

Chapter 3

Results

1. Effects of Indole Alkaloids on Isolated Rabbit Jejunum

Primary screening of the actions of the indole alkaloids was performed on isolated rabbit jejunum by measuring amplitude, tone and rate of contraction. The results obtained were shown in the Fig. 3: each point represents means and \pm S.E. mean of the response to each dose of the indole alkaloids.

Spontaneous movements of rabbit jejunum when exposed to different concentrations of the four indole alkaloids (2×10^{-6} M - 4.4×10^{-5} M) were measured in Tyrode's solution for 5 minutes after the addition of each alkaloid to the bath fluid. Administration of indole alkaloids in a cumulative regimen caused a concentration dependent reduction of the spontaneous movements of isolated rabbit jejunum. Figure 3 shows that at the dose 1.4×10^{-6} M to 2.7×10^{-6} M all indole alkaloids caused slight reduction of spontaneous movement of isolated rabbit jejunum. Additionally, I-1 at the concentration of 5.5×10^{-6} M sharply reduced spontaneous movement. In the cases of I-2, O-1 and O-2 at the same concentration were less pronounced. (Fig. 4A, 4B). The EC_{50} values i.e. the concentrations of the alkaloids which produced 50% inhibition of the control spontaneous contraction were summarized in Table 1. It can be seen from Table 1 that I-1 was the most potent alkaloid producing 50% inhibition of spontaneous movements with as a low concentration as 13.09×10^{-6} M. At higher

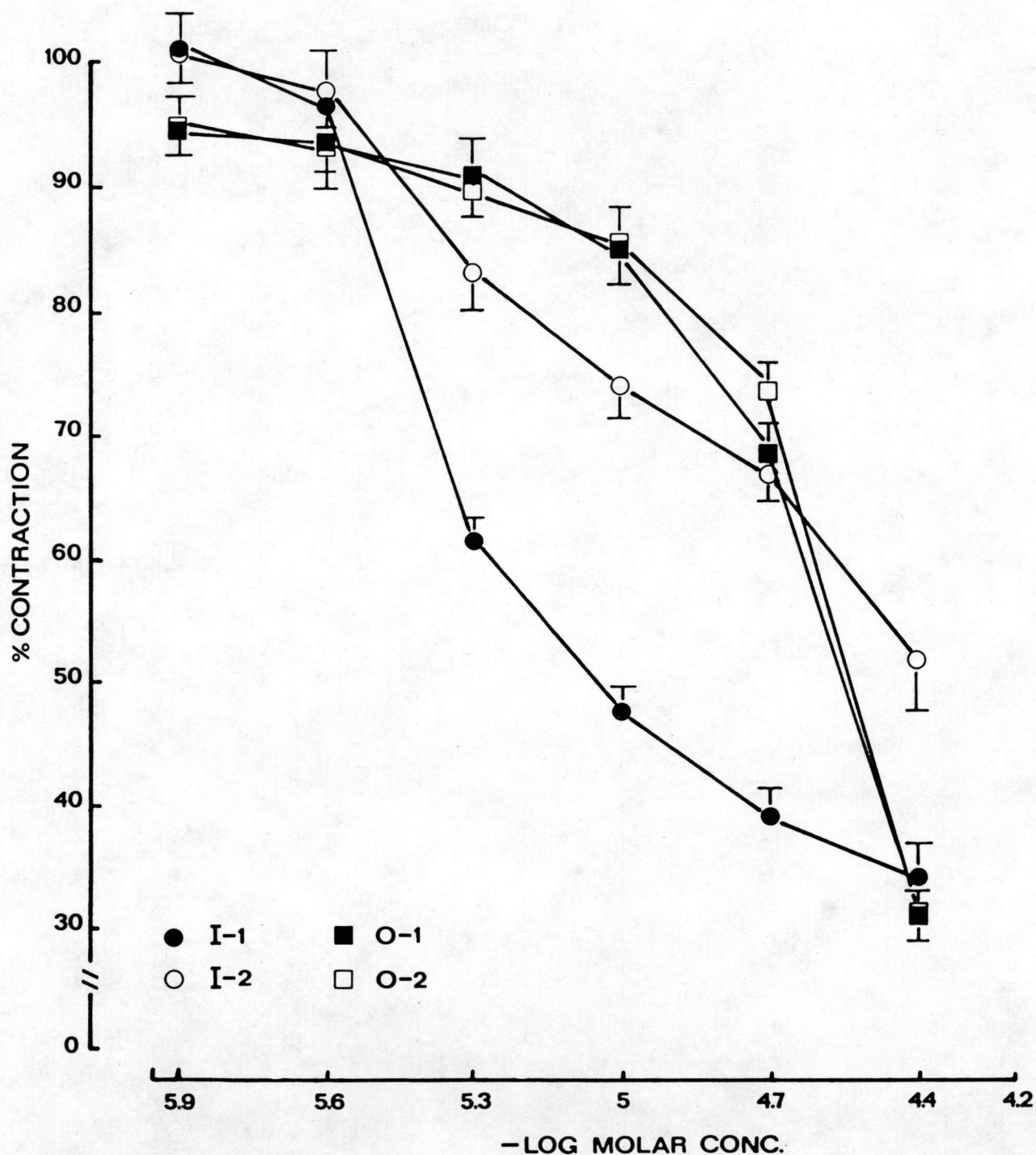


Figure 3. Cumulative log concentration-response curves of four indole alkaloids (I-1, I-2, O-1 and O-2) on spontaneous contraction of isolated rabbit jejunum following preincubation for 5 min with the alkaloids at indicated concentrations. Each point is the mean response of at least 8 measurements; vertical lines show standard error of the mean. The ordinate scale shows response as percentage of control. The abscissa scale is the negative logarithm in molar concentration of indole alkaloids.

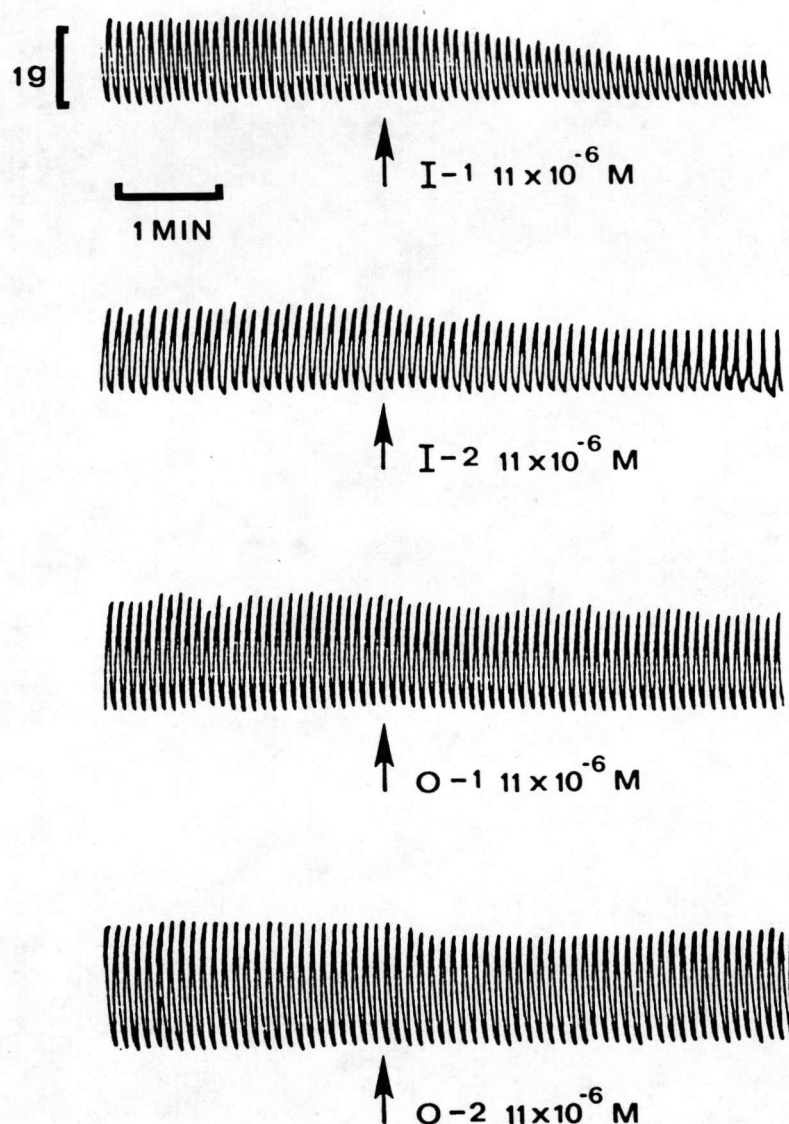


Figure 4. A. Trace of dose-response relationship of four indole alkaloids (I-1, I-2, O-1 and O-2) at concentration $11 \times 10^{-6} \text{ M}$ on isolated rabbit jejunum. The record shows the depressive effect of indole alkaloids on amplitude of contraction. I-1 depressed the curve more than the others. Tracings were obtained from a curvilinear pen recorder. The calibration of 1 g tension is shown on the vertical line and time scale under the first row of recordings and is identical for all cases and the concentration of four indole alkaloids shown are expressed in molar.

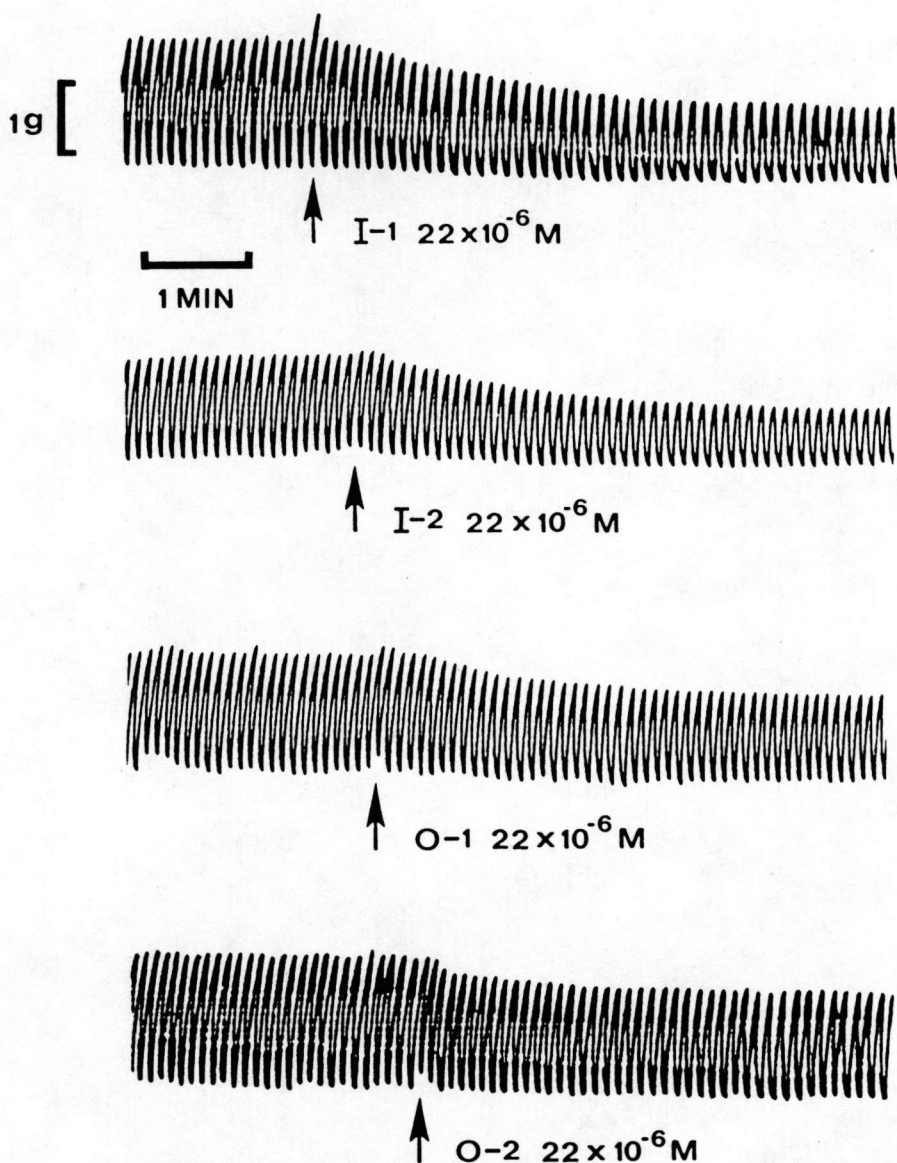


Figure 4. B. Trace of dose-response relationship of four indole alkaloids (I-1, I-2, O-1 and O-2) at concentration $22 \times 10^{-6} \text{ M}$ on isolated rabbit jejunum. The record shows the depressive effect of indole alkaloids on amplitude of contraction. I-1 was the most potent indole alkaloids. Tracings were obtained from a curvilinear pen recorder. The calibration of 1 g tension is shown on the vertical line and timescale under the top panel. The concentrations of indole alkaloids shown are expressed in molar.

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| INDOLE ALKALOIDS | EC 50 (x 10 ⁻⁶ M) |
|------------------|--------------------------------|
| I-1 | 13.09 |
| I-2 | 37.05 |
| O-1 | 43.89 |
| O-2 | 54.08 |

Table 1. Concentration of indole alkaloids which produced 50% inhibition (EC_{50}) of spontaneous contraction of isolated rabbit jejunum. The EC_{50} values were calculated from linear regression lines of the dose-response data.

concentration, cessation of spontaneous movements was observed with all alkaloids (I-1, I-2, O-1 and O-2 $> 4.4 \times 10^{-5}$ M). Spontaneous movement could be recovered after washing the tissues with Tyrode solution about six times.



2. Effects of Indole Alkaloids on Isolated Guinea-pig Ileum

The alkaloids were tested for their further effects on isolated guinea-pig ileum. Since this tissue did not have spontaneous movement, the contraction in form of contracture was induced by either carbachol, barium chloride, histamine or 5-hydroxytryptamine (5-HT). Contractile responses of each agonist were made for construction of the control dose-response curve. The dose-response of each agonist was subsequently measured in the presence of each of four indole alkaloids at doses 8.5×10^{-6} M, 11×10^{-6} M and 22×10^{-6} M 10 minutes exposure. The results from the control and drugged conditions were then compared.

2.1 Effects of Indole Alkaloids on Contraction of Guinea-pig Ileum Induced by 5-Hydroxytryptamine (5-HT)

It was frequently found that 5-HT administered in a cumulative doses regimen caused tachyphylaxis in the guinea-pig ileum therefore, in this experiment administration of 5-HT was made in a sequential doses regimen (10^{-9} M - 10^{-4} M), which produced less tachyphylaxis. The tracing of sequential concentration-response relationship for 5-HT was showed in Fig.5. Fig. 6 demonstrates that I-1 at 8.5×10^{-6} M, 11×10^{-6} M and 22×10^{-6} M produced dose-dependent reduction of the maximum effect of 5-HT contraction by 40%,

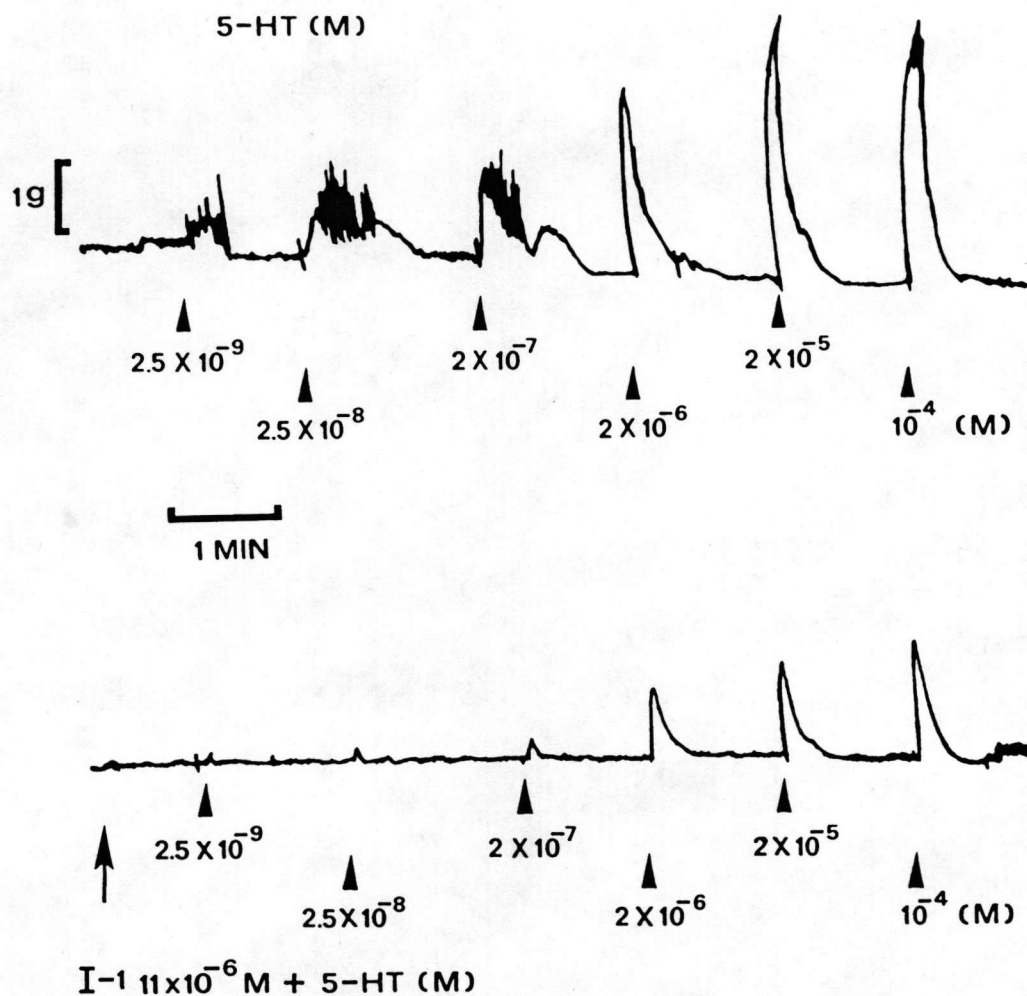


Figure 5. Trace of sequential concentration-response relationship of 5-hydroxytryptamine (5-HT) on isolated guinea-pig ileum preparation. Upper trace show response of the guinea-pig ileum to 5-HT alone, while lower trace represents the response following preincubating tissue in I-1 at concentration 11×10^{-6} M for 10 minutes. The vertical scale represents an isometric contraction equivalent to a standard change in load of 1 g. The horizontal scale represents 1 minute.

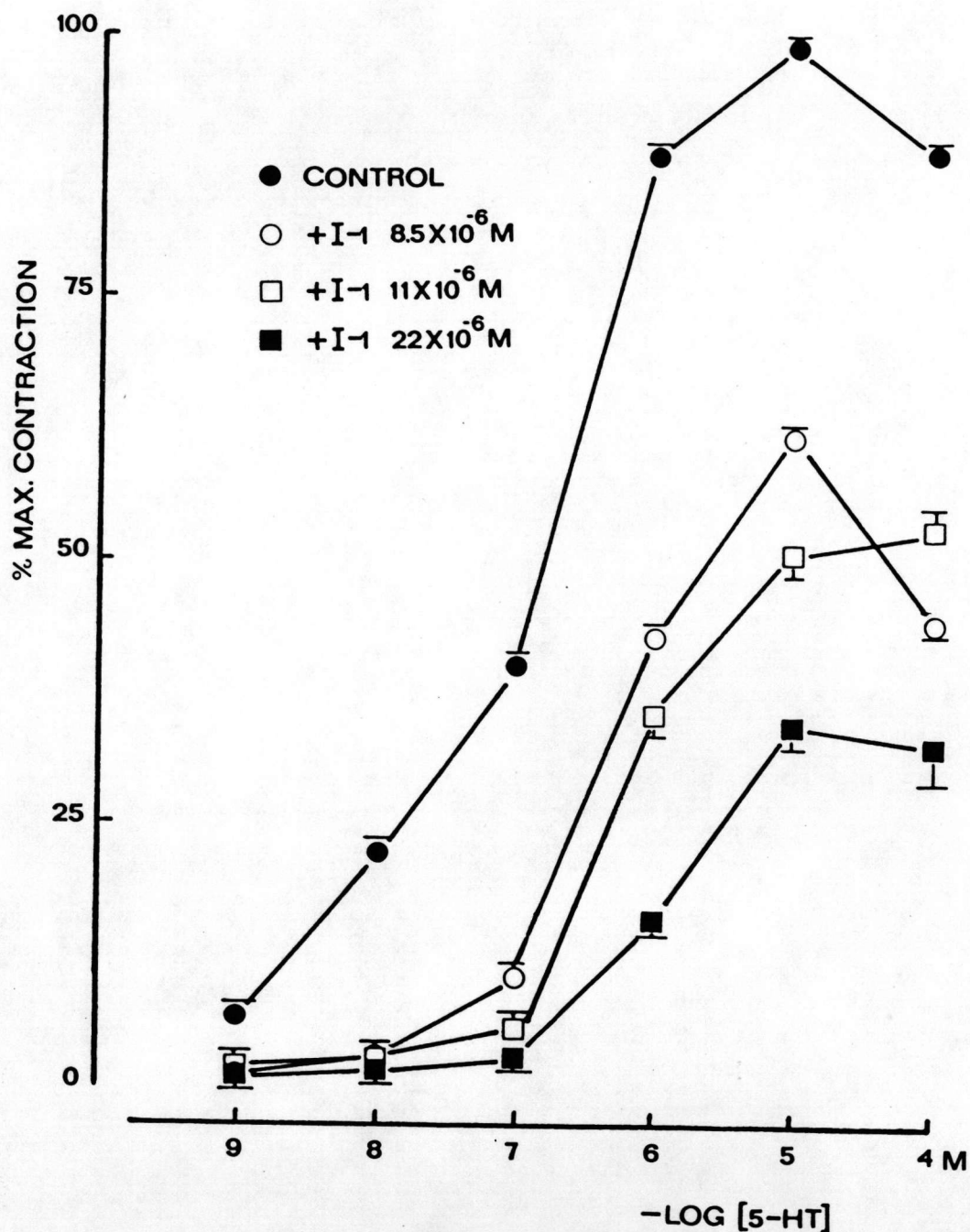


Figure 6. Log concentration-response curves of I-1 plotted from inhibition of contraction of isolated guinea-pig ileum induced by 5-HT. In this and the following figure 7-9 the control curves are shown by solid circles. The other symbols indicate responses in the presence of indole alkaloids. Each point is the mean of at least 6 responses; vertical bars show S.E. mean. The ordinate scale is percentage of maximal contraction; the abscissa scale is the negative logarithm in molar concentration. All points on the curves from I-1 treated condition are significantly different from the control ($p < 0.001$; Student's t test).

50% and 65% respectively ($p < 0.001$). pd_2 values of this antagonism are summarized in Table 2.

Fig. 7, I-2 at a low dose (8.5×10^{-6} M), could not alleviate the contraction induced by 5-HT. At higher doses, e.g. 11×10^{-6} M and 22×10^{-6} M however, this alkaloid caused dose-dependent reduction of the maximum effect of 5-HT induced contraction by 30% and 80% respectively ($p < 0.001$). The affinity of these non-competitive antagonism were listed in Table 2. By contrast, O-1 and O-2 at dose 11×10^{-6} M did not cause any reduction of dose-response curves of 5-HT (Fig. 8,9).

The calculated mean pd_2 values obtained for two indole alkaloids are summarized in Table 2. These data indicate that the affinity of I-1 at 11×10^{-6} M was stronger than I-2, while at the dose 22×10^{-6} M I-2 became stronger than I-1.

2.2 Effects of Indole Alkaloids on Sustained Contraction of Guinea-pig Ileum Induced by Carbachol.

Application of carbachol in a cumulative dose regimen (10^{-7} M - 10^{-5} M) produced contracture of isolated guinea-pig ileum. Fig. 10 indicates that I-1 at 8.5×10^{-6} M and 11×10^{-6} M did not modify the control dose-response curve of carbachol, while at 22×10^{-6} M this alkaloids showed significant reduction carbachol induced contraction ($p < 0.001$). pd_2 derived from these results were summarized in Table 3. Similar results were observed in the cases of I-2 (Fig 11).

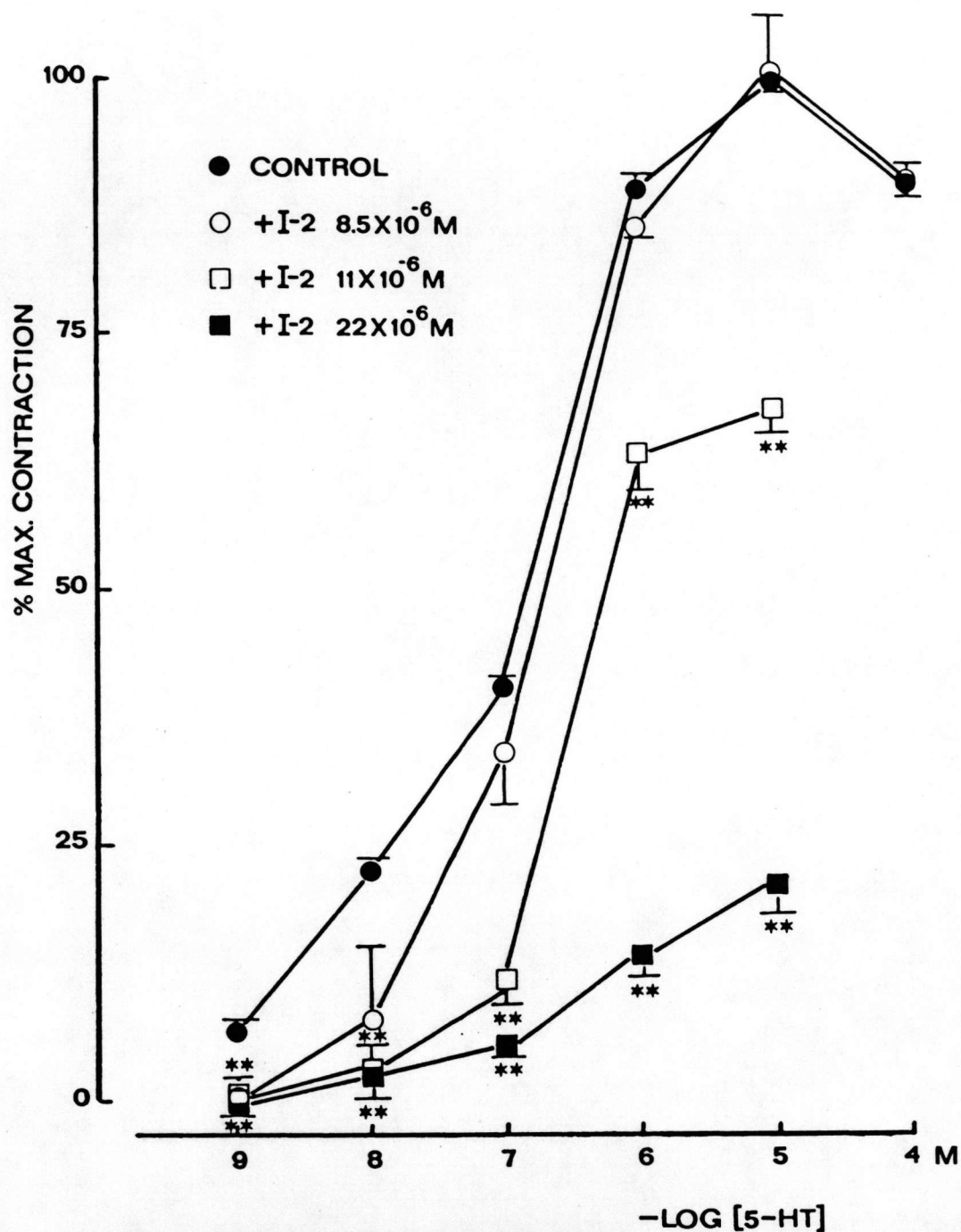


Figure 7. Log concentration-response curves of I-2 plotted from inhibition of contraction of isolated guinea-pig ileum induced by 5-HT. Each point is the mean of at least 6 responses; vertical bars show S.E. mean. The ordinate scale is percentage of maximal contraction; the abscissa scale is the negative logarithm in molar concentration. All points on the curves from I-2 treated condition are significantly different from the control, (** = $p < 0.001$, Student's t test).

| AGONIST | ANTAGONIST | PD ₂ VALUE |
|---------|-------------------------------|-------------------------|
| 5-HT | 8.5 x 10 ⁻⁶ M I-1 | 4.84 ± 0.01 (n = 5) |
| | 11 x 10 ⁻⁶ M I-1 | 4.90 ± 0.02 (n = 10) |
| | 22 x 10 ⁻⁶ M I-1 | 4.91 ± 0.02 (n = 9) |
| 5-HT | 8.5 x 10 ⁻⁶ M I-2* | - |
| | 11 x 10 ⁻⁶ M I-2 | 4.62 ± 0.04 (n = 6) |
| | 22 x 10 ⁻⁶ M I-2 | 5.23 ± 0.07 (n = 6) |

Table 2. pD₂ Values of indole alkaloids, I-1 and I-2, agonist spasmodic action of 5-hydroxytryptamine (5-HT) on isolated guinea-pig ileum. The number of observations (n) is given in parenthesis. The Table shown mean pD₂ values and standard errors of means.

*This concentration cannot block effect of 5-HT

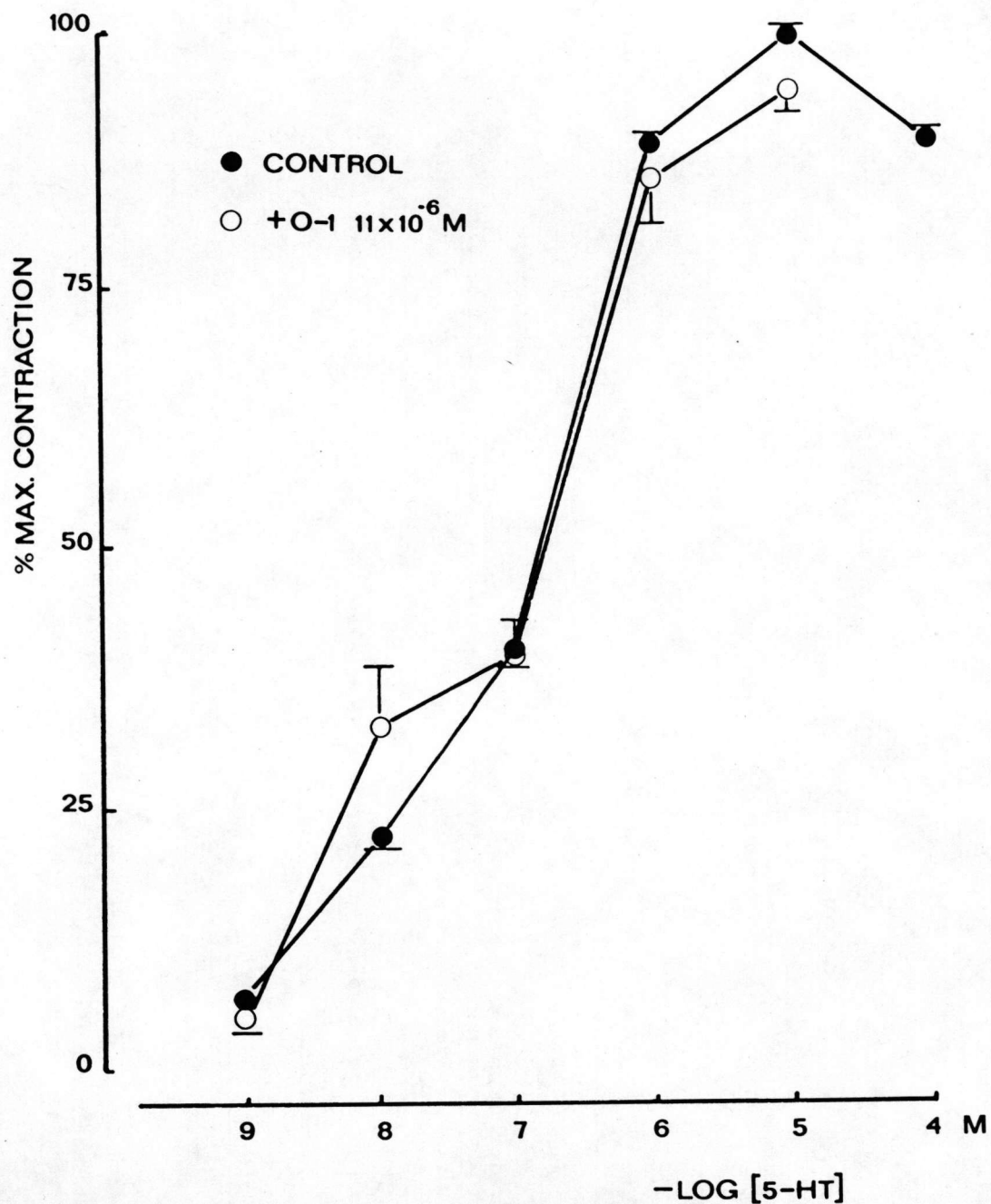


Figure 8. Log concentration-response curves of O-1 plotted from inhibition of contraction of isolated guinea-pig ileum induced by 5-HT. O-1 at $11 \times 10^{-6} \text{ M}$ did not produced any significantly deviation of the dose-response curve of 5-HT as compare to the control.

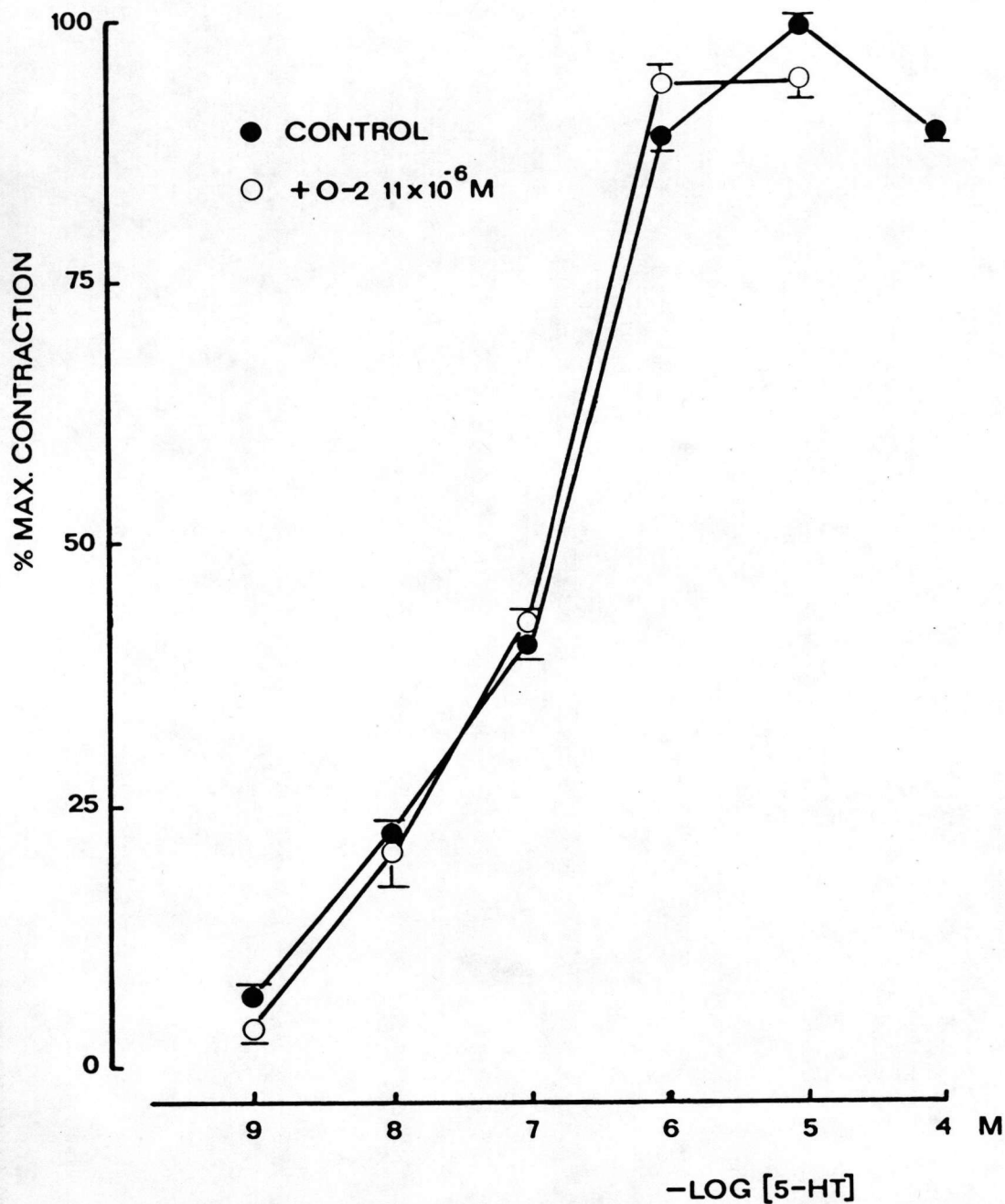


Figure 9. Log concentration-response curves of O-2 plotted from inhibition of concentration of isolated guinea-pig ileum induced by 5-HT. O-2 at $11 \times 10^{-6} \text{ M}$ did not produced any significantly deviation of the dose-response curve of 5-HT as compare to the control.

Fig. 12 shows that O-1 at 11×10^{-6} M and 22×10^{-6} M caused variable reduction of contraction induced by carbachol. However at the dose of 22×10^{-6} M a slight reduction of maximum effect of carbachol was observed. O-2 at 11×10^{-6} M (Fig. 13), shows higher potency in reducing of the effect of carbachol at low doses, as compared to the antagonism at concentration of O-2 22×10^{-6} M. It is noteworthy that at higher dose of carbachol both produced the same reduction effect ($p < 0.001$).

2.3 Effects of Indole Alkaloids on Contraction of Guinea-pig Ileum Induced by Histamine.

Administration of histamine in cumulative dose regimen (10^{-8} M - 10^{-5} M) to the bath fluid caused sustained contraction of the isolated guinea-pig ileum. Results illustrated in Fig. 14, 15, 16 and 17 indicate that addition of either I-1, I-2, O-1 and O-2 (11×10^{-6} M), did not alter the dose-response curve of histamine significantly.

2.4 Effects of Indole Alkaloids on Contraction of Guinea-pig Ileum Induced by Barium Chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$)

Application of barium chloride in cumulative dose regimen (10^{-4} M - 10^{-2} M), to the bath fluid caused contraction of the isolated guinea-pig ileum. Fig. 18, 19, 20 and 21 indicate that I-1, I-2, O-1 and O-2 (11×10^{-6} M), did not modify the control dose-response curve of barium chloride.

3. Effects of Indole Alkaloids on Isolated Aortic Strip of Rabbit

Studying effects of indole alkaloids on isolated rabbit aortic strip to confirm the mitigation of contraction effect induced by

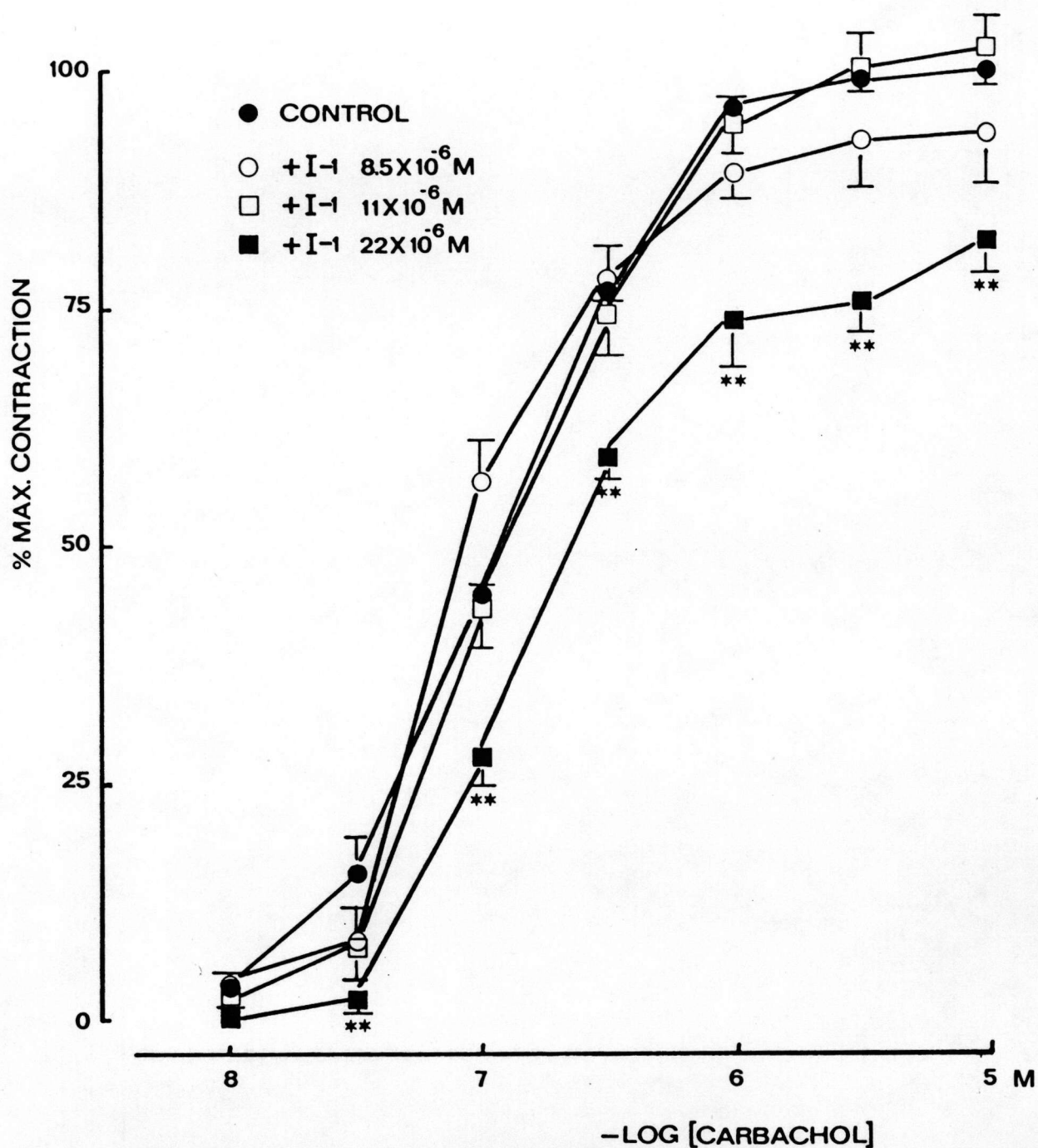


Figure 10. Cumulative log concentration-response curves of carbachol on isolated guinea-pig ileum in the absence (●) and presence of I-1 at concentration which was indicated (○, □, ■), in this figure and the following 11-13. Carbachol induced sustained contraction of isolated guinea-pig ileum, incubation of the tissue with I-1 (concentration $8.5 \times 10^{-6} \text{ M}$ and $11 \times 10^{-6} \text{ M}$) did not produce any significant alteration of the responses. Whereas all points in $22 \times 10^{-6} \text{ M}$. I-1 curves are reduced significantly different (** = $p < 0.01$, Student's t test).

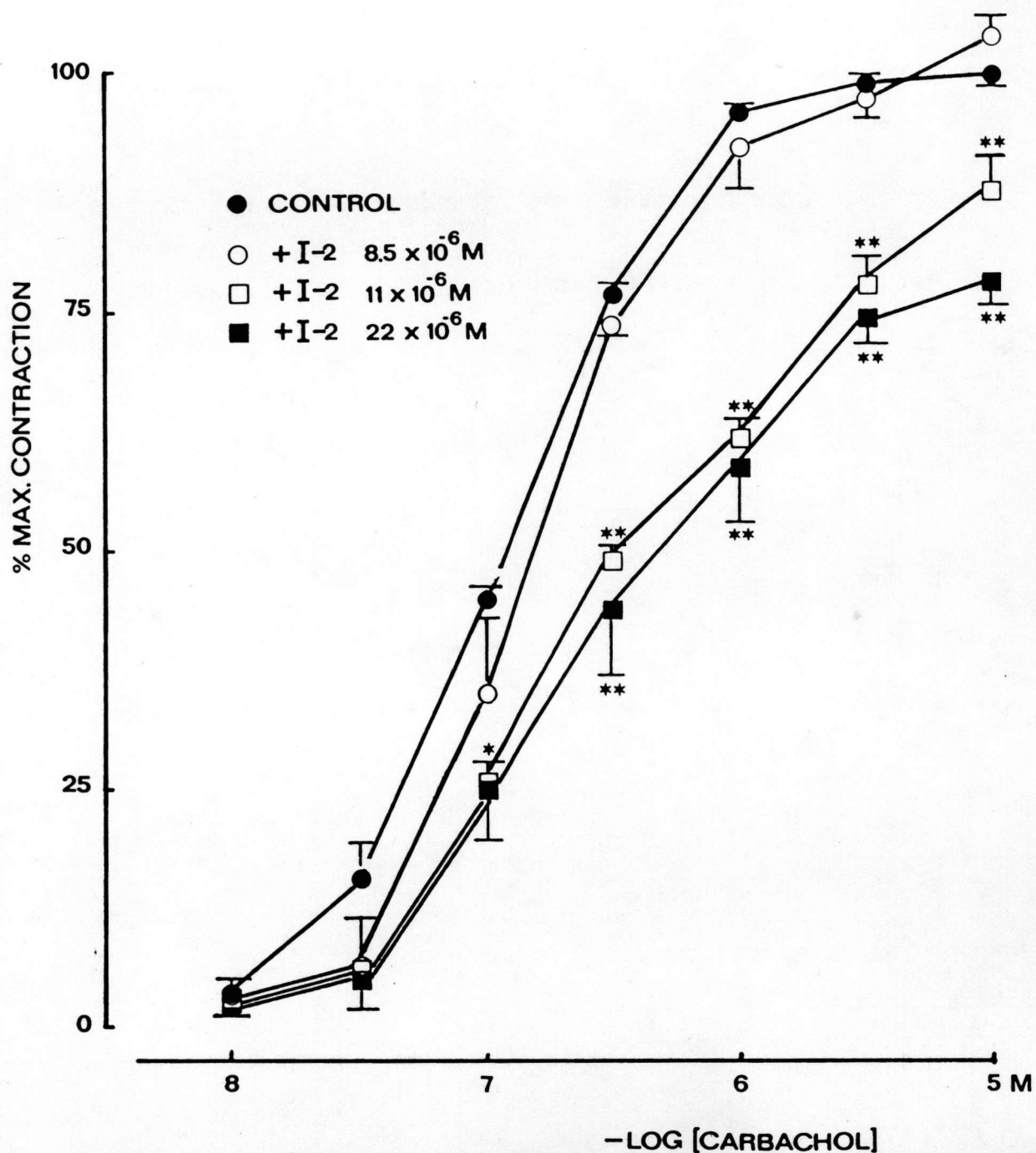


Figure 11. Cumulative log concentration-response curves of carbachol on isolated guinea-pig ileum. Carbachol induced sustained contraction of isolated guinea-pig ileum, incubation of the tissue with I-2 at $8.5 \times 10^{-6} \text{ M}$ did not affect the response of carbachol, whereas at the concentration $11 \times 10^{-6} \text{ M}$ and $22 \times 10^{-6} \text{ M}$ the alkaloid produced significant shift of the concentration-response curves to the right (* = $p < 0.01$, ** = $p < 0.001$, Student's t test).

| AGONIST | ANTAGONIST | pD ₂ VALUS |
|-----------|-------------------------------|------------------------|
| CARBACHOL | 8.5 x 10 ⁻⁶ M I-1* | - |
| | 11 x 10 ⁻⁶ M I-1* | - |
| | 22 x 10 ⁻⁶ M I-1 | 3.96 ± 0.11 (n = 4) |
| CARBACHOL | 8.5 x 10 ⁻⁶ M I-2* | - |
| | 11 x 10 ⁻⁶ M I-2 | 4.05 ± 0.17 (n = 3) |
| | 22 x 10 ⁻⁶ M I-2 | 4.09 ± 0.05 (n = 4) |

Table 3. pD₂ Values of indole alkaloids, I-1 and I-2, against spasmodic action of carbachol on isolated guinea-pig ileum. The number of observation (n) is given in parenthesis. The Table shows mean pD₂ values and standard errors of means.

* This concentration cannot block effect of carbachol.

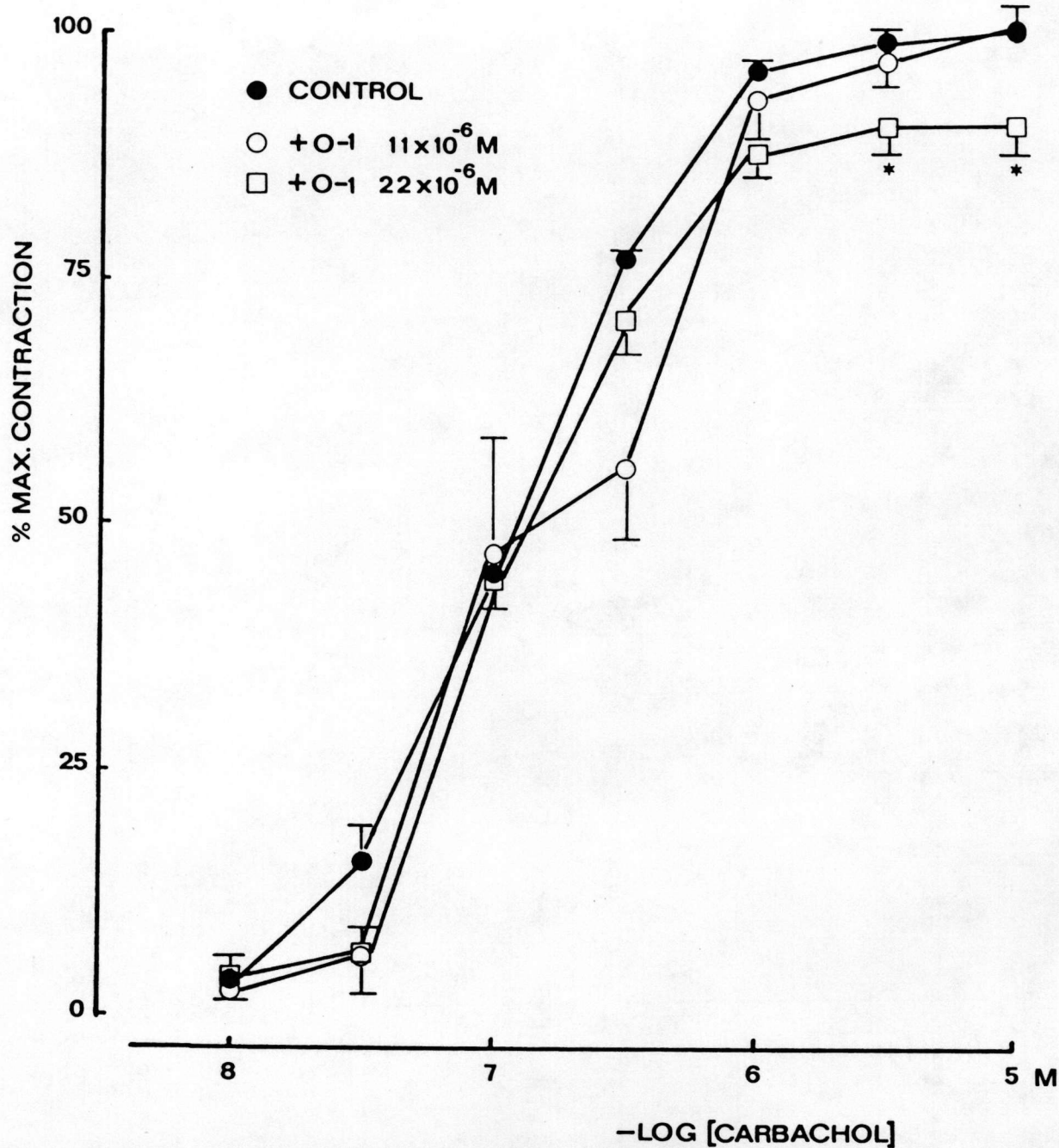
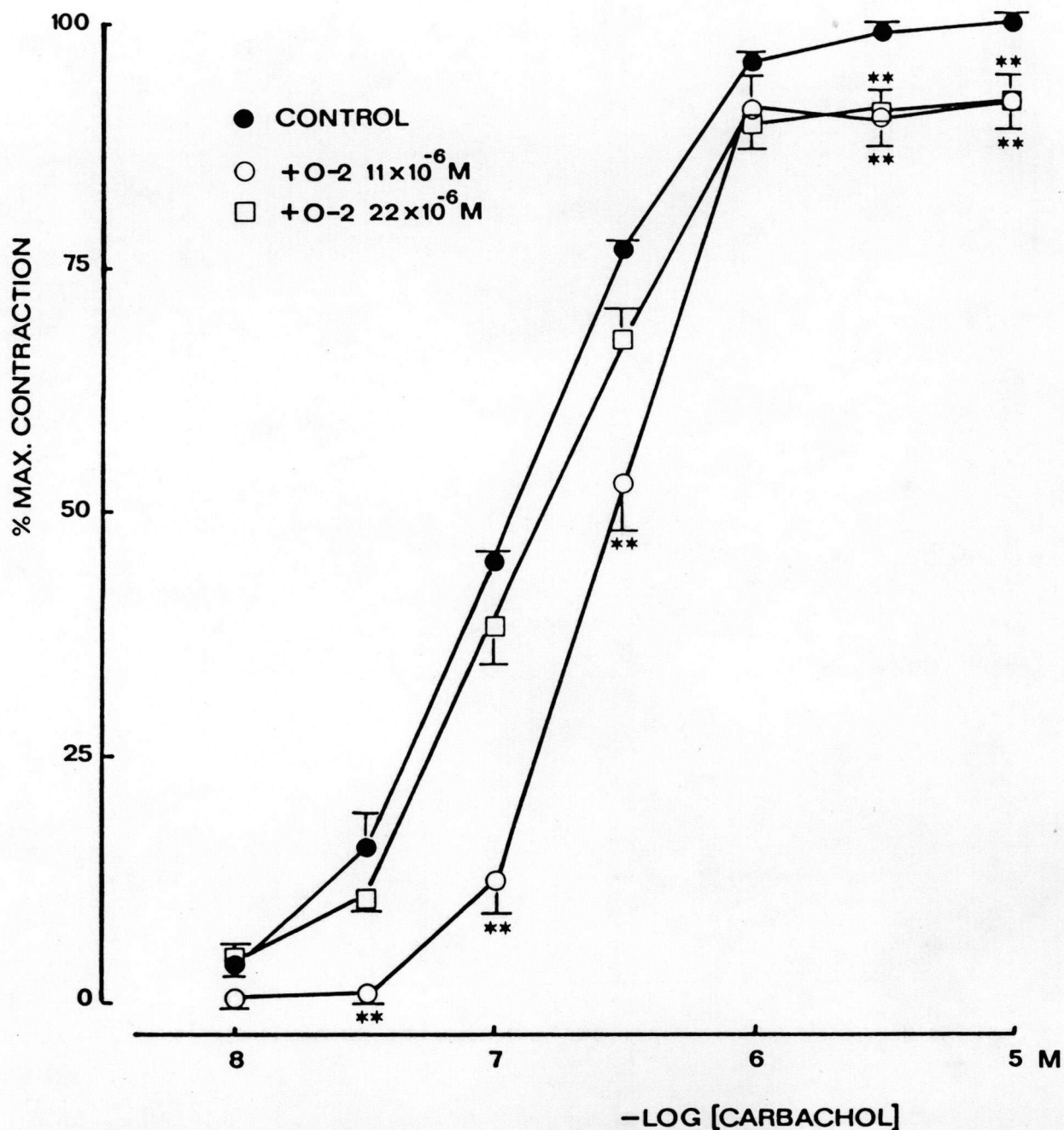


Figure 12. Cumulative log concentration-response curves of carbachol on isolated guinea-pig ileum. Carbachol induced sustained contraction of isolated guinea-pig ileum, incubation of the tissue with O-1 at concentration 11×10^{-6} M did not produce any significant alteration of the responses. Whereas the two highest points of 22×10^{-6} M O-1 are significantly different (* = $p < 0.01$, Student's t test).



Figures 13. Cumulative log concentration-response curves of carbachol on isolated guinea-pig ileum. Carbachol induced sustained contraction of isolated guinea-pig ileum. Incubation of the tissue with O-2 at concentration 22×10^{-6} M did not produce any significant alteration of the responses except the two highest points are significantly different. All of the points on the control and 11×10^{-6} M O-2 curves are significantly different except the fifth points (** = $p < 0.001$, Student's t test).

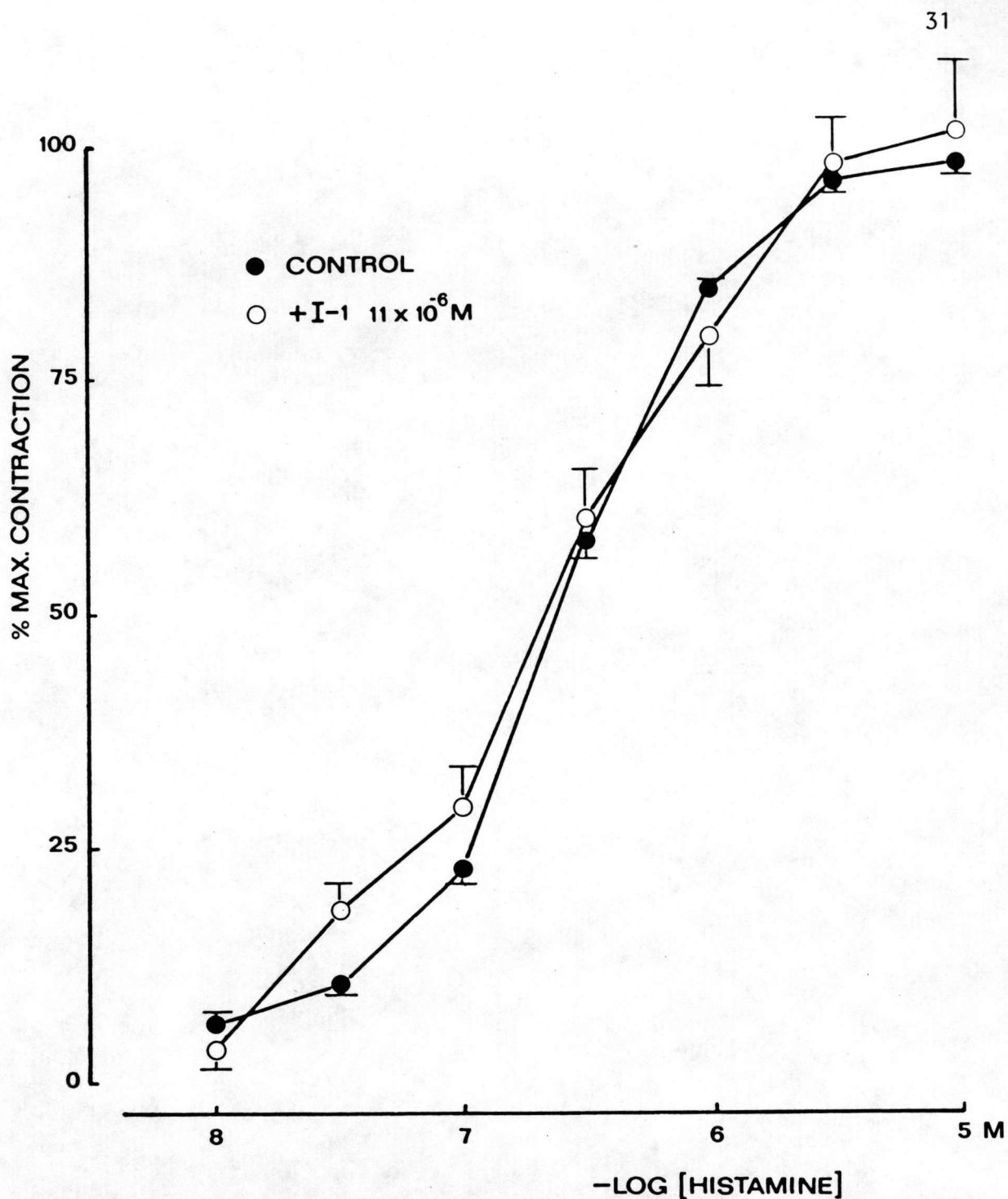


Figure 14. Cumulative log concentration-response curves of I-1 plotted from inhibition of contraction of isolated guinea-pig ileum induced by histamine. In this and the following figure 15-17 the control curves are shown by solid circles. The other symbol indicate responses in the presence of indole alkaloids. Each point is the mean of 4 responses; vertical bars show S.E. mean. The abscissa scale is the negative logarithm in molar concentration. None of the points in this figure is significantly different ($p > 0.4$).

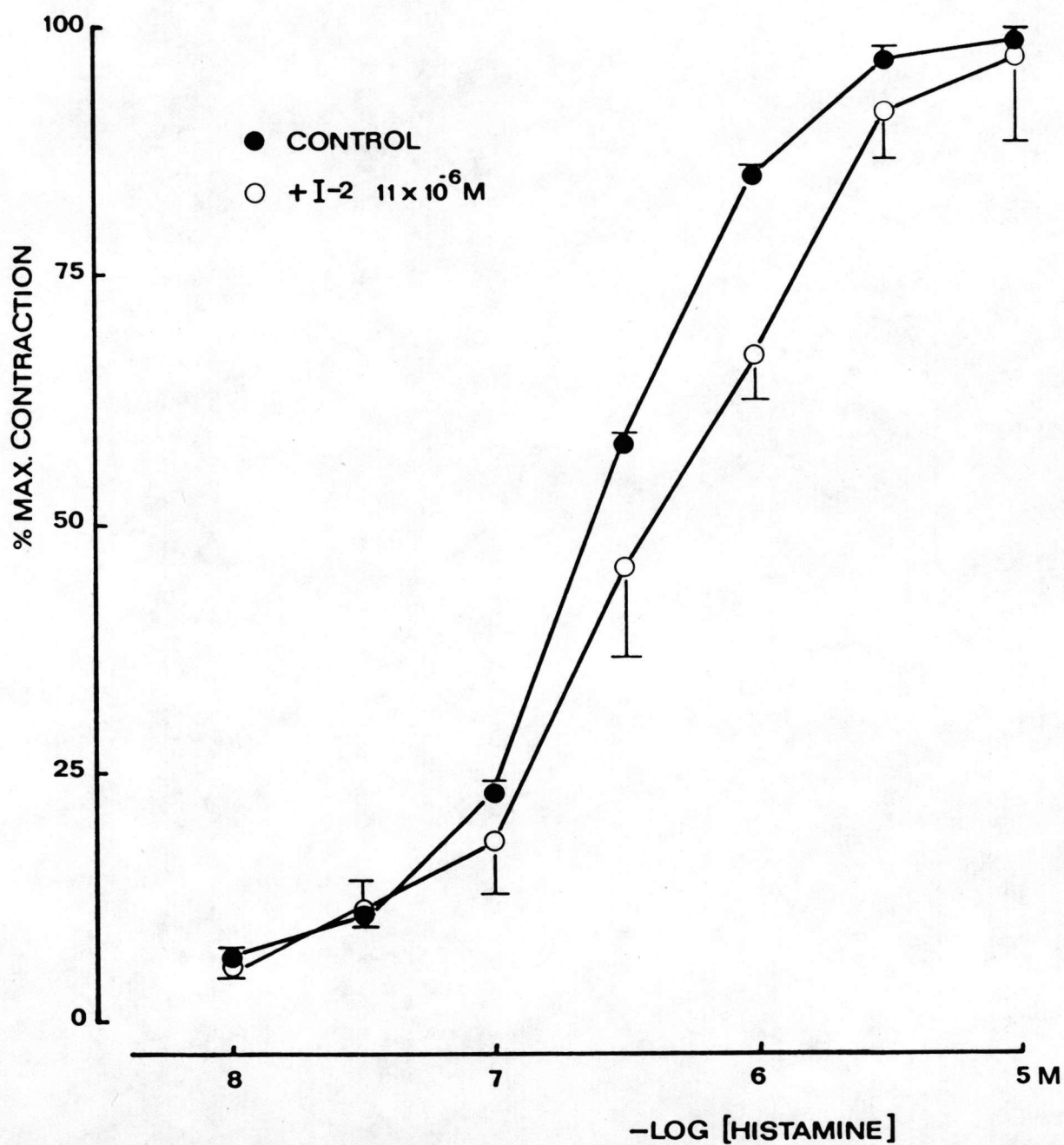


Figure 15. Cumulative log concentration-response curves of I-2 plotted from inhibition of contraction of isolated guinea-pig ileum induced by 5-HT. Each point is the mean of 4 responses. None of the corresponding points in the two curves are significantly different ($p > 0.3$).

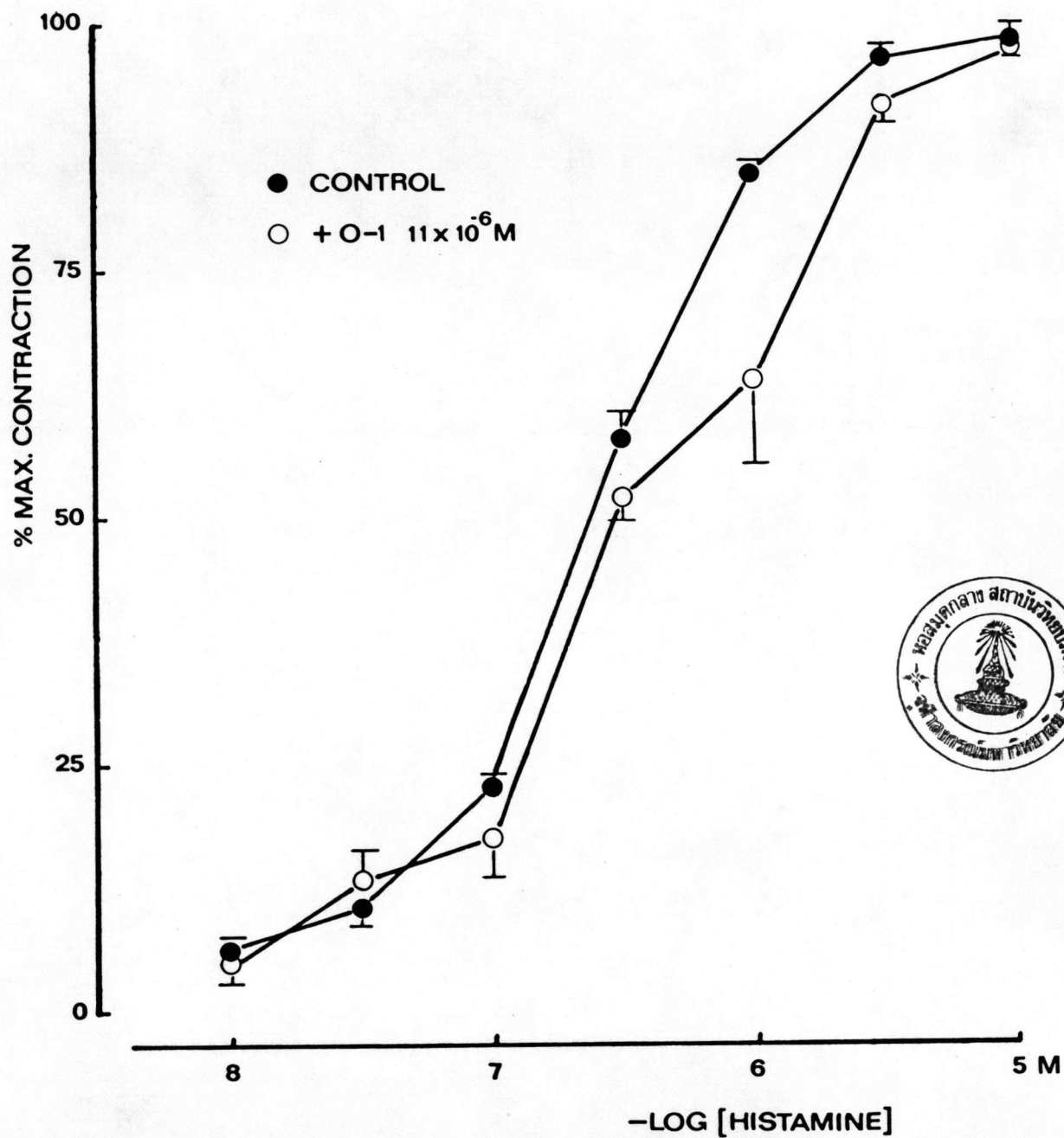


Figure 16. Cumulative log concentration-response curves of O-1 plotted from inhibition of contraction of isolated guinea-pig ileum induced by 5-HT. Each point is the mean of 4 responses. None of the points in this figure is significantly different ($p > 0.3$).

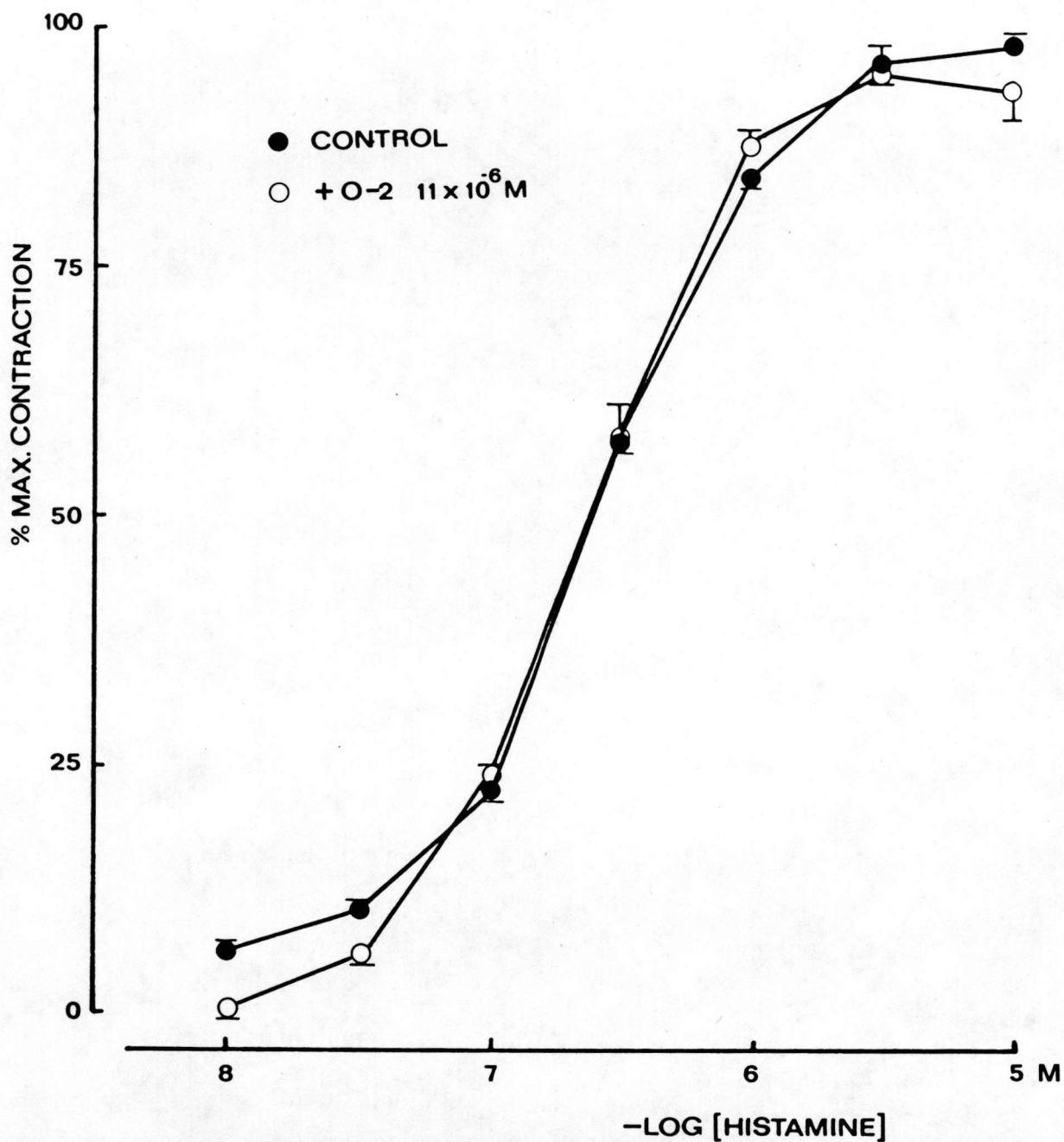


Figure 17. Cumulative log concentration-response curves of O-2 plotted from inhibition of contraction of isolated guinea-pig ileum induced by 5-HT. Each point is the mean of 4 responses. None of the corresponding points in the two curves are significantly different ($p > 0.5$).

5-HT on isolated guinea-pig ileum. Isolated aortic strip have distinct receptors for 5-HT and noradrenaline (Apperley *et al*, 1976) and they have no spontaneous movement so the contractions were induced by 5-HT and noradrenaline.

3.1 Effect of Indole Alkaloids on Sustained Contraction of Aortic Strip Induced by 5-Hydroxytryptamine (5-HT)

5-HT induced contractions of the isolated aortic strip at the concentration range 10^{-8} M - 10^{-4} M. Tracing of aortic contraction induced by administration of 5-HT in a cumulative dose regimen is shown in Fig.22. Fig.23 (I-1) and Fig. 24 (I-2) indicate that incubation of the strip with both I-1 and I-2 at 11×10^{-6} M and 22×10^{-6} M shifted dose-response curves of 5-HT to the right in a parallel manner ($p < 0.001$). Linear regression analysis of these results show coefficient of correlations of 0.71 and 0.87 for I-1 and 0.67 and 0.83 for I-2 respectively for the two concentrations. Contrary to the cases of guinea-pig ileum, maximum responses of aortic strip to 5-HT was less affected by either I-1 or I-2. By contrast, O-1 and O-2 (11×10^{-6} M) did not modify significantly the control curve of 5-HT (Figs. 25 and 26).

The calculated mean pA_2 values obtained for I-1 and I-2 against 5-HT were summarized in Table 4. It can be seen from Table 4 that at 11×10^{-6} M the affinity of I-1 expressed as pA_2 (5.89) was stronger than that of I-2 (4.80), whereas at 22×10^{-6} M affinity of I-2 became greater than that of I-1.

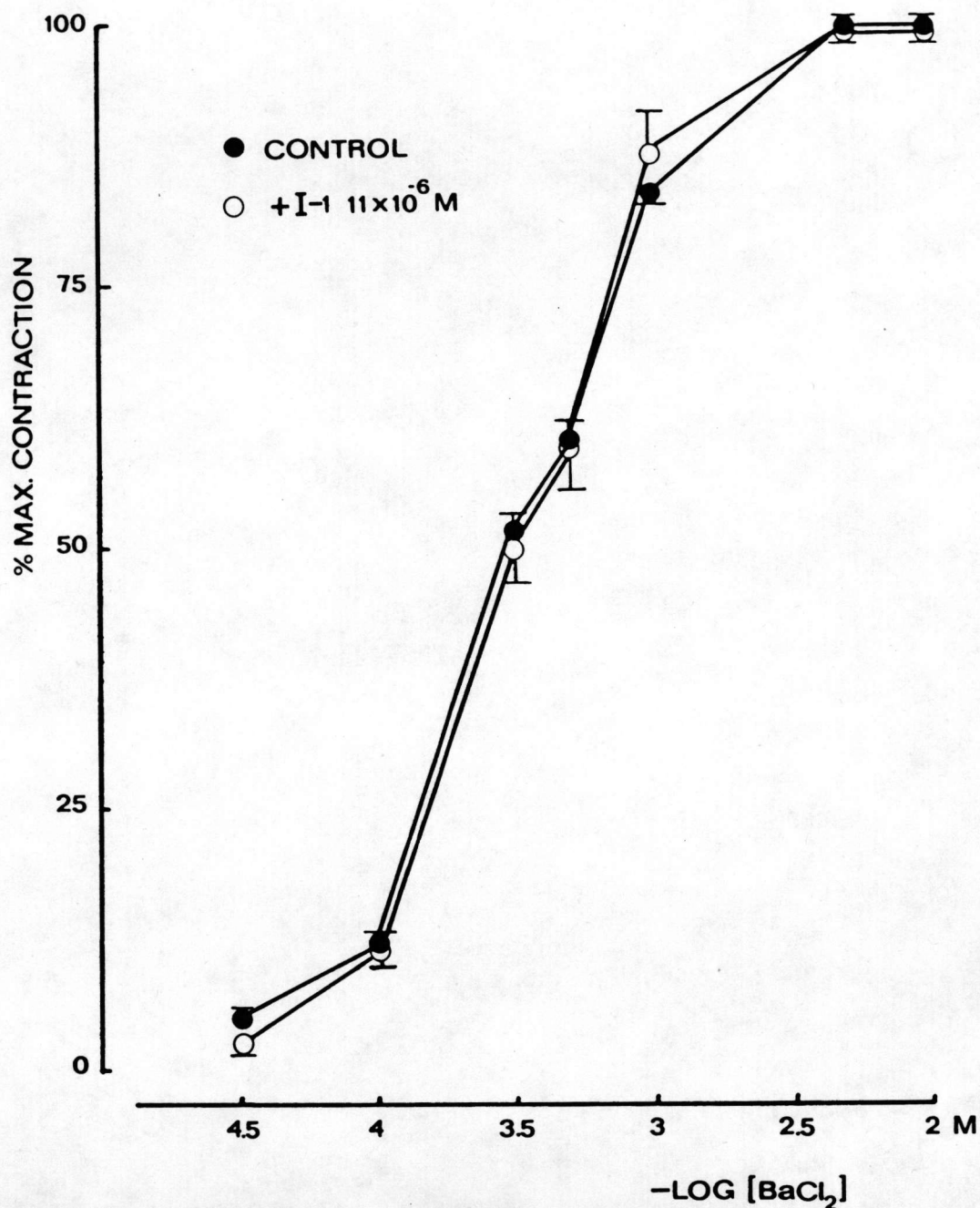


Figure 18. Cumulative log concentration-response curves of barium chloride on isolated guinea-pig ileum. Barium chloride induced sustained contraction of isolated guinea-pig ileum, incubation of the tissue with I-1 at concentration $11 \times 10^{-6} \text{ M}$ did not produce any significant alteration of the responses. In this and the following figure 19-21 the control curves are shown by solid circles. The other symbols indicate responses in the presence of indole alkaloids. Each point is the mean of 4 responses; vertical bars show S.E. mean. The abscissa scale is the negative logarithm in molar concentration ($p > 0.5$).

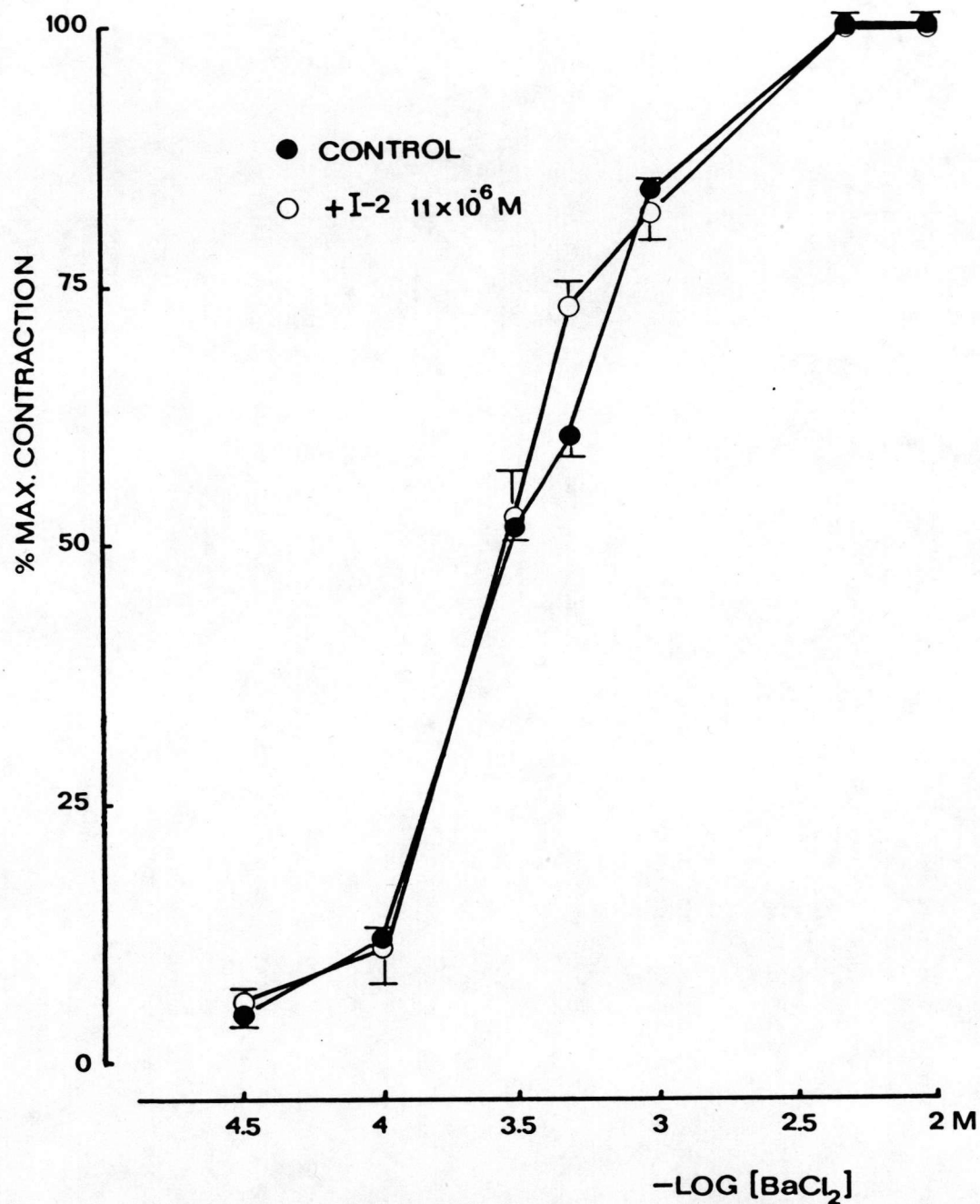


Figure 19. Cumulative log concentration-response curves of barium chloride on isolated guinea-pig ileum. Barium chloride induced sustained contraction of isolated guinea-pig ileum, incubation of the tissue with I-2 at concentration $11 \times 10^{-6} \text{ M}$ did not produce any significant alteration of the responses ($p > 0.5$).

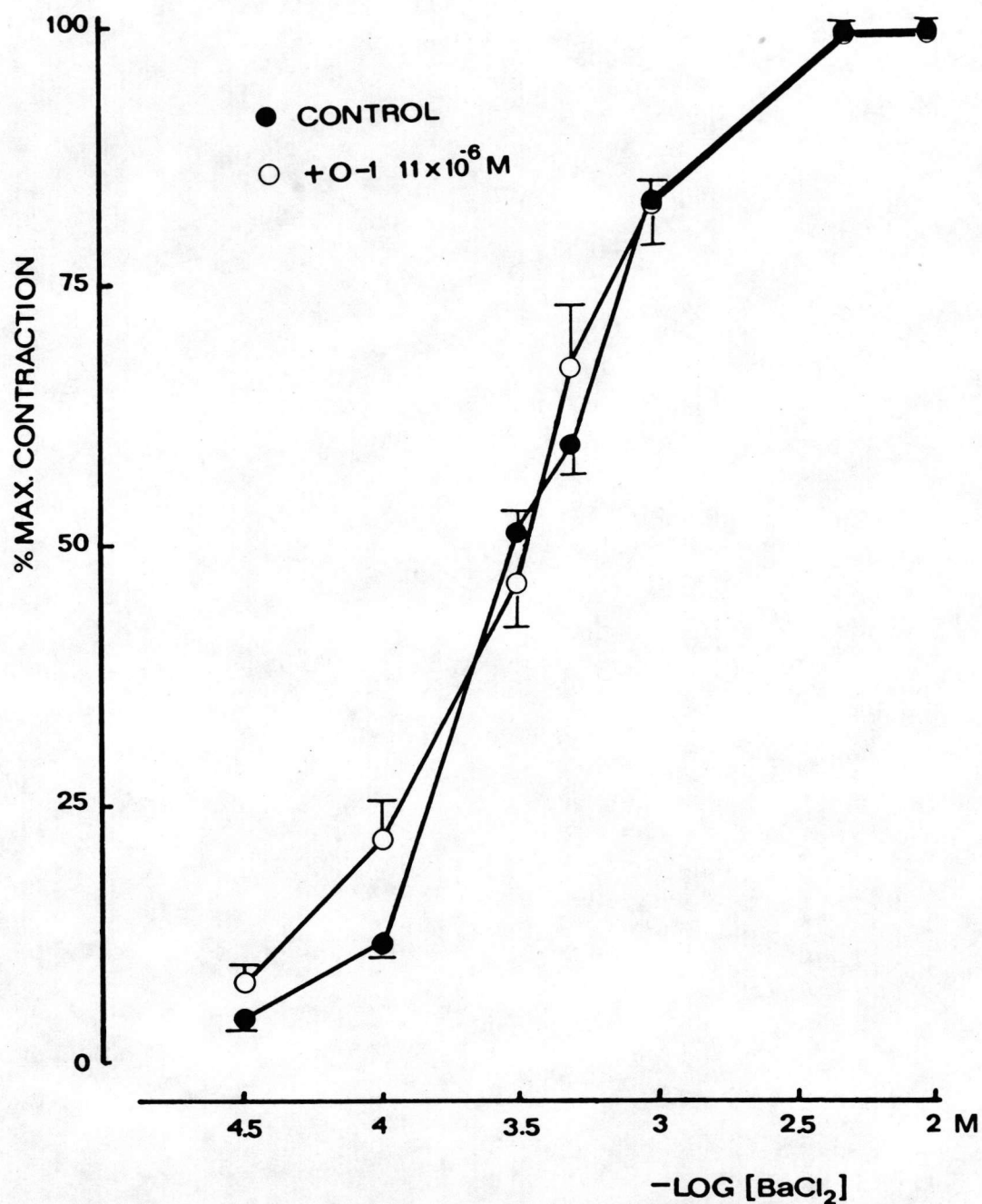


Figure 20. Cumulative log concentration-response curves of barium chloride on isolated guinea-pig ileum. Barium chloride induced sustained contraction of isolated guinea-pig ileum, incubation of the tissue with O-1 at concentration $11 \times 10^{-6} \text{ M}$ did not produce any significant alteration of the responses ($p > 0.5$).

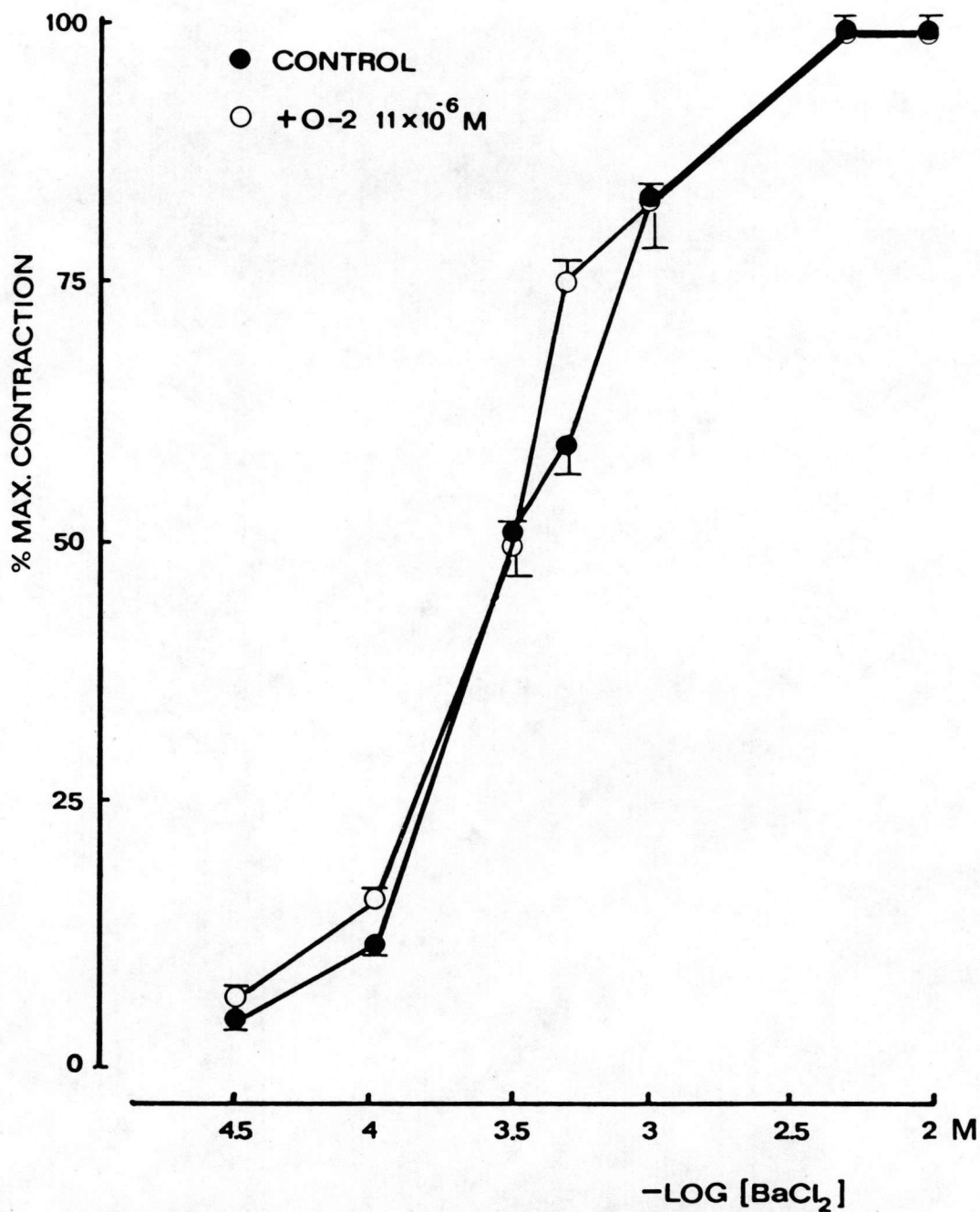


Figure 21. Cumulative log concentration-response curves of barium chloride on isolated guinea-pig ileum. Barium chloride induced sustained contraction of isolated guinea-pig ileum, incubation of the tissue with O-2 at concentration 11×10^{-6} M did not produce any significantly different of the responses ($p > 0.5$).

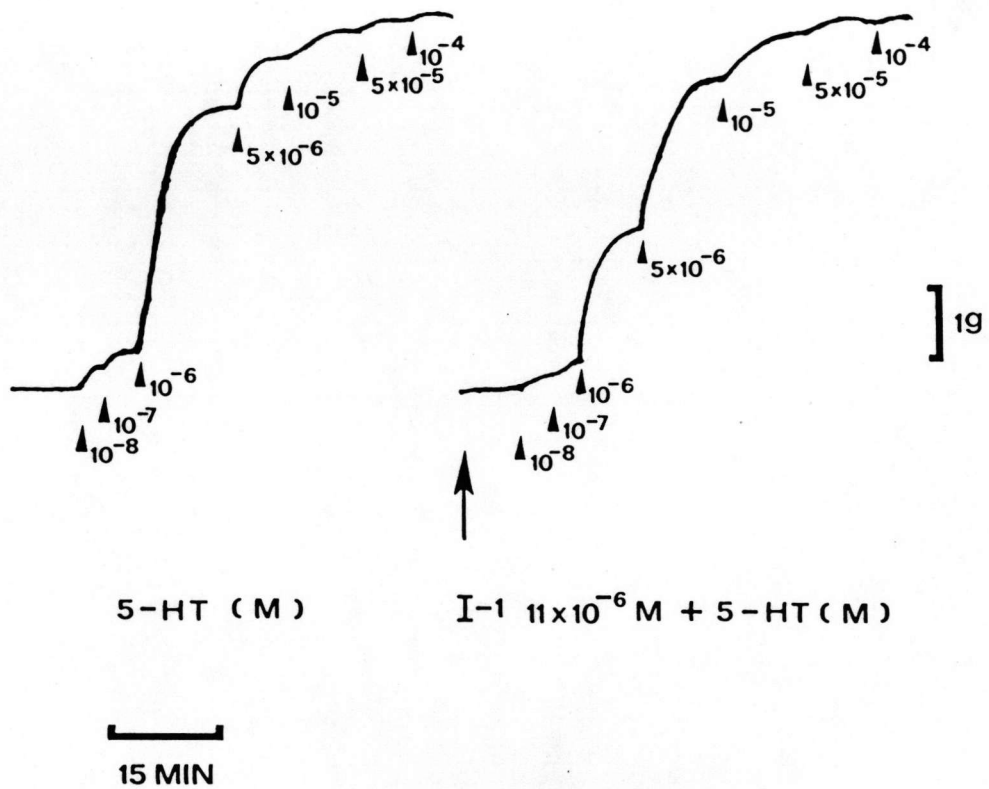


Figure 22. Trace of cumulative concentration-response relationship of 5-hydroxytryptamine (5-HT) on isolated rabbit aortic strip preparation. Left trace shows response of the rabbit aortic strip to 5-HT alone, while right trace represents the response following preincubating tissue in I-1 at concentration 11×10^{-6} M for 10 minutes. 5-HT was added at \blacktriangle ; the concentrations shown represent the cumulative total concentration. The vertical scale represents an isometric contraction equivalent to a standard change in load of 1 g. The horizontal scale represents 1 minute.

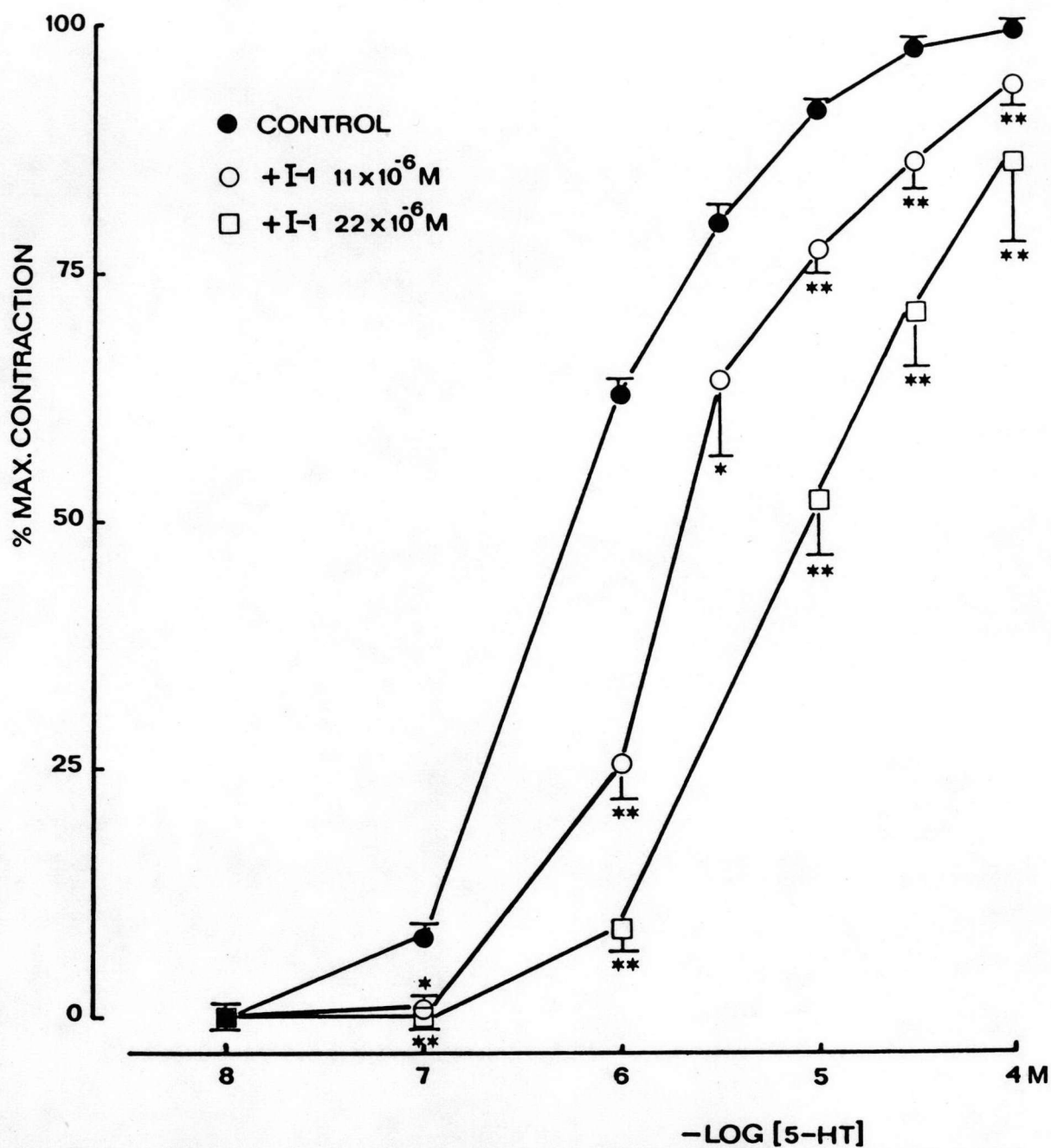


Figure 23. Cumulative log concentration-response curves of I-1 plotted from inhibition of contraction of isolated rabbit aortic strip induced by 5-HT. In this and the following figure 24-26 the control curves are shown by solid circles. The open symbols indicate responses in the presence of indole alkaloids. Each point is the mean of 5 responses; vertical bars show S.E. mean. The ordinate scale is percentage of maximal contraction; the abscissa scale is the negative logarithm in molar concentration. All points on the curves from I-1 treated condition are significantly different from the control (* = $p < 0.01$ and ** = $p < 0.001$, Student's t test).

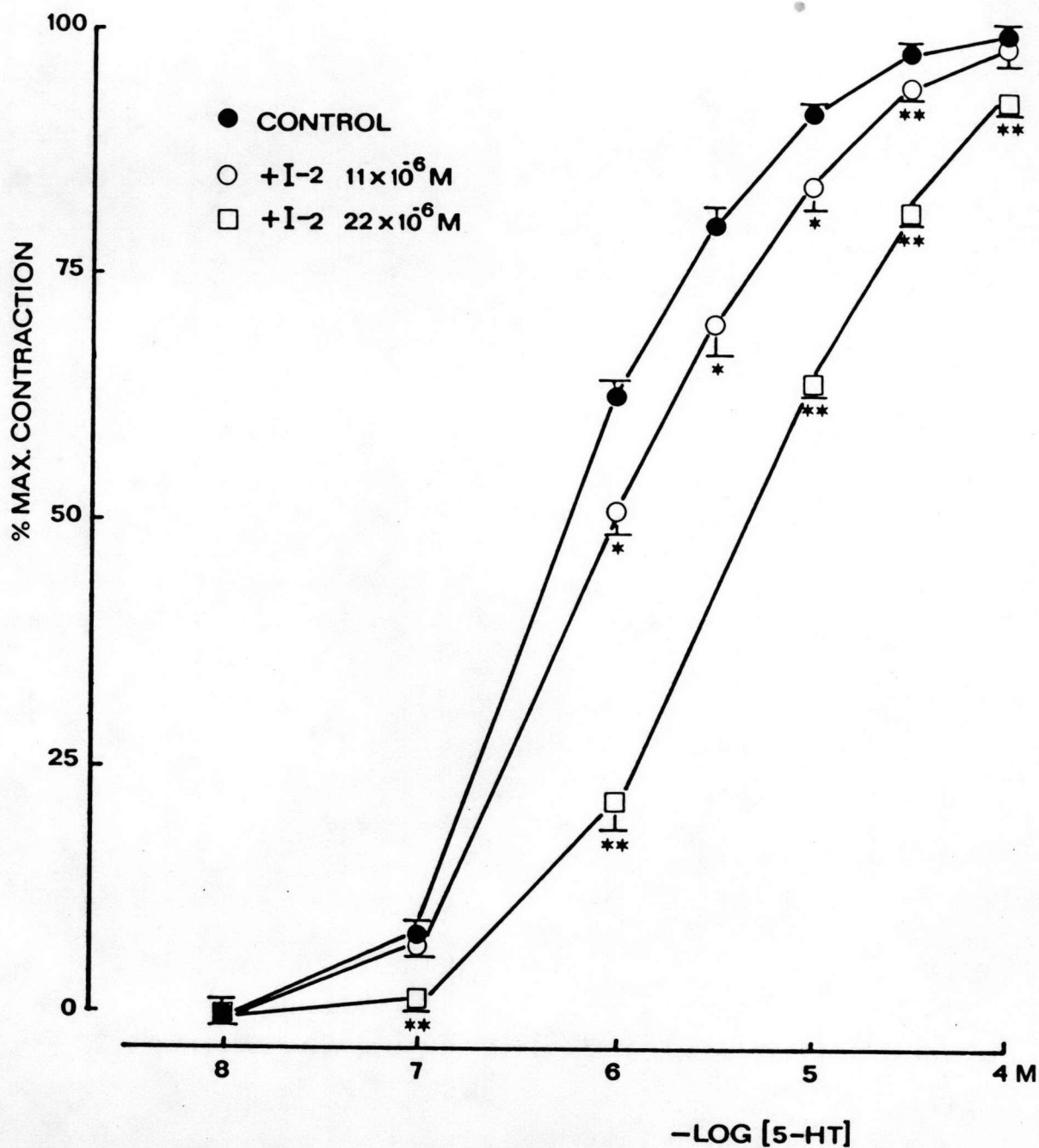


Figure 24. Cumulative log concentration-response curves of I-2 plotted from inhibition of contraction of isolated rabbit aortic strip induced by 5-HT. Each point is the mean of 5 responses. All points on the curves from I-2 treated condition are significantly different from the control (* = $p < 0.01$ and ** = $p < 0.001$, Student's t test).

3.2 Effects of Indole Alkaloids on Contraction of Aortic Strip Induced by Noradrenaline (NA)

Application of noradrenaline in a cumulative dose regimen (10^{-9} M - 10^{-4} M) to the bath fluid produced a dose dependent contraction of the isolated aortic strip. This NA induced contraction was not antagonized by any of the indole alkaloids tested as suggested by Fig. 27, 28, 29 and 30.

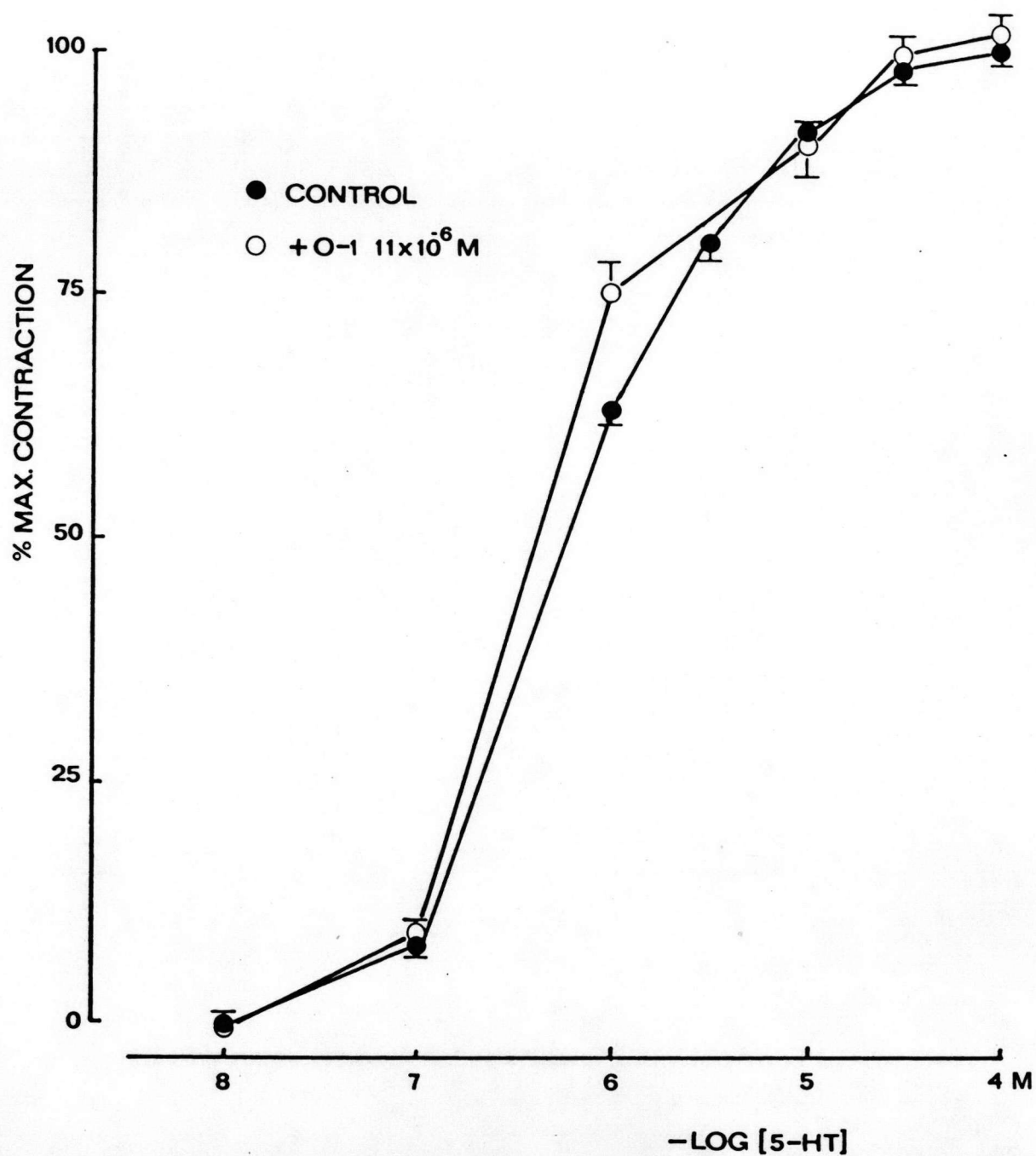


Figure 25. Cumulative log concentration-response curves of O-1 plotted from inhibition of contraction isolated rabbit aortic strip induced by 5-HT. Each point is the mean of 5 responses. None of the points in this figure are significantly different ($p > 0.2$).

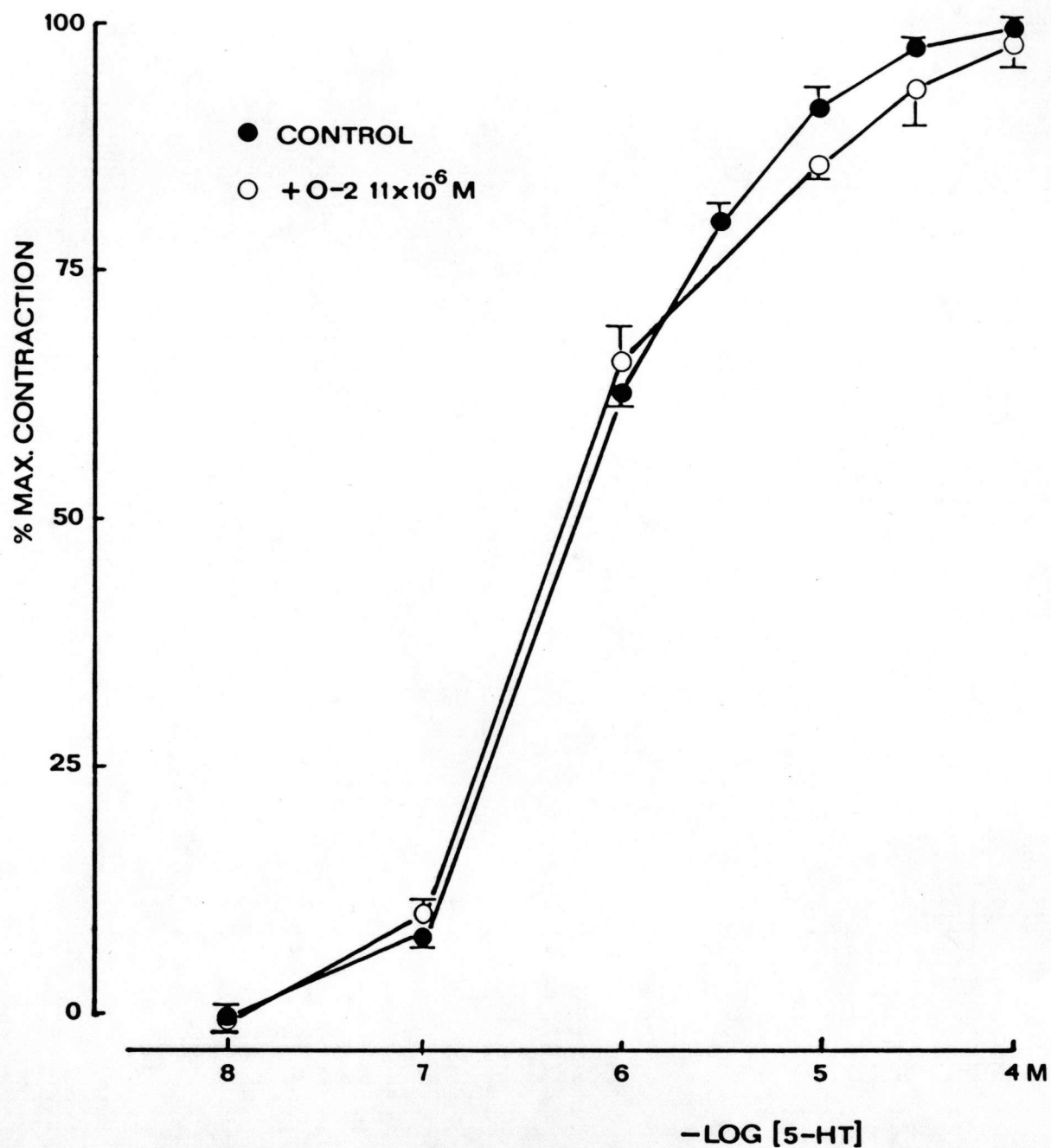


Figure 26. Cumulative log concentration-response curves of O-2 plotted from inhibition of contraction of isolated rabbit aortic strip induced by 5-HT. Each point is the mean of 5 responses. None of the corresponding points in the two curves are significantly different ($p > 0.3$).

| AGONIST | ANTAGONIST | | pA ₂ VALUE |
|---------|-------------------------|-----|-----------------------|
| 5-HT | 11 x 10 ⁻⁶ M | I-1 | 5.89 |
| | 22 x 10 ⁻⁶ M | I-1 | 5.79 |
| 5-HT | 11 x 10 ⁻⁶ M | I-2 | 4.80 |
| | 22 x 10 ⁻⁶ M | I-2 | 6.15 |

Table 4. pA₂ Values of indole alkaloids, I-1 and I-2, calculated from data of inhibitory action of the alkaloid on 5-HT induced contraction of the aortic strip.

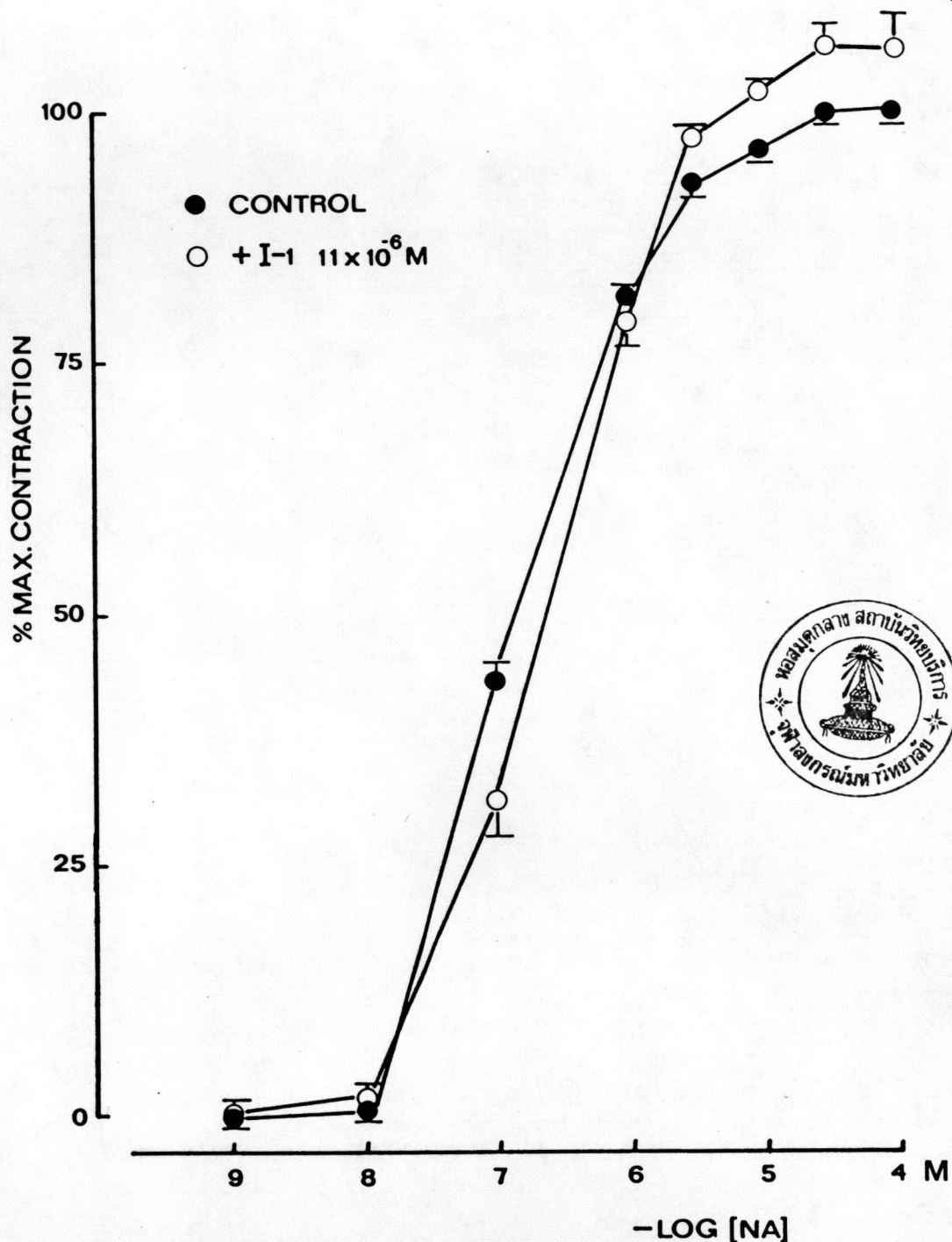


Figure 27. Cumulative log concentration-response curves of noradrenaline on isolated rabbit aortic strip. Noradrenaline induced sustained contraction of isolated rabbit aortic strip, incubation of the tissue with I-1 at concentration $11 \times 10^{-6} \text{ M}$ did not produce any significant alteration of the responses. In this and the following figure 28-30 the control curves are shown by solid circles. The open circles indicate responses in the presence of indole alkaloids. Each point is the mean of 4 responses; vertical bars show S.E. mean. The abscissa scale is the negative logarithm in molar concentration ($p > 0.3$).

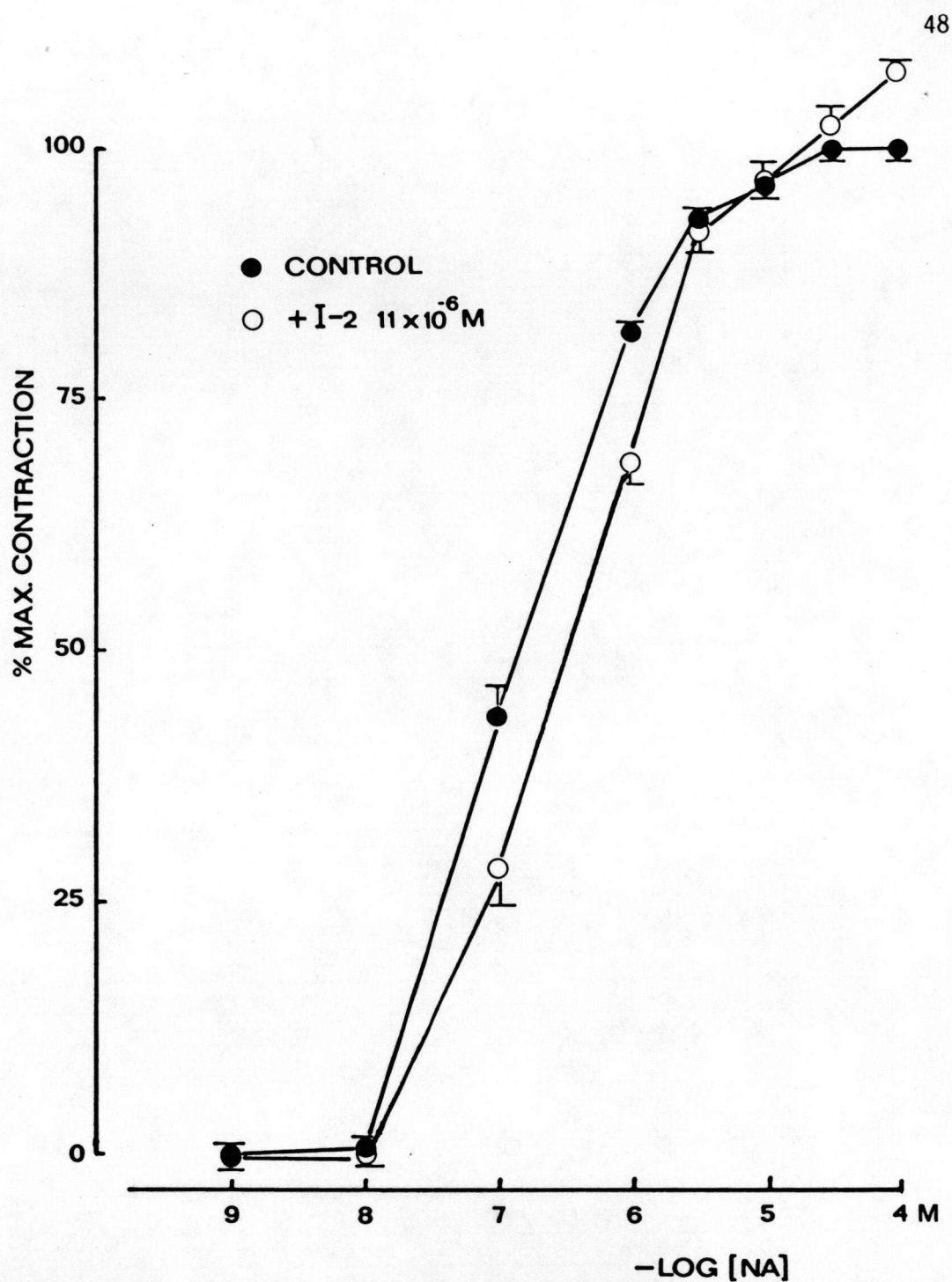


Figure 28. Cumulative log concentration-response curves of noradrenaline on isolated rabbit aortic strip. Noradrenaline induced sustained contraction of isolated rabbit aortic strip, incubation of the tissue with I-2 at concentration $11 \times 10^{-6} \text{ M}$ did not produce any significant alteration of the responses. Each point is the mean of 4 responses ($p > 0.2$).

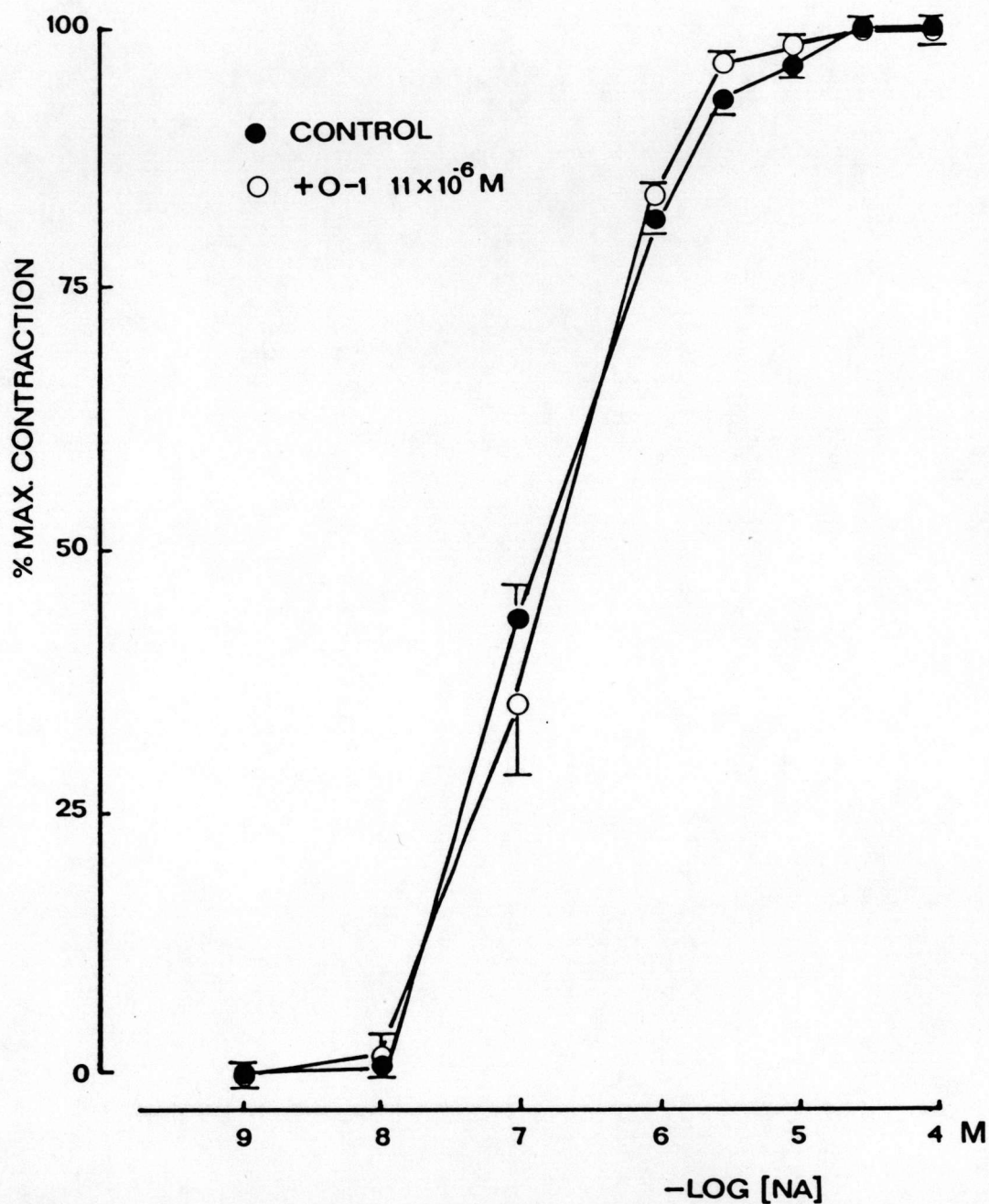


Figure 29. Cumulative log concentration-response curves of O-1 plotted from inhibition of contraction of isolated rabbit aortic strip induced by noradrenaline. Each point is the mean of 4 responses. None of the corresponding points in the two curves are significantly different ($p > 0.5$).

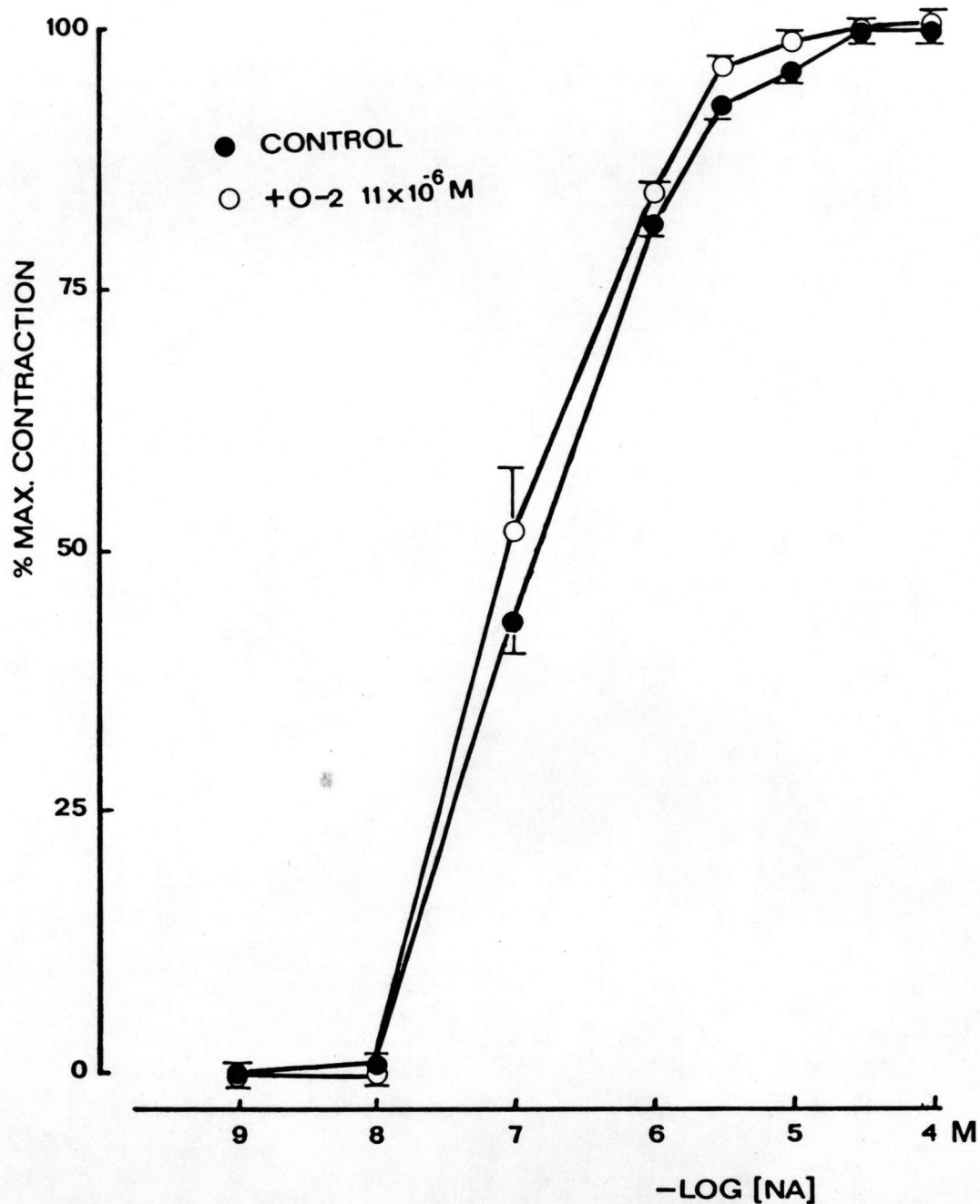


Figure 30. Cumulative log concentration-response curves of O-2 plotted from inhibition of contraction of isolated rabbit aortic strip induced by noradrenaline. Each point is the mean of 4 responses. None of the corresponding points in the two curves are significantly different ($p > 0.5$).