RESULTS

RABBIT AORTIC STRIP:

Cumulative administration of increasing doses of adrenaline and noradrenaline (1×10^{-8}, 2×10^{-8}, 4×10^{-8}, 8×10^{-8} and 1.6×10^{-7}) elicited increasing contractile responses from the rabbit aoric strip. The sensitivity of the aortic strip to adrenaline and noradrenaline varied in different experiments. However, when the height of contraction in mm was plotted against log dose of the stimulant drugs a statistically linear relationship was invariably observed. Addition of chlorpromazine to the bath 15 min before the cumulative administration of the stimulant drugs, caused a parallel shift of the dose-response line to the right indicating an antagonism of the responses (Fig.1). The lines shown in Fig. 1 are statistically parallel . Statistical tests for linearity and parallelism applied here were similar to those described by Finney (1952).

The antagonistic effect of different concentrations of chlorpromazine was expressed in terms of the dose-ratio This is the ratio of equiactive doses of the stimulant drug before and after the addition of the antagonist. (Gaddum, Hameed, Hathway and Stephens, 1955). Dose ratios for five different concentrations of chlorpromazine were determined in triplicate. The results are summarized in Fig. 1 and Table 1.

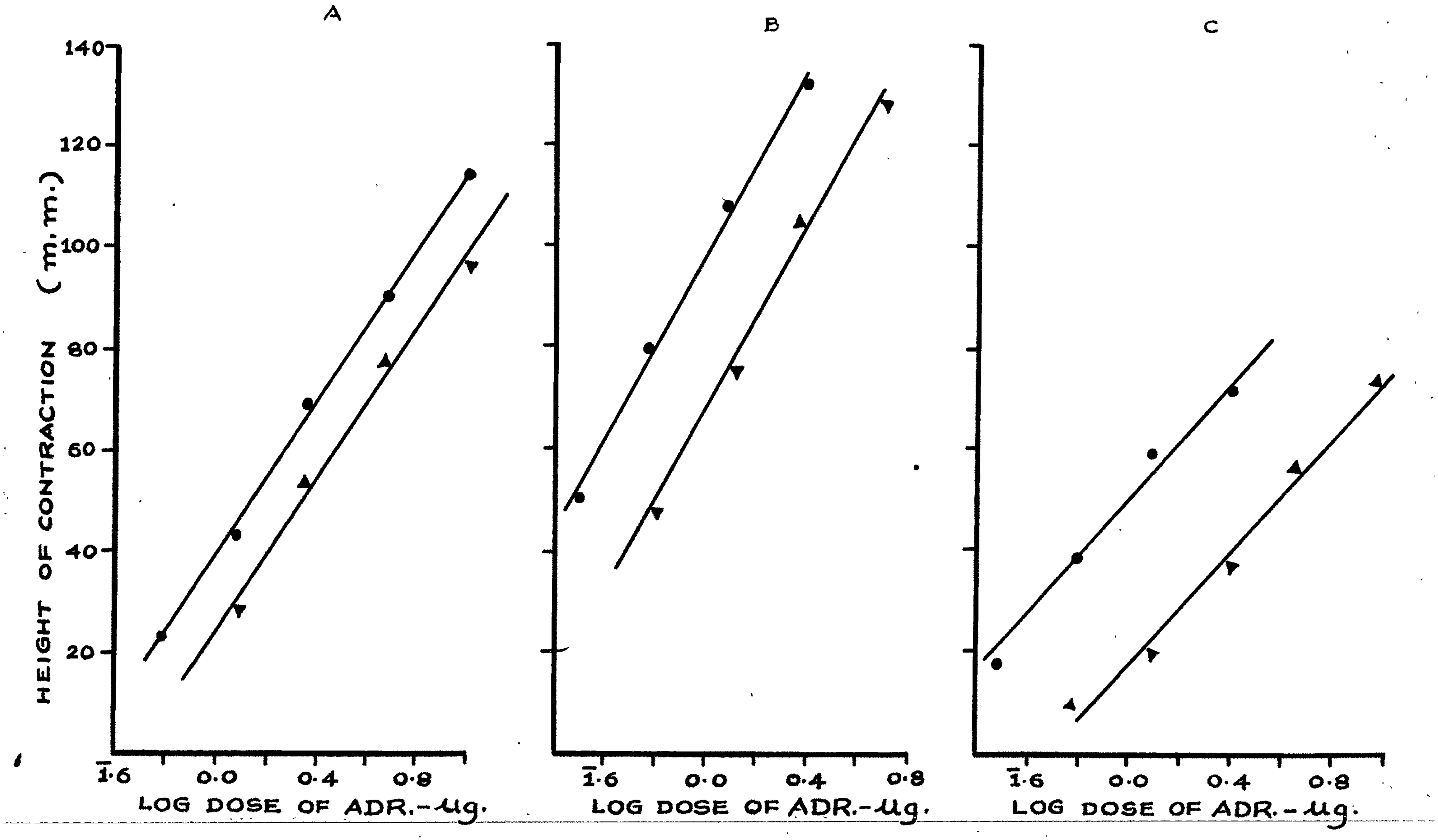
Arunlakshana and Schild (1959) have shown that if

LEGEND TO FIG. 1

Rabbit aortic strip: Dose - response curves of adrenaline alone (● - ●) and in presence of chlorpromazine (▲ - ▲) 2×10^{-15} (in A); 5×10^{-15} (in B); 1×10^{-14} (in C); 2×10^{-14} (in D) and 4×10^{-14} (in E).

The points represent the mean of three observations. Note the parallel shift of the dose - response lines to the right in the presence of chlorpromazine.

FIG : 1



A
B
C
HEIGHT OF CONTRACTION (m.m.)
140
120
100
80
60
40
20
$\bar{1}$·6
0·0
0·4
0·8
LOG DOSE OF ADR.-μg.

FIG : 1

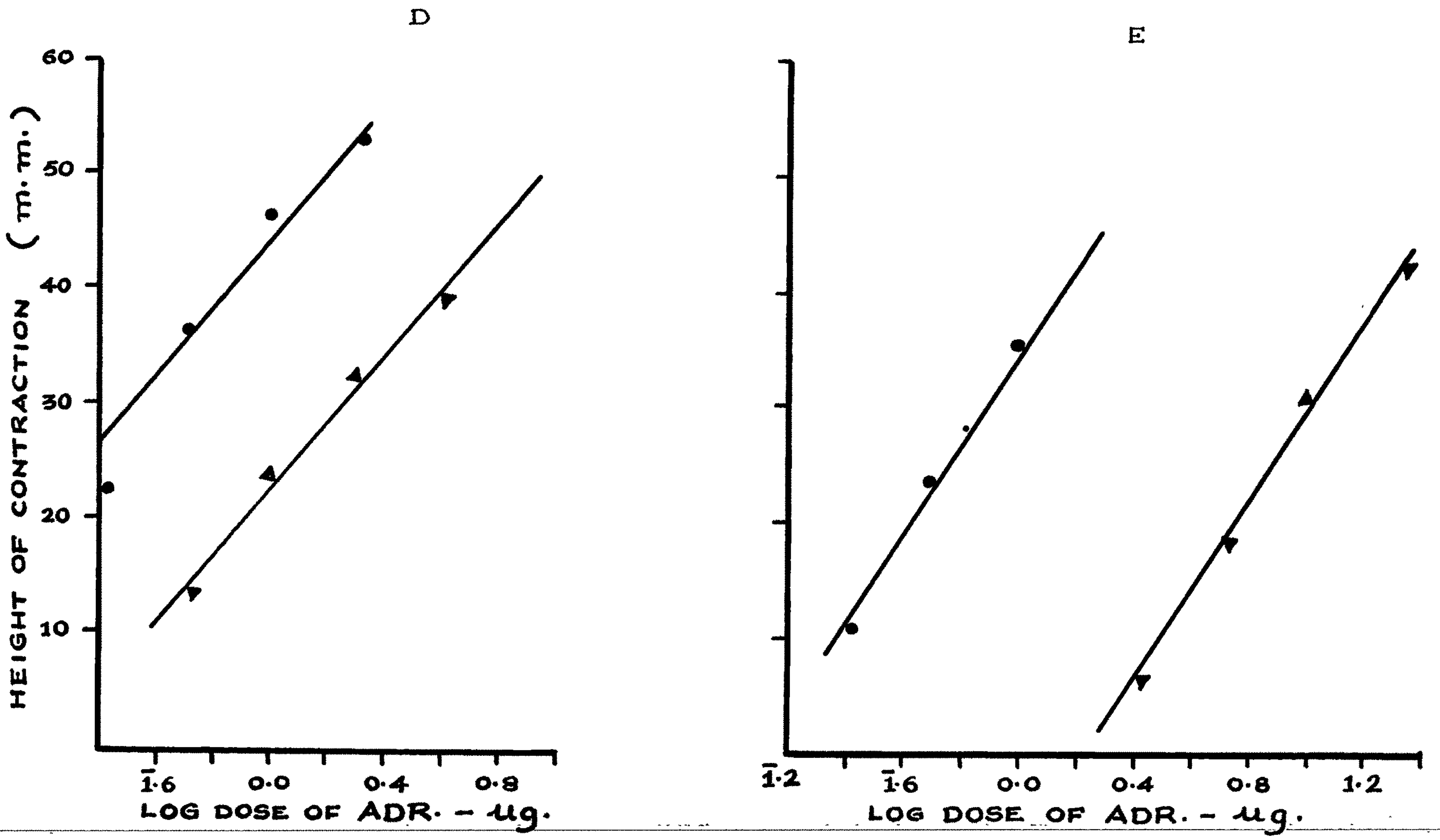

D
E
HEIGHT OF CONTRACTION (m.m.)
60
50
40
30
20
10
$\bar{1}$·2
$\bar{1}$·6
0·0
0·4
0·8
1·2
LOG DOSE OF ADR. – ug.
LOG DOSE OF ADR. – ug.

TABLE. 1

Antagonism of the responses of the rabbit aortic strip to adrenaline and noradrenaline by chlorpromazine. Antagonism is expressed in terms of the dose - ratio.

Treatment	Mean dose - ratio ± S.E.	
	Adrenaline	Noradrenaline
Chlorpromazine 1×10^{-15}	-	1.479
Chlorpromazine 2×10^{-15}	1.576 ± 0.145	-
Chlorpromazine 5×10^{-15}	2.171 ± 0.030	2.416
Chlorpromazine 1×10^{-14}	3.745 ± 0.057	3.467
Chlorpromazine 2×10^{-14}	5.956 ± 0.354	5.495
Chlorpromazine 4×10^{-14}	12.896 ± 1.216	-

an antagonist shifts the dose-response curve of an agonist without altering the slope of the curve (as in Fig. 1), this effect is consistent with competitive antagonism. This, however, does not provide complete proof of competitive antagonism.

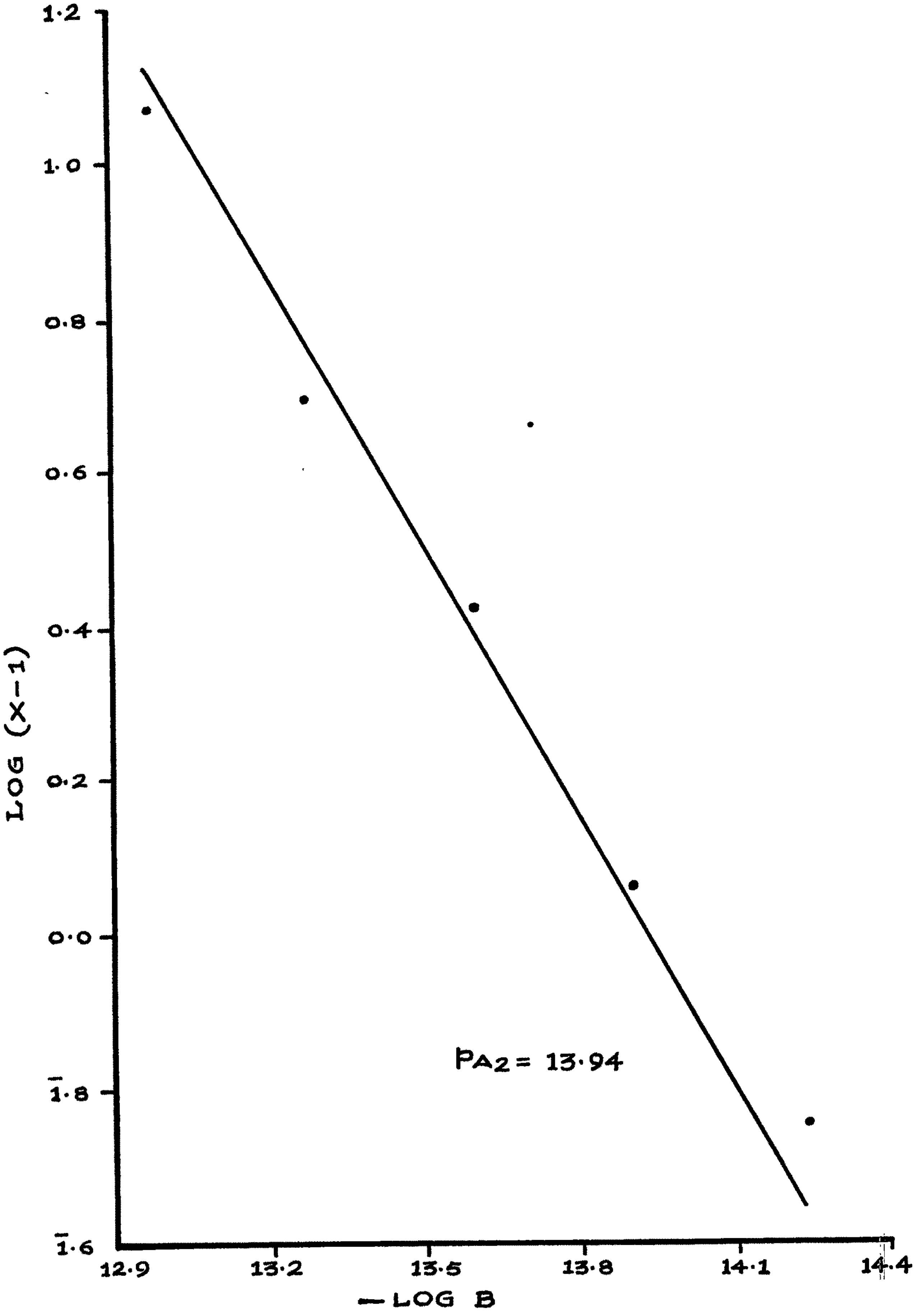
The present data was, therefore, examined more precisely to see whether they fit the hypothesis of competitive antagonism.

Logarithm of (x-1) was plotted against negative logarithm of B where x is the dose ratio and B the corresponding molecular concentration of chlorpromazine. The results from 5 sets of determinations are plotted in Fig. 2 and Fig. 3 in which the lines are the calculated regression lines. The intercepts of these lines with the abscissa (at 0 level) give the pA_2 values for the antagonist. pA_{10} values can also be determined from the regression lines. Under the conditions of competitive antagonism $pA_2 - pA_{10} = 0.95$. The pA_2 value for chlorpromazine in antagonising adrenaline was found to be 13.94; in antagonizing noradrenaline the pA_2 value was 14.1. Value for $pA_2 - pA_{10}$ was 0.82 for antagonism of adrenaline responses by chlorpromazine; for antagonism of noradrenaline the value was 1.2. These values for $pA_2 - pA_{10}$ are in good agreement with the theoretical value of 0.95 for competitive antagonism. This agreement indicates that the antagonism of adrenaline and noradrenaline by chlorpromazine is competitive. The log K_2

LEGEND TO FIG. 2.

Rabbit aortic strip: Data shown in Table 1 is plotted by the method of Arunlakshana and Schild (1959) to determine the value of pA_2 and $pA_2 - pA_{10}$. The ordinate log (x - 1), where x is the ratio of equiactive doses of adrenaline in the absence and in the presence of chlorpromazine. The abscissa is negative log B, where B is the molar concentration of chlorpromazine. The line is calculated regression line. It intercepts the abscissa (at o level) at 13.94 which is the pA_2 value. $pA_2 - pA_{10} = 0.82$. (which is in good agreement with the theoretical value of 0.95 for competitive antagonism).

FIG : 2

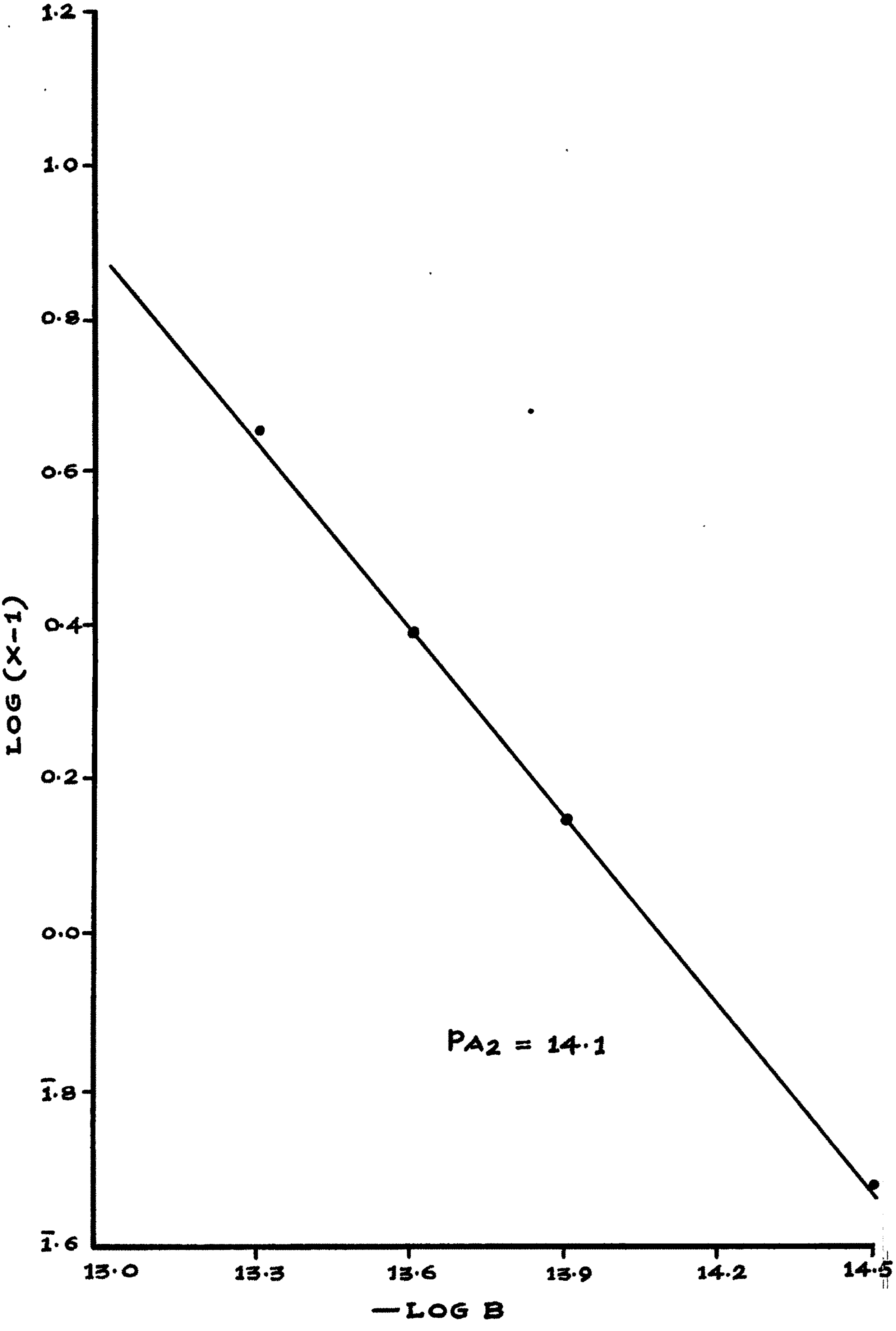


1·2
1·0
0·8
0·6
0·4
0·2
0·0
$\bar{1}$·8
$\bar{1}$·6
LOG (X−1)
12·9
13·2
13·5
13·8
14·1
14·4
—LOG B
PA2 = 13·94

LEGEND TO FIG. 3:

Rabbit aortic strip: Data shown in Table 1 is plotted by the method of Arunlakshana and Schild (1959) to determine the value of pA_2 and $pA_2 - pA_{10}$. The ordinate log (x - 1), where x is the ratio of equiactive doses of noradrenaline in the absence and in the presence of chlorpromazine. The abscissa is negative log B, where B is the molar concentration of chlorpromazine. The line is the calculated regression line. It intercepts the abscissa (at o level) at 14.1 which is the pA_2 value. $pA_2 - pA_{10} = 1.2$ (which is in good agreement with the theoretical value of 0.95 for competitive antagonism).

FIG :3


1·2
1·0
0·8
0·6
0·4
0·2
0·0
$\bar{1}$·8
$\bar{1}$·6
LOG (X−1)
13·0
13·3
13·6
13·9
14·2
14·5
—LOG B
pA_2 = 14·1

values for each response in the presence of chlorpromazine were calculated by using the formula $\log \frac{x-1}{B} = \log K_2$, for $n = 1$. The mean $\log K_2$ value was determined. The values are given in table II. (See "Resume of literature for theoretical considerations).

RECEPTOR PROTECTION:

Six pairs of aortic strips were used for the receptor protection technique. In three experiments adrenaline 1×10^{-5} protected the receptors from blockade by chlorpromazine (5×10^{-7}). In three other experiments 1×10^{-5} of noradrenaline was used for protecting the receptor. Adrenaline (1×10^{-8}) was used as the stimulant drug in those experiments where adrenaline was employed to protect the receptor while noradrenaline (1×10^{-8}) was used in experiments where noradrenaline was used to protect the receptors. When the sensitivity of the strips was tested 1½ hr after the removal of chlorpromazine, the protected strips were found to respond to the stimulant drugs whereas the unprotected strips were completely insensitive. The findings of a typical experiment are illustrated in Fig. 4. The results are summarised in Table III.

RAT SEMINAL VESICLE:

Administration of increasing doses of adrenaline and noradrenaline elicited increasing contractile

TABLE II

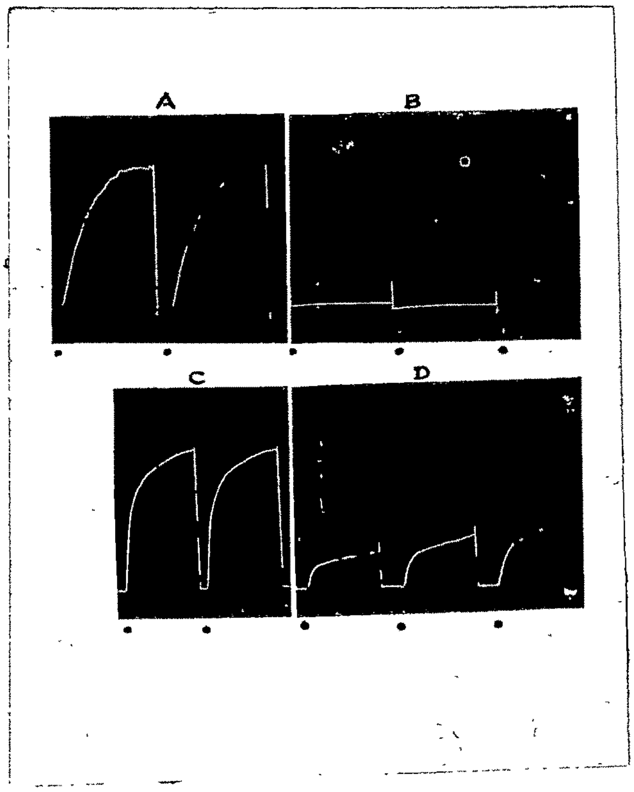
Values of log $\frac{x-1}{B}$ = log K_2 for adrenaline - chlorpromazine on rabbit aortic strip.

No. of expt.	-log conc. chlorpromazine	log K_2	Mean log K_2
3	14.24	14.00	
3	13.90	13.96	
3	13.60	14.03	13.99
3	13.27	13.96	
3	12.97	14.04	

LEGEND TO FIG. 4:

Responses (at dots) of the unprotected (in A and B) and the protected (in C and D) rabbit aortic strip to adrenaline 1×10^{-8}, before (in A and C) and 60 min after (in B and D) a 6 min exposure to chlorpromazine 5×10^{-7}.

FIG: 4



T A B L E. III

Effects of adrenaline and noradrenaline receptor protection against chlorpromazine blockade on rabbit aortic strip.

Without protection by adrenaline or noradrenaline.				Protected by adrenaline or noradrenaline. (1×10^{-5}).			
Control		After chloropromazine (5×10^{-7})		Control		After chloropromazine (5×10^{-7})	
Adr. (1×10^{-8})	Noradr. (1×10^{-8})	Adr. (1×10^{-8})	Noradr. (1×10^{-8})	Adr. (1×10^{-8})	Noradr. (1×10^{-8})	Adr. (1×10^{-8})	Noradr. (1×10^{-8})
19	58.5	0	0	23.5	33.5	10	16
30	39	0	0	55	41.5	21	21
59.5	26	0	0	55.5	42	25	21

Note: Adr. : Adrenaline.

Noradr: Noradrenaline.

Figures in the table indicate contractions of the strip in mm.

responses from the seminal vesicles. The sensitivity of the seminal vesicles to adrenaline and noradrenaline varied to a considerable degree in different experiments. As a result, the doses of adrenaline and noradrenaline used in each experiment varied according to the sensitivity of the tissue. However, when the height of contraction in mm was plotted against the log dose of the stimulant drugs a statistically linear relationship was invariably observed. Chlorpromazine caused a parallel shift of the dose-response curve to the right indicating an antagonism of the responses.

The antagonistic effect of different concentrations of chlorpromazine was expressed in terms of the dose-ratio. Dose-ratios for five different concentrations of chlorpromazine were determined in triplicate. The results are summarised in Table IV. Logarithm of (x-1) was plotted against the negative logarithm of B where x is the dose ratio and B the corresponding molecular concentration of chlorpromazine. The results from 5 sets of determinations are plotted in Fig. 5 and 6 in which the lines are the calculated regression lines. The pA_2 value for chlorpromazine in antagonising adrenaline was found to be 14.15; in antagonizing noradrenaline the pA_2 value was 14.06. Value for pA_2 - pA_{10} was 1.05 for antagonism of adrenaline responses by chlorpromazine; for antagonism of noradrenaline the value was 1.06. These values for pA_2 - pA_{10} are in good agreement with the theoretical value of 0.95 for

TABLE. IV

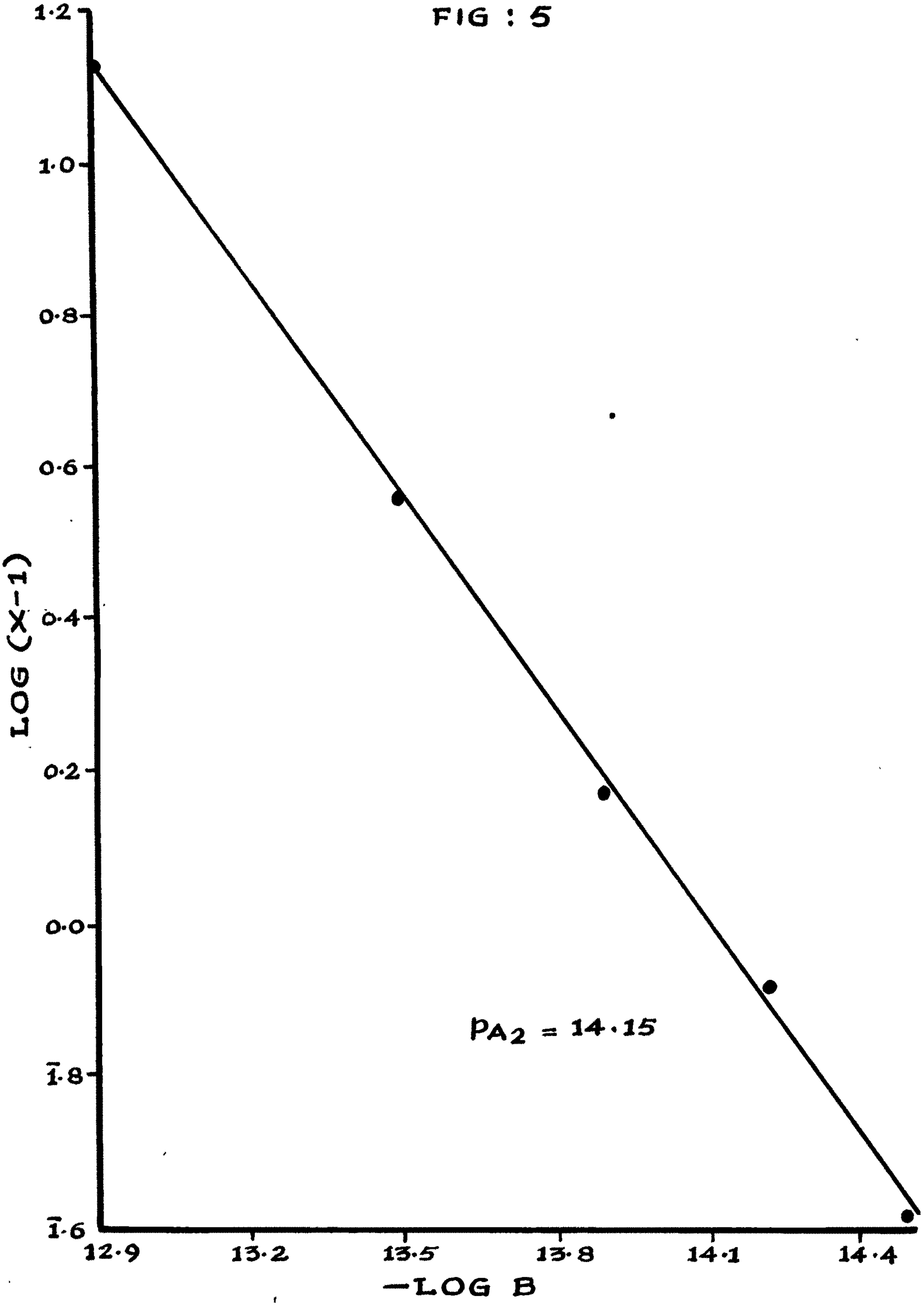
Antagonism of the responses of the rat seminal vesicles to adrenaline and noradrenaline by chlorpromazine. Antagonism is expressed in terms of the dose - ratio.

Treatment	Mean dose - ratio $\pm$ S.E.	
	Adrenaline	Noradrenaline.
Chlorpromazine 1×10^{-15}	1.412 ± 0.133	-
Chlorpromazine 2×10^{-15}	1.853 ± 0.022	1.742
Chlorpromazine 4×10^{15}	2.518 ± 0.102	2.290
Chlorpromazine 8×10^{-15}	-	3.508
Chlorpromazine 1×10^{-14}	4.654 ± 0.129	4.074
Chlorpromazine 2×10^{-14}	-	6.918
Chlorpromazine 4×10^{-14}	14.493 ± 0.77	-

LEGEND TO FIG. 5:

Rat seminal vesicle: Data shown in Table IV is plotted by the method of Arunlakshana and Schild (1959) to determine the value of pA_2 and $pA_2 - pA_{10}$. The ordinate log (x - 1), where x is the ratio of equiactive doses of adrenaline in the absence and in the presence of chlorpromazine. The abscissa is negative log B, where B is the molar concentration of chlorpromazine. The line is the calculated regression line. It intercepts the abscissa (at o level) at 14.15 which is the pA_2 value. $pA_2 - pA_{10} = 1.05$ (which is in good agreement with the theoretical value of 0.95 for competitive antagonism).

FIG : 5

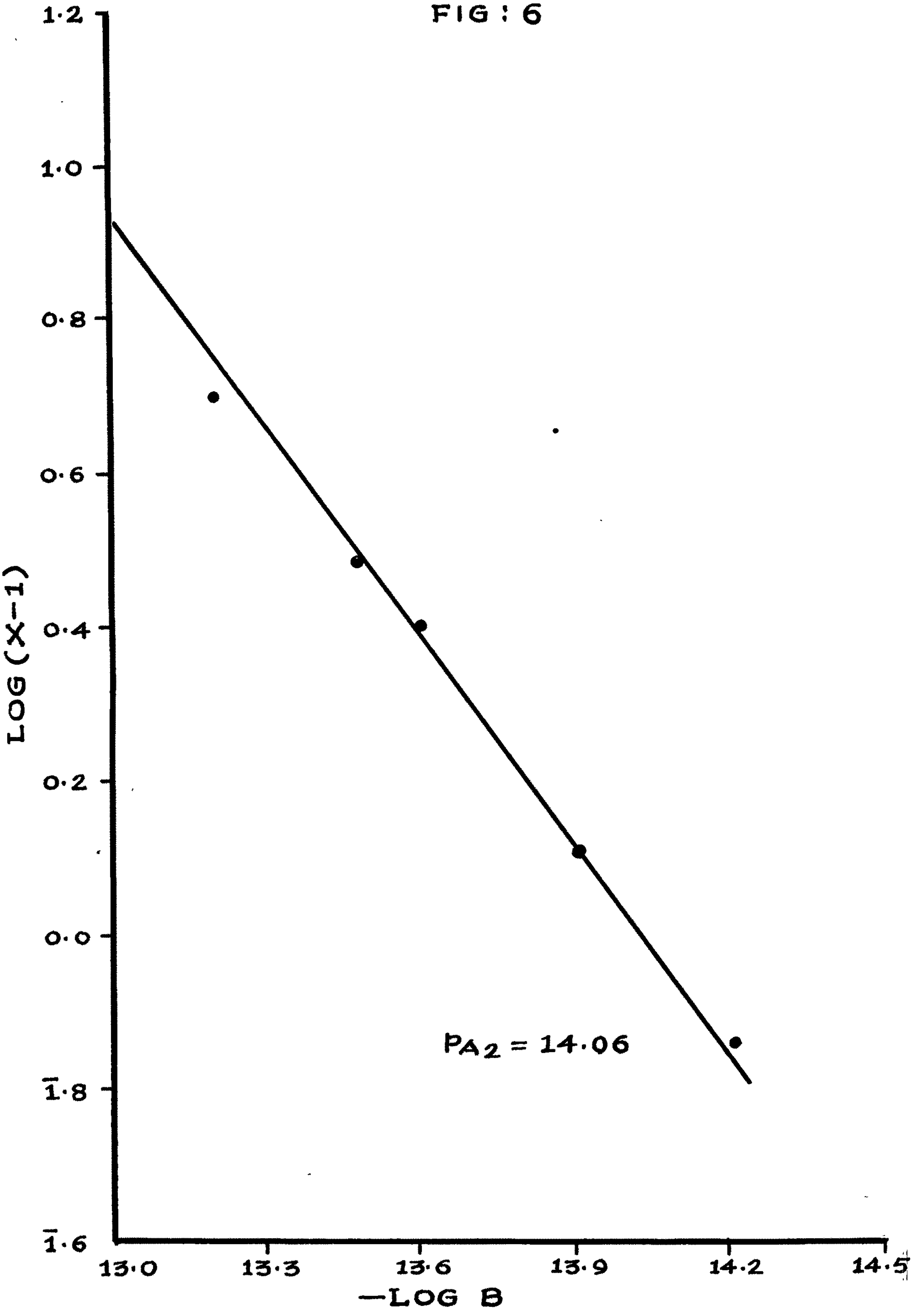


1·2
1·0
0·8
0·6
0·4
0·2
0·0
$\bar{1}$·8
$\bar{1}$·6
LOG (X-1)
12·9
13·2
13·5
13·8
14·1
14·4
—LOG B
pA_2 = 14·15

LEGEND TO FIG. 6:

Rat seminal vesicle: Data shown in Table IV is plotted by the method of Arunlakshana and Schild (1959) to determine the value of pA_2 and $pA_2 - pA_{10}$. The ordinate log (x - 1), where x is the ratio of equiactive doses of noradrenaline in the absence and in the presence of chlorpromazine. The abscissa is negative log B, where B is the molar concentration of chlorpromazine. The line is the calculated regression line. It intercepts the abscissa (at o level) at 14.06 which is the pA_2 value. $pA_2 - pA_{10} = 1.06$ (which is in good agreement with the theoretical value of 0.95 for competitive antagonism).

FIG: 6


1·2
1·0
0·8
0·6
0·4
0·2
0·0
$\bar{1}$·8
$\bar{1}$·6
LOG (X−1)
13·0
13·3
13·6
13·9
14·2
14·5
—LOG B
pA_2 = 14·06

competitive antagonism. This agreement indicates that the antagonism of adrenaline and noradrenaline by chlorpromazine is competitive. The log K_2 values for each response in the presence of chlorpromazine were calculated by using the formula log $\frac{x-1}{B}$ = log K_2 presuming n (slope) = 1. The mean log K_2 value was determined. The values are given in Table V.

CAT BLOOD PRESSURE:

In 12 experiments, chlorpromazine in doses of 2 mg/kg 4 mg/kg and 8 mg/kg severely depressed and many times reversed the pressor effect of adrenaline (5 to 20 μg/kg). The pressor response to noradrenaline (5 to 10 μg/kg) was either unaltered or slightly potentiated (Fig.7). In these doses chlorpromazine enhanced the depressor effect of isoprenaline (1.5 μg/kg).

Effect of pretreatment with hexamethonium and cocaine:

Circulating adrenaline and noradrenaline are taken up, bound and retained at or near sympathetic nerve endings (Axelrod,Weil-Malherbe and Tomchik, 1959;Whitby, Axelrod and Weil-Malherbe, 1961). Chlorpromazine inhibits the uptake of the free hormones at these sites (Hertting et al.,1961; Axelrod,Hertting and Potter, 1962; and Hertting et al., 1962). Of the two amines,tissues binding plays a more significant part in the inactivation of noradrenaline. It was therefore, thought that in the case of noradrenaline the potentiation caused by inhibition of uptake might be .. ..

T A B L E. V

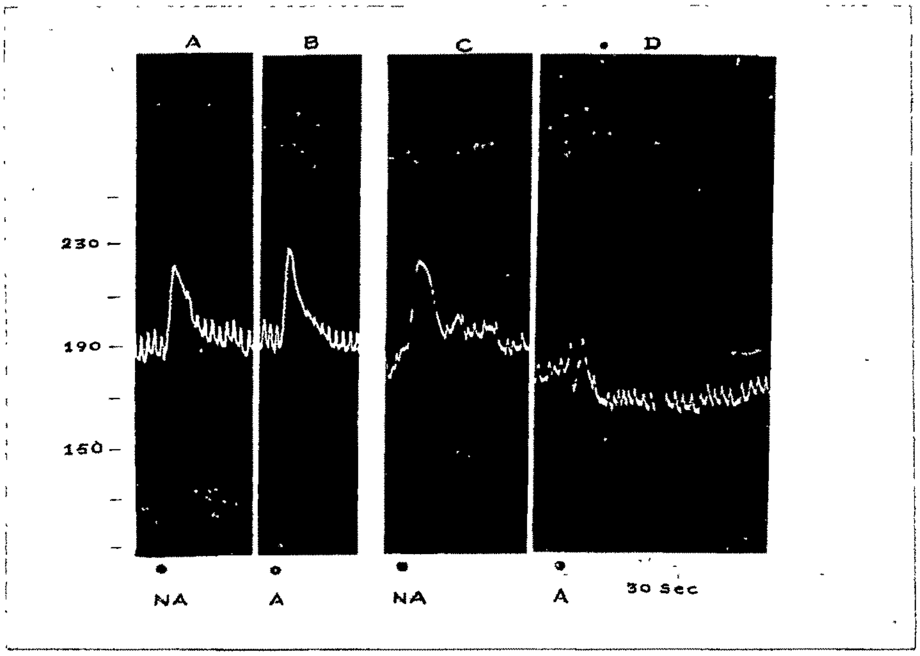
Values of log $\frac{x-1}{B}$ = log K_2 for adrenaline - chlorpromazine on rat seminal vesicle.

No. of Expt.	-log conc. chlorpromazine	Log K_2	Mean log K_2
3	14.51	14.13	
3	14.21	14.14	
3	13.91	14.09	14.09
3	13.51	14.07	
3	12.91	14.04	

LEGEND TO FIG. 7.

Cat, 1.8 kg. Chloralose (60 - 80 mg/kg) anaesthesia. Record of carotid blood pressure. Responses at dots to noradrenaline (5 µg/kg at NA) and adrenaline (15 µg/kg at A) alone (in A and B) and 10 min after an injection (8 mg/kg) of chlorpromazine (in C and D). Time 30 sec. Injections were intravenous.

FIG: 7



masking the adrenergic blocking action of chlorpromazine.

In order to test this possibility the effect of chlorpromazine on the pressor responses to the amines was studied in animals in whom the uptake of the amines was already blocked by hexamethonium or cocaine.

<u>Hexamethonium:-</u> Hexamethonium (10 mg/kg) was administered slowly intravenously in divided doses, over a period of thirty minutes. In these animals chlorpromazine in a dose of 4 mg/kg reversed the pressor effect of adrenaline (5 µg/kg); pressor responses to noradrenaline (3 µg/kg) were potentiated (Fig.8).

<u>Cocaine:-</u> Cocaine (5 mg/kg) was administered slowly intravenously over a period of 5 minutes. In these animals chlorpromazine in a dose of 4 mg/kg reversed the pressor effect of adrenaline (5 µg/kg); the pressor responses to noradrenaline (3 µg/kg) were potentiated (Fig. 9.).

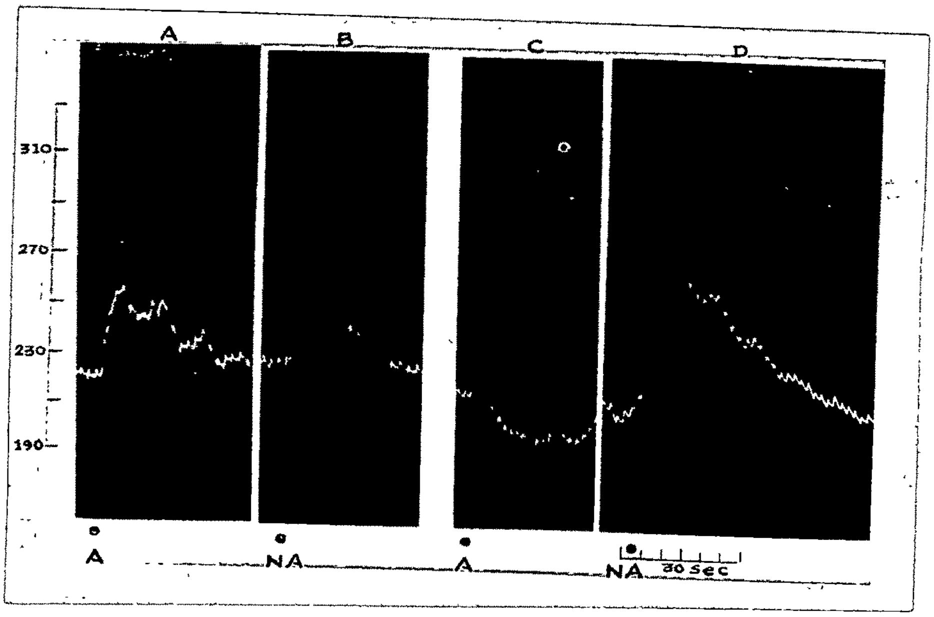
<u>Effect of treatment with dichloroisoprenaline:</u>

During earlier experiments it was observed that chlorpromazine considerably potentiates the depressor effect of isoprenaline. In contrast to noradrenaline which exerts an almost purely vasoconstrictor effect adrenaline has both vasoconstrictor and vasodilator components of action. The selective depression of the pressor effects of adrenaline by small doses (2-8 mg/kg) of chlorpromazine could be due to a potentiation of the

LEGEND TO FIG. 8.

Cat, 2.5 kg. Chloralose (60 - 80 mg/kg) anaesthesia. Record of carotid blood pressure. Responses at dots to adrenaline (10 µg/kg at A) and noradrenaline (4 µg/kg at NA) alone (in A and B) and 10 min after an injection (4 mg/kg) of chlorpromazine (in C and D). The animal was treated with hexamethonium (10 mg/kg in three divided doses) 15 min before the start of the experiment. Time 30 sec. Injections were intravenous.

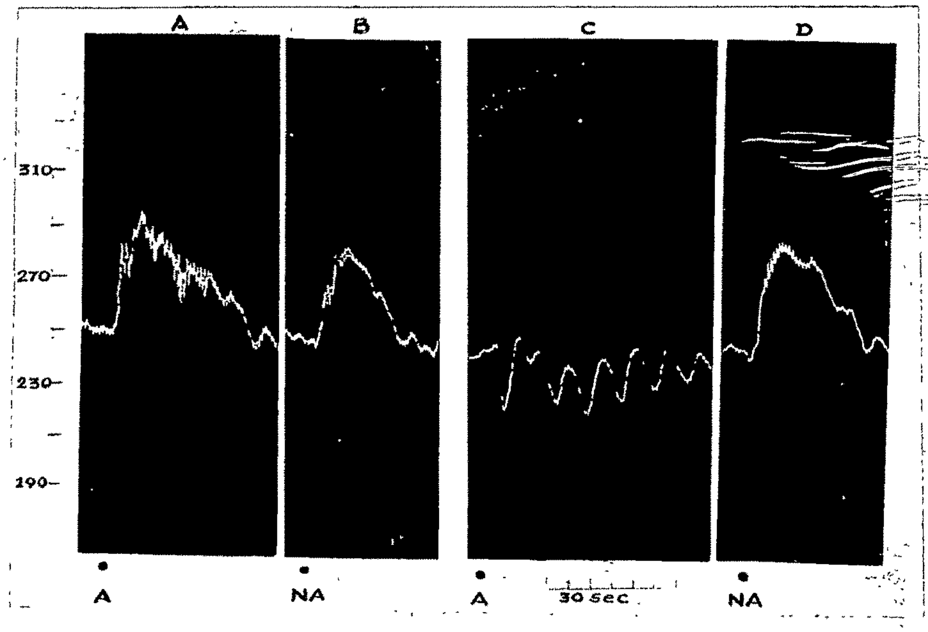
FIG : 8



LEGEND TO FIG. 9.

Cat, 2.7 kg. Chloralose (60 - 80 mg / kg) anaesthesia. Record of carotid blood pressure. Responses at dots to adrenaline (6 µg/kg at A) and noradrenaline (4 µg/kg at NA) alone (in A and B) and 10 min after an injection (4 mg/kg) of chlorpromazine (in C and D). The animal was treated with cocaine (5 mg/kg) 15 min before the start of the experiment. Time 30 sec. Injections were intravenous.

FIG: 9



vasodilator component of the action of adrenaline. If this supposition is correct, dichlorisoprenaline which selectively blocks the vasodepressor action of catecholamines, should reverse the inhibition of pressor effect of adrenaline by chlorpromazine.

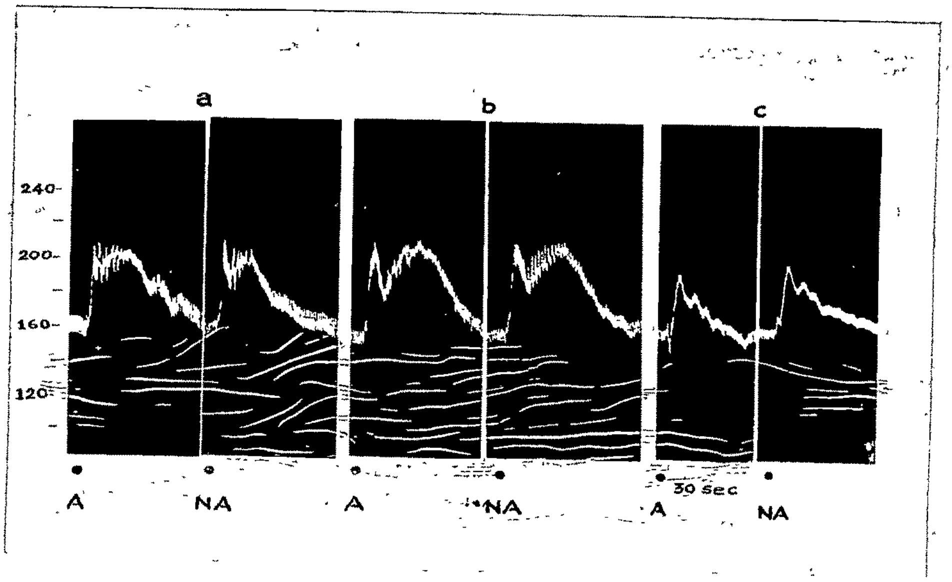
In experiments designed to test this possibility dichloroisoprenaline (10 mg/kg) was administered intravenously in three divided doses given at five minutes intervals. The blocking action of dischlorisoprenaline was confirmed by injection of isoprenaline (2.5 µg/kg) which produced a good depressor effect before dichloroisoprenaline administration but had no effect thereafter. In these animals chlorpromazine (10 mg/kg) failed to reduce the pressor effects of adrenaline or noradrenaline. To the contrary a slight potentiation of the responses was observed. A large dose of chlorpromazine (50 mg/kg) however, depressed the responses both to adrenaline and noradrenaline to the same extent (Fig.10).

In another set of experiments chlorpromazine (32 mg/kg) dramatically reversed the pressor effect of adrenaline, slightly reduced the pressor effect of noradrenaline and potentiated the depressor effect of isoprenaline. Dichlorisoprenaline (10 mg/kg) administered at this stage converted the depressor response to adrenaline to a pressor effect which was only slightly smaller than the control response to adrenaline. The slight inhibition of noradrenaline response was unaffected by dichloroisoprenaline while

LEGEND TO FIG. 10.

Cat, 2.0 kg, Chloralâse (60 - 80 mg/kg) anaesthesia. Record of carotid blood pressure. Responses at dots to adrenaline (15 µg/kg at A) and noradrenaline (10 µg/kg at NA). Chlorpromazine (10 mg/kg) was injected between a and b and (32 mg/kg) of chlorpromazine was injected between b and c. The animal was treated with dichloroisoprenaline (10 mg/kg in three divided doses) 15 min before the start of the experiment. Time 30 sec. Injections were intravenous.

FIG: 10



the depressor response to isoprenaline was converted to a slight pressor effect (Fig.11).

Quantitation of the antagonism of the pressoreffect of noradrenaline by chlorpromazine:

The results of the above experiments with dichloroisoprenaline indicated that chlorpromazine in small doses (2 to 8 mg/kg) has no true adrenergic blocking action. The adrenergic blocking action of large doses (8 to 32 mg/kg) of chlorpromazine against noradrenaline was further analysed by using the method of Chen and Russell (1950). According to these authors, in competitive inhibition the $\frac{1}{V}$ versus $\frac{1}{S}$ lines for various concentrations of an antagonist will have different slopes with the same intercept (S : dose, V : response). Dose-response lines for the pressor effect of noradrenaline were determined before and after three different concentration of chlorpromazine (8 mg/kg, 16 mg/kg and 32 mg/kg). In Fig.12 are shown plots of $\frac{1}{V}$ versus $\frac{1}{S}$ in the presence of different concentrations of chlorpromazine where V is the rise of blood pressure in mm of Hg and S is the dose of noradrenaline in ug per kg. It can be seen that the various lines intercept at the same point.

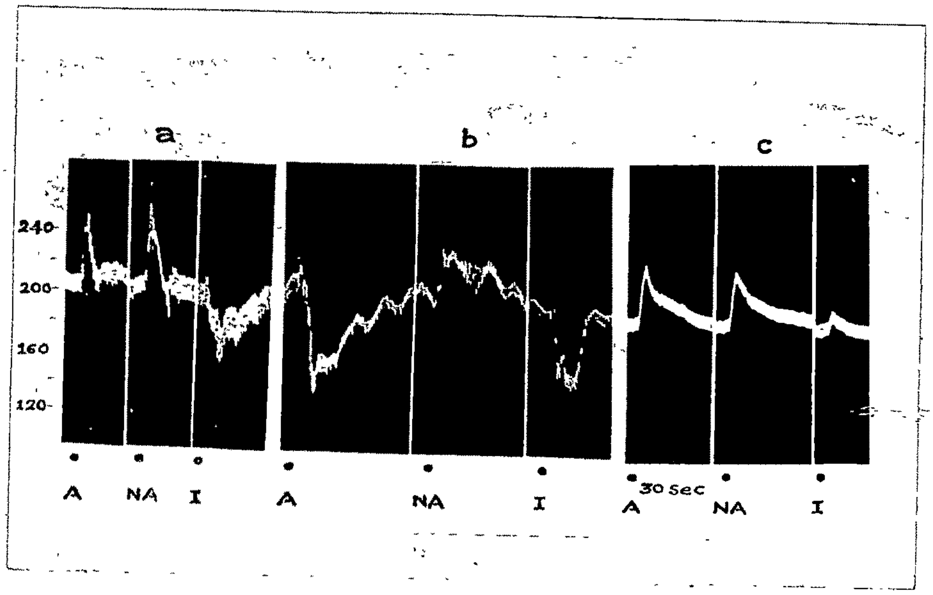
RABBIT BLOOD PRESSURE:

In experiments with the rabbit aortic strips it was observed that chlorpromazine exhibits a powerful adrenergic blocking action (pA_2 = 13.95). On the other

LEGEND TO FIG 11.

Cat, 2.3 kg. Chloralose (60 - 80 mg/kg) anaesthesia. Record of carotid blood pressure. Responses at dots to adrenaline (10 µg/kg at A), noradrenaline (10 µg/kg at NA) and isoprenaline (2 µg/kg at I). Chlorpromazine (16 mg/kg) was injected between a and b. Dichloroisoprenaline (10 mg/kg in three divided doses) was injected between b and c. Time 30 sec. Injections were intravenous.

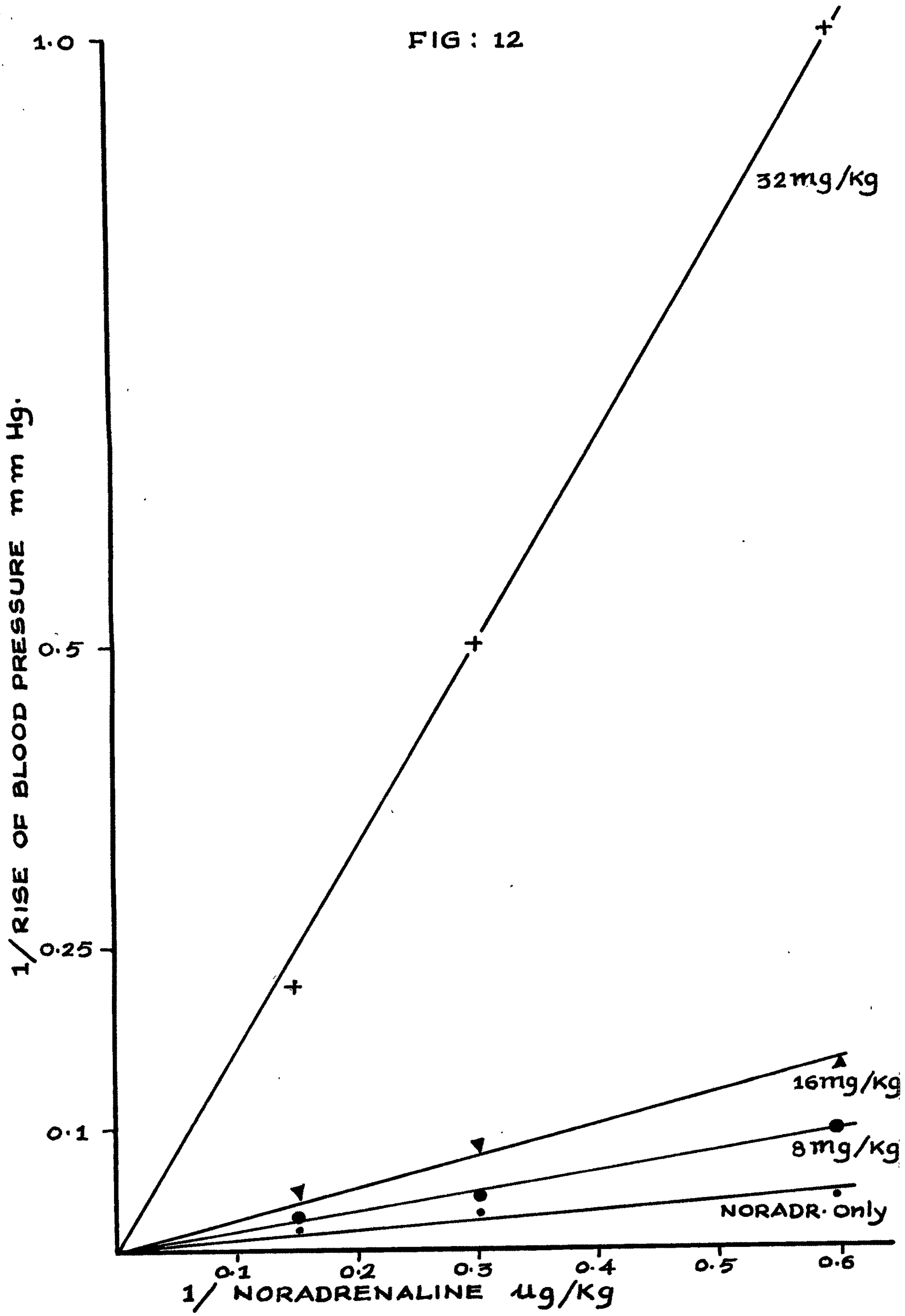
FIG: 11



LEGEND TO FIG. 12.

Figure shows the reciprocals of the rise in blood pressure plotted against the reciprocals of dosage of noradrenaline (1.6 µg/kg, 3.2 µg/kg and 6.4 µg/kg) alone and after three different doses of chlorpromazine (8mg/kg, 16 mg/kg and 32 mg/kg). Note that the lines have different slopes but the same intercept.

FIG: 12



1.0
0.5
0.25
0.1
1/RISE OF BLOOD PRESSURE mm Hg.
32mg/Kg
16mg/Kg
8mg/Kg
NORADR. only
0.1
0.2
0.3
0.4
0.5
0.6
1/ NORADRENALINE μg/Kg

hand, large doses of chlorpromazine were required to block pressor effect of noradrenaline in cats. These could be due to species difference. In order to confirm this impression the effect of chlorpromazine on pressor responses to adrenaline and noradrenaline was studied in rabbits. Moreover, the rabbit blood pressure was particularly suitable for testing the adrenergic blocking action of chlorpromazine against adrenaline pressor effect,as in this species adrenaline does not produce a vasodilator effect.

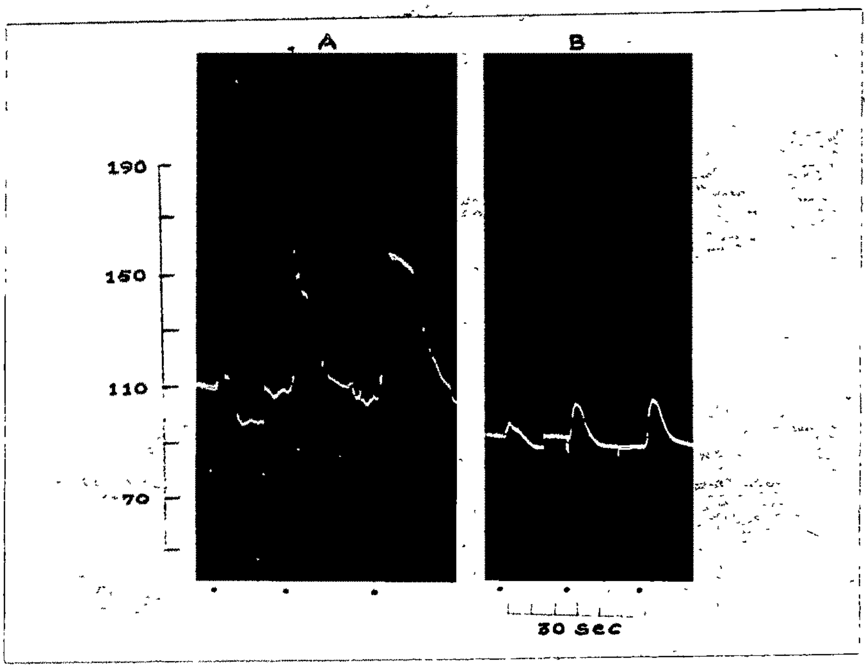
Dose-response lines for adrenaline (10 µg/kg, 20 µg/kg and 40 µg/kg) and noradrenaline (10 µg/kg, 20 µg/kg and 40 µg/kg) pressor effect were constructed before and after chlorpromazine (1 mg/kg). After the administration of chlorpromazine the dose-response lines were shifted to the right and the dose ratios for adrenaline and noradrenaline were 22.39 and 25.12 respectively. Thus it can be seen that in this species chlorpromazine antagonizes the pressor effects of adrenaline and noradrenaline more or less to the same extent.

Figures 13 and 14 show the blocking action of chlorpromazine (1 mg/ kg) against adrenaline and noradrenaline respectively.

LEGEND TO FIG. 13.

Rabbit 1.5 kg. Urethane (1.5 g/kg) anaesthesia subcutaneously. Record of carotid blood pressure. Response to increasing doses (10 μg/kg, 20 μg/kg and 40 μg/kg) of adrenaline before (in A) and after 1 mg/kg of chlorpromazine (in B). Time 30 sec. Injections were intravenous.

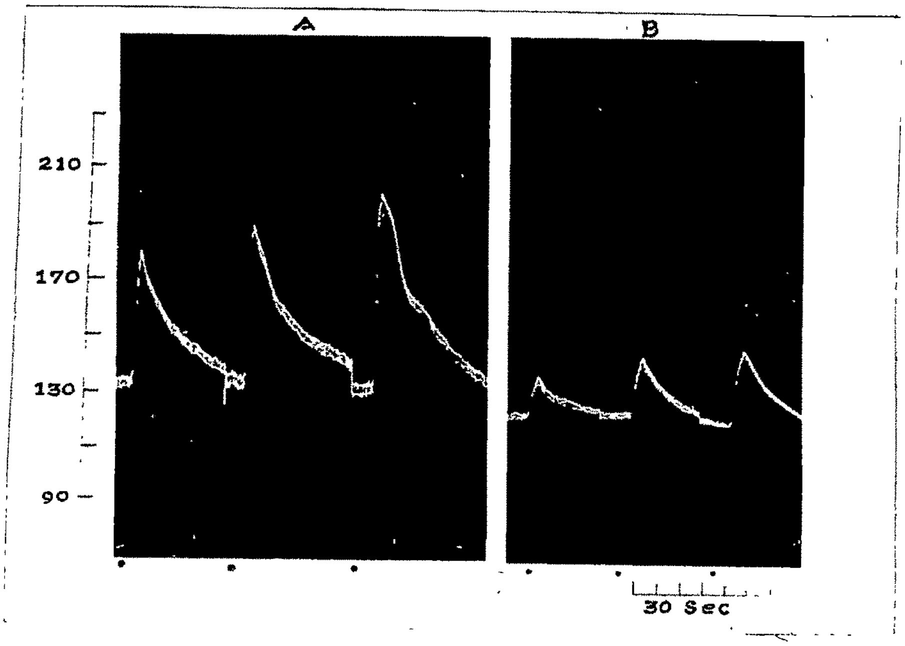
FIG:13



LEGEND TO FIG. 14.

Rabbit, 1.7 kg. Urethane (1.5 g/kg) anaesthesia subcutanenusly. Record of carotid blood pressure. Response to increasing doses (10 μg/kg, 20 μg/kg and 40 μg/kg) of noradrenaline before (in A) and after 1 mg/kg of chlorpromazine (in B). Time 30 sec. Injections were intravenous.

FIG: 14



RABBIT ISOLATED AURICLES:

Adrenaline 1×10^{-8} produced an increase in the amplitude of contractives of the rabbit auricle. Chloropromazine 1.6×10^{-11} and 3.2×10^{-11} added 10 min before adrenaline potentiated the positive inotropic action of the latter drug. Fig. 15 illustrates the finding of a representative experiment.

LEGEND TO FIG. 15.

Rabbit isolated auricle: Responses (at dots) to adrenaline (1×10^{-8}). Chlorpromazine (1.6×10^{-11}) was added at arrow 1; 3.2×10^{-11} was added at arrow 2. Contact time: 10 min.

FIG: 15