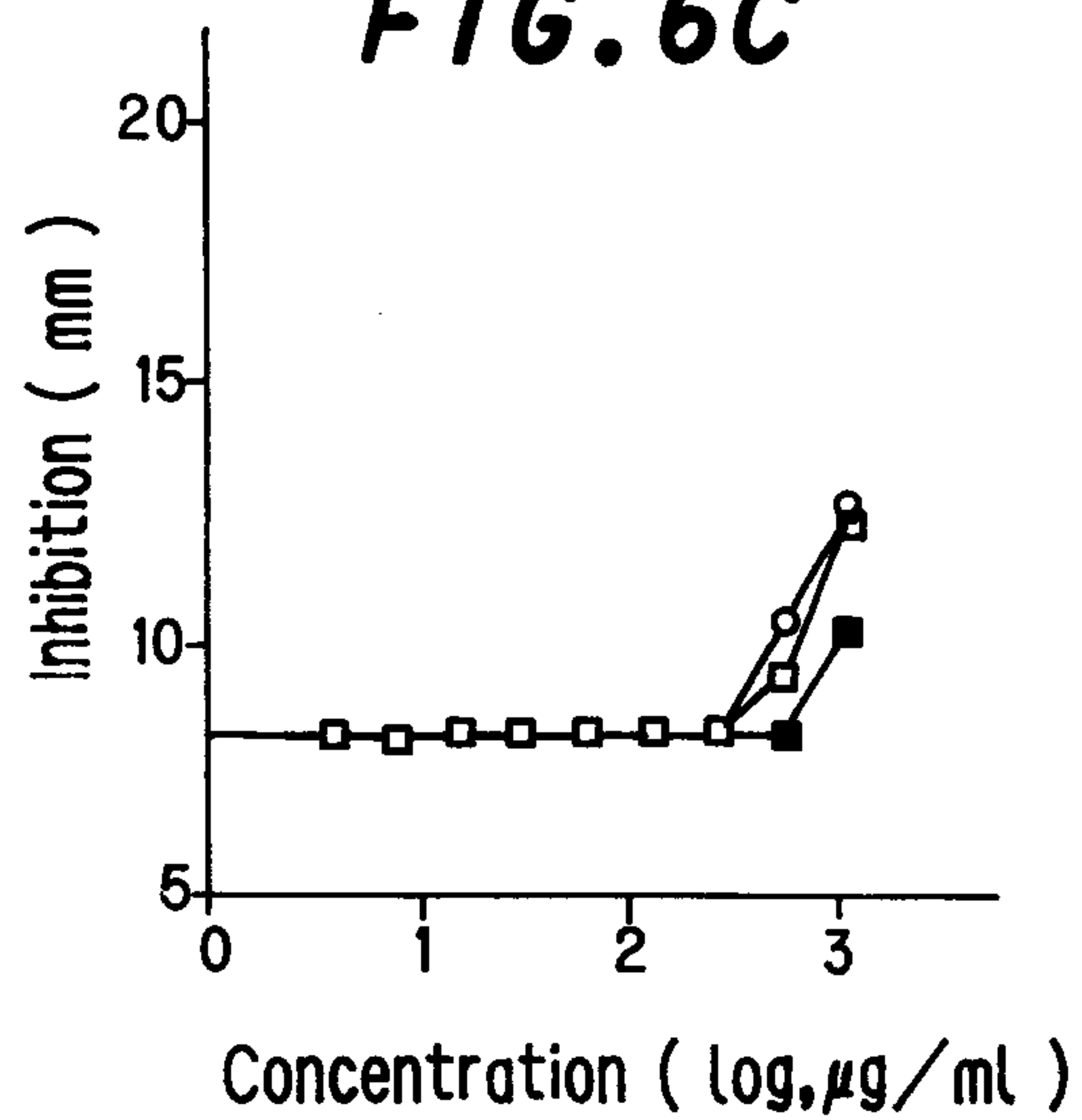
FIG. 6A**FIG. 6B****FIG. 6C**

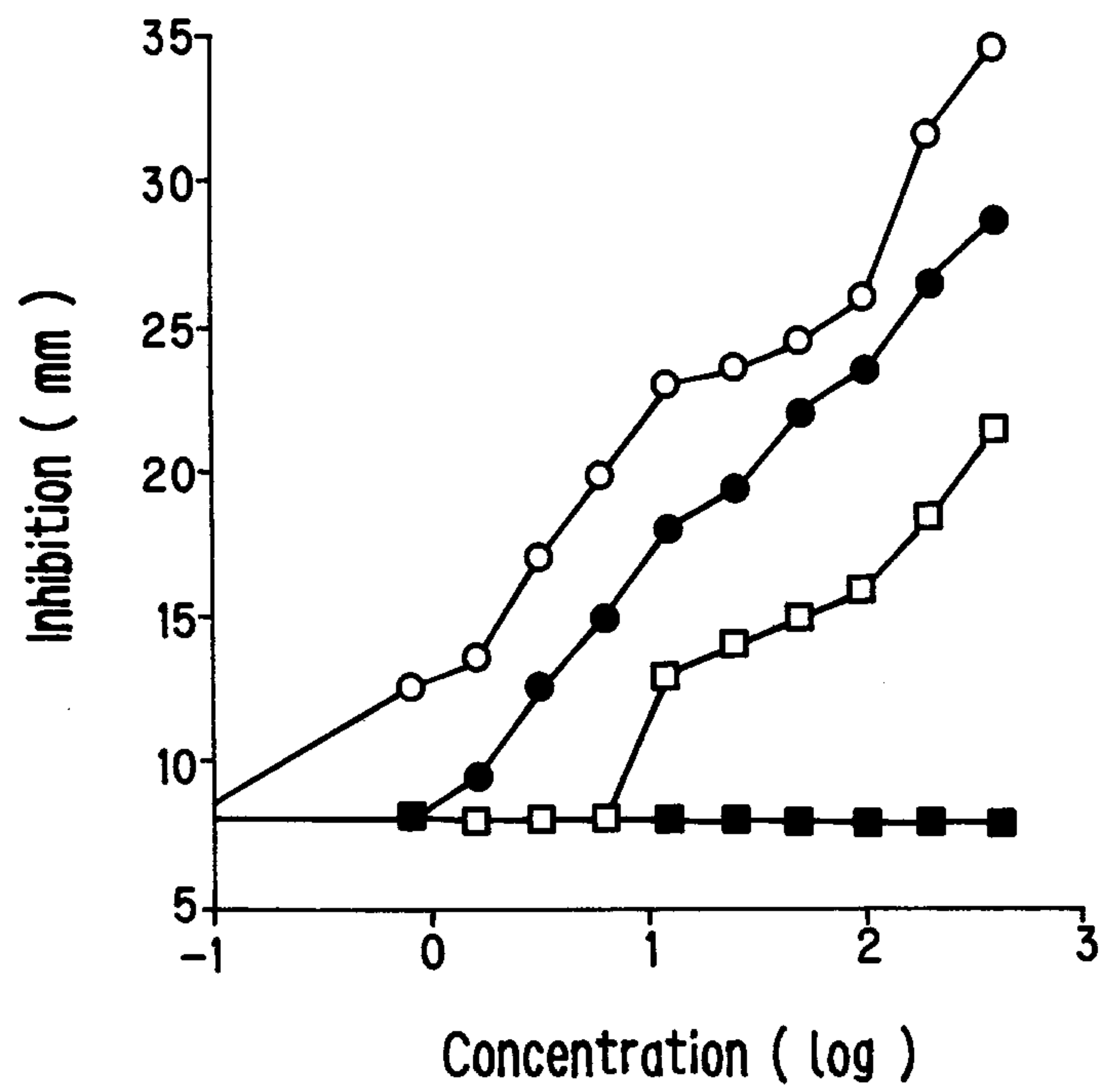
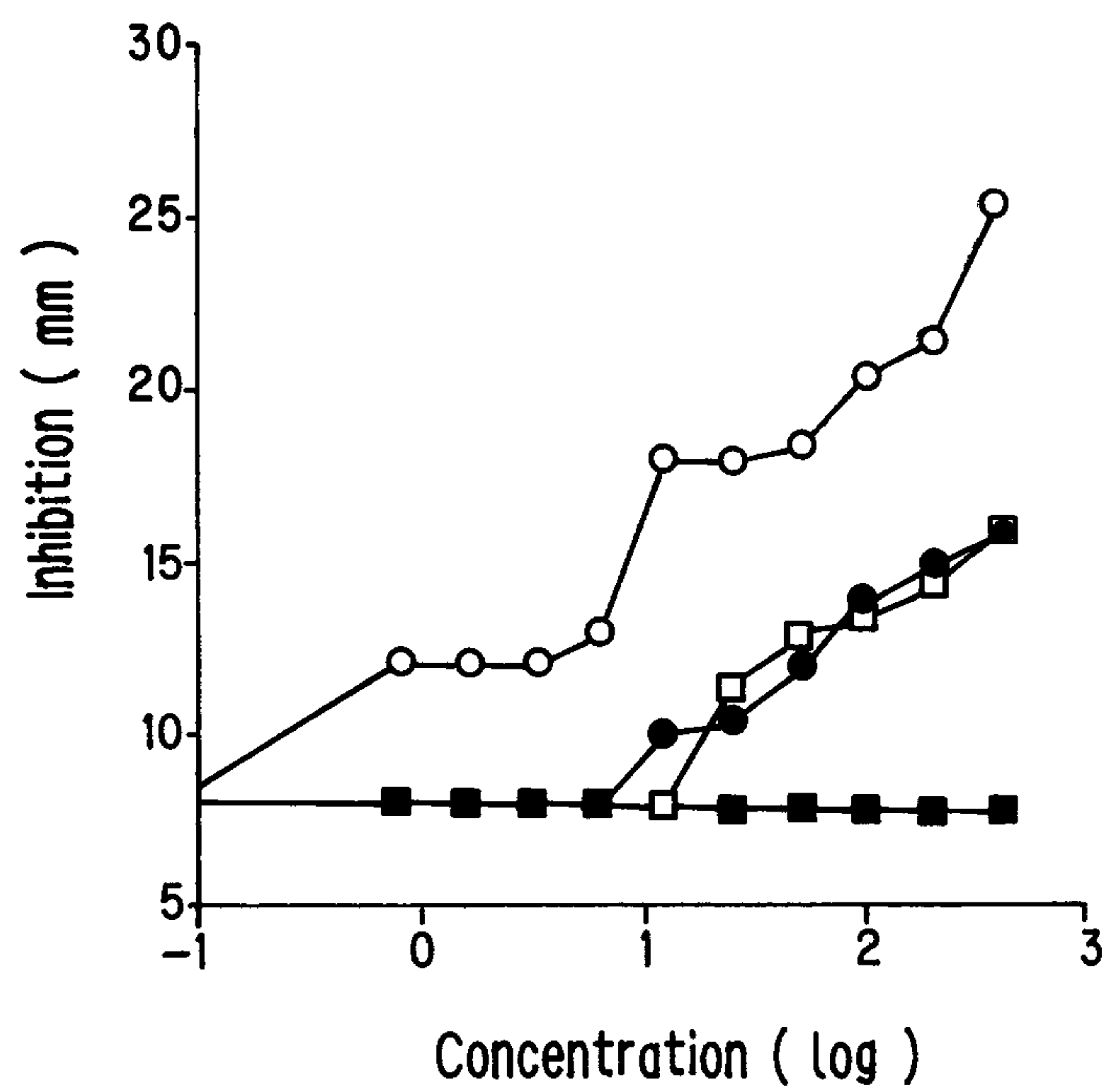
FIG. 7**FIG. 8**

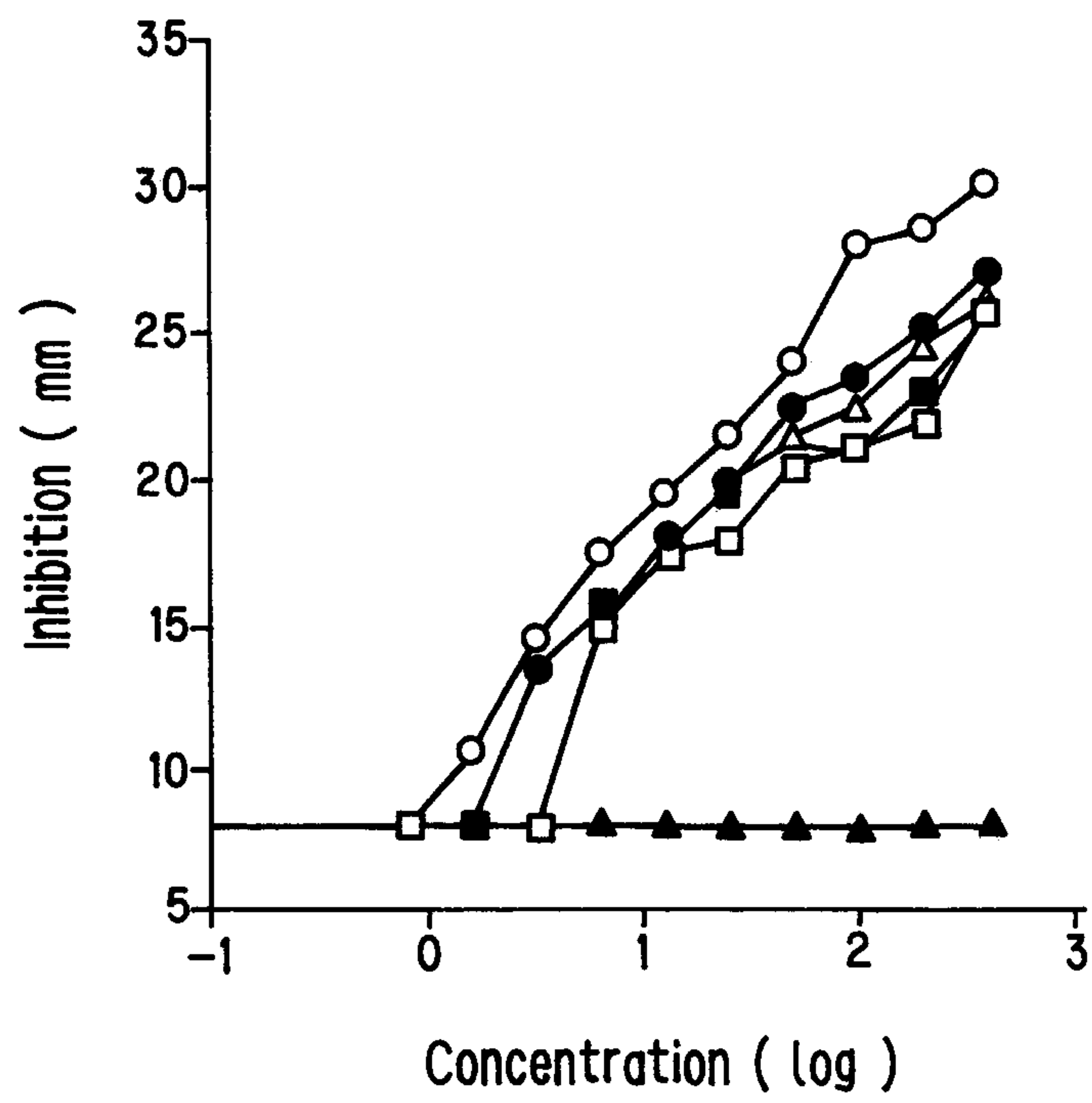
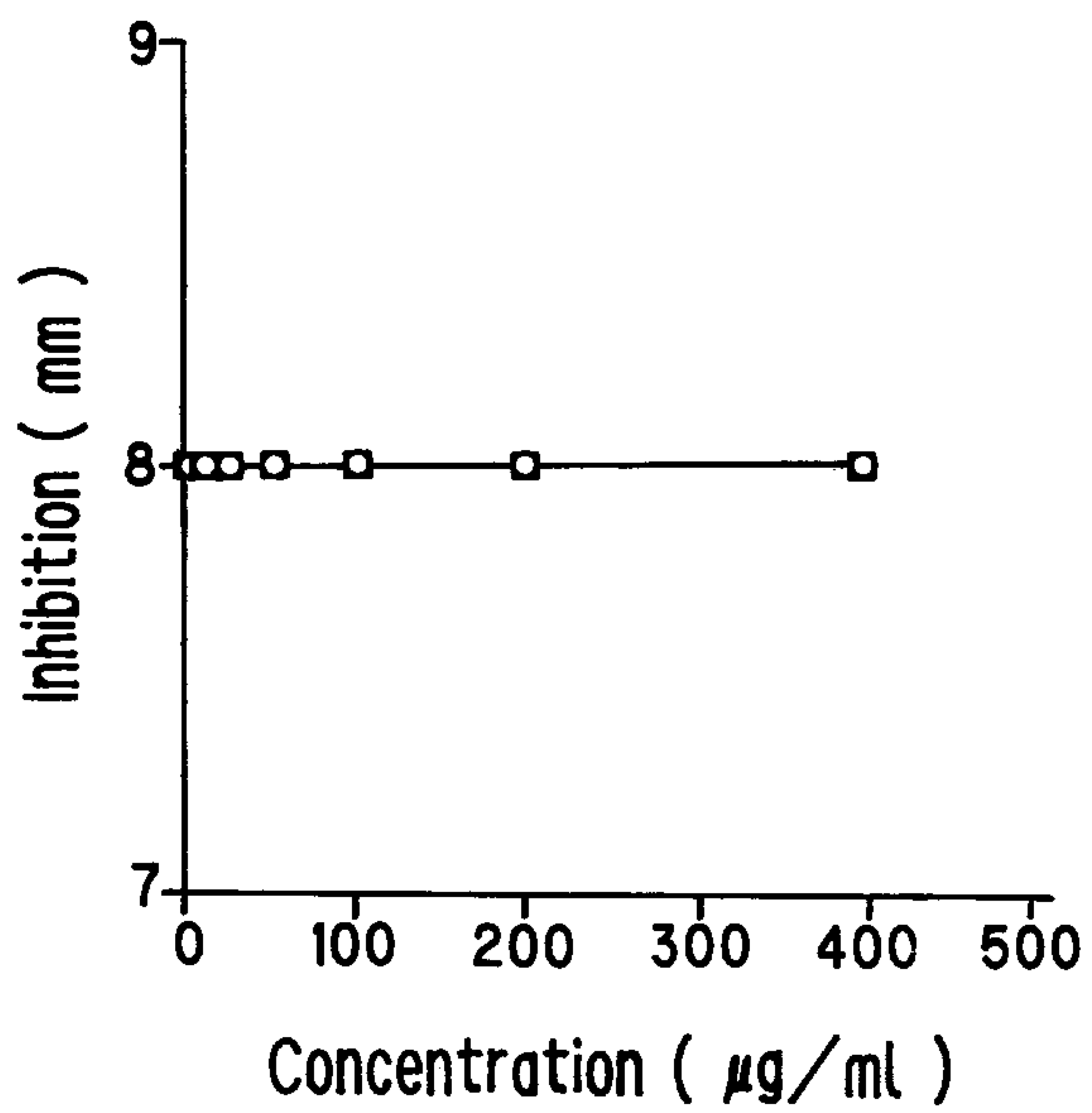
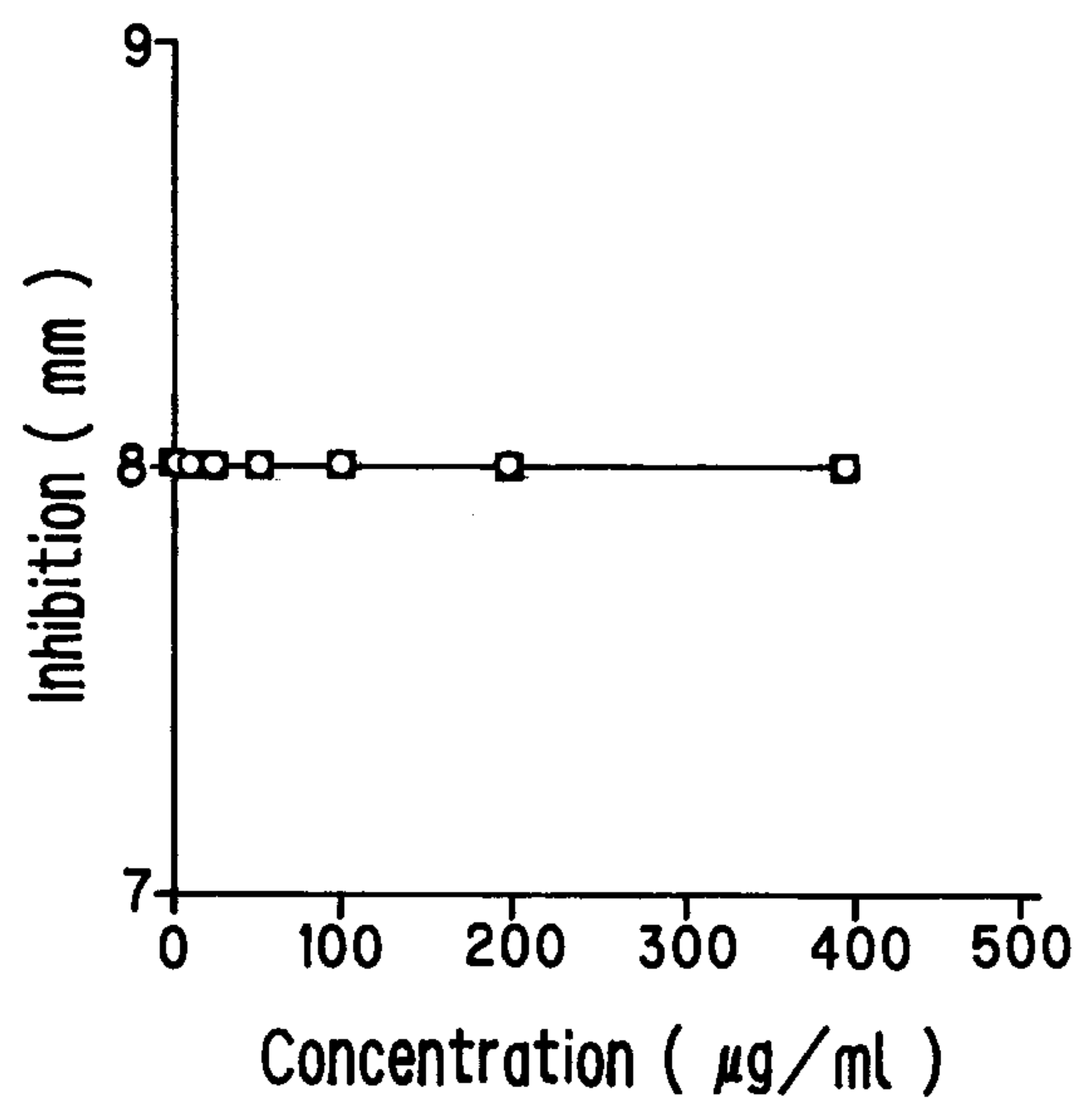
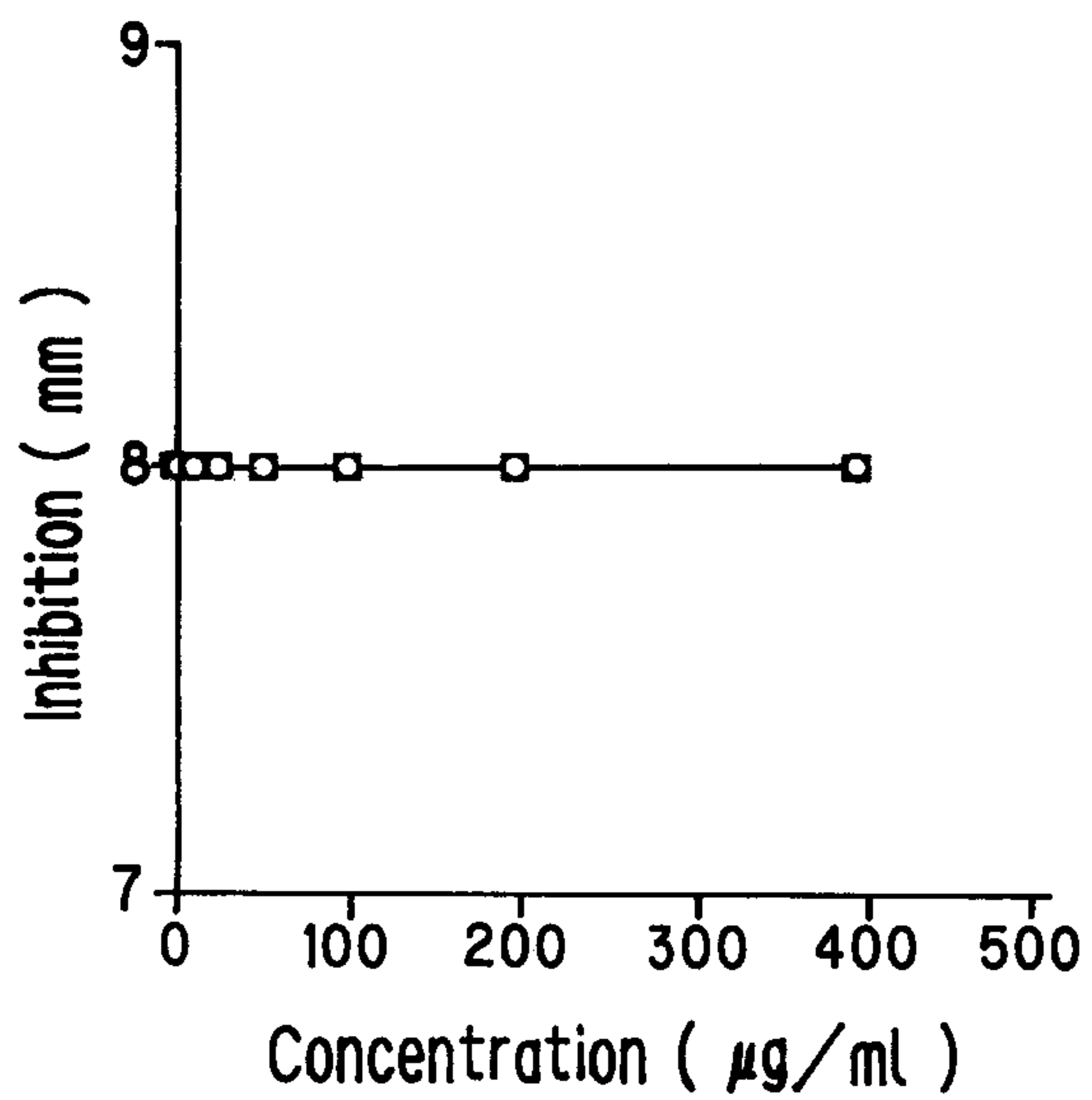
FIG. 9

FIG. 10A**FIG. 10B****FIG. 10C****FIG. 10D**