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Anti-inflammatory and some other pharmacological effects of 3,4-*trans*-2,2-dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)-phenyl]-7-methoxy-chroman (Centchroman)

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Summary

1. Anti-inflammatory, analgesic, antipyretic and other pharmacological activities of a new chroman derivative, Centchroman, have been described.

2. Centchroman was found to possess significant anti-inflammatory action in the carrageenin-induced oedema test in mice and rats. It inhibited granuloma formation in the cotton pellet test. The anti-arthritic activity was also evident in formaldehyde-induced arthritis and adjuvant-induced arthritis in rats.

3. Centchroman had a lower ulcerogenic index than phenylbutazone.

4. The mechanism of the anti-inflammatory action of Centchroman seemed to be independent of endogenous adrenocortical hormones or of its weak oestrogenic activity.

5. Centchroman antagonized phenylquinone writhing and bradykinin-induced bronchospasm but was devoid of any antipyretic activity.

Introduction

The post-coital anti-fertility activity of 3,4-*trans*-2,2-dimethyl-3-phenyl-4-[*p*-(*β*-pyrrolidinoethoxy)-phenyl]-7-methoxy-chroman (Centhroman, Fig. 1) was described by Kamboj, Chandra, Kar, Ray, Grover & Anand (1971) of our Institute. The compound was found to possess weak oestrogenic (half that of oestrone), anti-oestrogenic and anti-progestational properties (Kamboj, Chandra, Kar, Ray, Grover & Anand, personal communication). It was subjected to detailed pharmacological examination and was found to possess potent anti-inflammatory activity. This has been investigated in detail. A brief report of part of this work has appeared (Srimal & Dhawan, 1972).

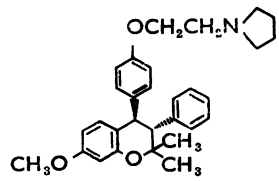

FIG. 1. Structure of Centchroman.

Methods

Hydrochloride of Centchroman was used as a freshly prepared aqueous solution in all the tests.

Anti-inflammatory activity

Graded doses of Centchroman were fed to groups of 5 male albino mice (15–20 g) or rats (90–110 g) of CDRI colony. In acute tests the compound was fed one hour prior to the induction of inflammation. In chronic tests it was fed once daily for the duration of the experiment. In every experiment one group served as control (without drug treatment) and another group received a standard drug for comparison. In view of the poor activity of phenylbutazone in the carrageenin oedema test in mice (Levy, 1969) cortisone was used as the standard compound in this test in mice and rats, whereas phenylbutazone was the standard compound in all other tests. Per cent inhibition compared with the control group was calculated and wherever possible the dose inhibiting inflammation by 50% (ED_{50}) was determined graphically. Standard error of the mean (S.E.) was calculated for each group. The significance of drug-induced changes was assessed by Student's *t* test wherever necessary.

Carrageenin-induced oedema in mice. Oedema was induced in the left paw of mice by a subplanter injection of 0·025 ml of 1·0% carrageenin solution in 0·9% w/v NaCl solution (saline) (Srimal & Dhawan, 1971). Both the paws were cut identically at the ankle joint after killing the mice by ether 4 h later. The oedema which developed in each mouse was calculated by subtracting the weight of the control limb from the weight of the injected limb.

Carrageenin-induced oedema in rats. The technique of Winter, Risley & Nuss (1962) was employed using 0·1 ml of 1·0% carrageenin solution in saline. The volume of the paw was measured plethysmographically (Harris & Spencer, 1962) immediately and 4 h after the injection of the irritant.

Formaldehyde-induced arthritis in rats. Arthritis in the left hind paw of rats was induced by subplanter injection of 0·1 ml of 2·0% formaldehyde solution on the first and the third day of the experiment (Selye, 1949). The volume of the paw was measured before the injection of the irritant and once daily for the succeeding 10 days. The mean increase in the paw volume of each group after 10 days was calculated and the inhibition compared with the control group was plotted as percentage.

Cotton pellet test in rats. The autoclaved cotton pellets weighing 50 ± 1 mg each were implanted under the dorsal skin of the rat by the method of Winter & Porter (1957). The rats were killed on the 7th day after which the pellets and the granulation tissue formed around them were dissected out. They were dried in an oven at 60° C to a constant weight.

Adjuvant arthritis in rats. Chronic arthritis in rats was induced by injection of 0·5 mg of killed *Mycobacterium tuberculosis* (Difco) suspended in 0·1 ml of liquid paraffin in the left paw. The volume of both the hind limbs was measured daily with a plethysmograph for 31 days.

Effect of local injection of the compound on inflammation. Oedema was produced in the left hind paw of rats by injection of 0·05 ml of 25% kaolin suspension or 0·05 ml of 1·0% carrageenin solution. In the control group 0·05 ml of the irritant was mixed with the same volume of saline before injection, while

in the treated groups the irritant was mixed with the test compound dissolved in 0·05 ml saline. The volume of the paw was measured plethysmographically immediately after and 4 h after the injection.

Effect on adrenal function. Bilateral adrenalectomy in rats was performed two days prior to the experiment by the method of Schultzer (1935). After the operation rats were given saline *ad libitum* in place of water. Acute oedema in groups of adrenalectomized as well as normal rats was induced by means of carrageenin as described above.

In another experiment Centchroman was fed to rats daily for 7 days before they were killed. Both adrenal glands were removed, freed from the surrounding fat and weighed. Ascorbic acid and cholesterol contents of the glands were estimated by the methods of Roe & Kuether (1943) and Zlatkis, Zak & Boyle (1953) respectively. The livers of these rats were also removed and ATPase activity was assayed in homogenate of pooled livers. A 10% (w/v) homogenate was prepared in 0·25 M sucrose with a Potter Elvehjem homogenizer. The reaction mixture consisted of 0·05 M Tris at pH 8·0, 1 mM ATP and 0·1 ml of 10% tissue homogenate in a final volume of 2 ml. Release of inorganic phosphorus (Pi) from ATP was measured according to the technique of Fiske & Subba Row (1925). The release of 1 μM of Pi/100 mg of tissue in 15 min at 37° C was considered as one unit of enzyme activity.

Effect on serum transaminases and white blood corpuscles. Formaldehyde arthritis was induced in rats as described above except in one group which was normal. The compounds were fed daily for 7 days and the rats were killed on the 8th day. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined by the method of Hanson (1959). One unit of the enzyme activity was equivalent to the formation of 0·047 (μg pyruvate/min)/ml.

Total and differential leucocyte counts were made from blood of each rat by the standard method.

Ulcerogenic index in rats. A modification of the method described by Thuillier, Bessin, Geffroy & Godfroid (1968) was used. Rats were fed the compound daily for 6 days, the food was withdrawn from 2 h before to 2 h after the treatment. Water was given *ad libitum*. The animals were killed on the 7th day, the stomach removed, cut along the lesser curvature and the gastric contents washed with saline. The stomach was placed on a convex surface with the mucosa on the outside. The mucosa of each stomach was examined under the low power (20×) of a dissecting microscope. The degree of single ulceration was determined for each stomach and scored according to the following scale:

Degree 0: absence of any detectable lesion.
0·5: small haemorrhagic effusion.
1·0: haemorrhagic effusion.
1·5: mucosal ulcerations of limited diffusion involving not more than $\frac{1}{3}$ of the whole surface.
2·0: mucosal ulcerations of limited diffusion involving not more than $\frac{2}{3}$ of the whole surface.
2·5: mucosal ulcerations of generalized diffusion.

3·0: deep ulcerations of limited diffusion.
3·5: deep ulcerations of generalized diffusion.
4·0: perforated ulcer.

The average degree of single ulceration (ADU) for each group was determined by adding together the degree of single ulceration (DSU) for the batch divided by the number of animals. On the basis of the percentage of rats with ulcerations (%RU) the Ulceration Index (UI) was calculated by means of the following formula:

$$\frac{(\text{ADU}) \times (\%\text{RU})}{100} = (\text{UI})$$

Antipyretic effect in rats

Rats were kept for 24 h at constant room temperature of 24° C before pyrexia was induced by subcutaneous injection of 2·0 ml of 15% yeast suspension in gum acacia. Rectal temperature of the rats was measured by means of a Tri-R Electronic thermometer. Compounds were administered when the temperature had reached its peak.

Analgesic activity in mice

Antagonism of phenylquinone writhing. The writhing syndrome in groups of 10 mice was induced by i.p. injection of 2·0 mg/kg phenylquinone dissolved in 5% ethanol, 30 min after administration of Centchroman. The mice were observed during the next 30 min for the appearance of writhing. Per cent of mice protected in each group was calculated.

Haffner's tail clip method. Mice pretreated with graded doses of the compound were tested for reaction to pain due to application of a clip at the tail base according to the technique of Bianchi & David (1960).

Antagonism of bradykinin-induced bronchospasm in guinea-pigs

Guinea-pigs (400–700 g) of either sex were anaesthetized with urethane (1·0–1·25 g/kg i.p.). A cannula with a side arm was inserted in the trachea. A fixed volume (4–6 ml) of air was delivered from a Palmer miniature respiration pump at the rate of 80/minute. Resistance of the lungs was recorded according to the technique of Konzett & Rossler (1940) through a Grass PT 5 volumetric transducer on a Grass model 7 Polygraph. Spasm of the bronchi was induced by 0·5–1·0 μg bradykinin i.v. or 2–5 μg histamine i.v. injected through a polythene cannula inserted in a jugular vein. Injections of both the spasmogens were repeated after the administration of Centchroman.

Effect on the gross behaviour and acute toxicity

Graded doses of the compound were administered to groups of 5 mice or rats. They were kept at a room temperature of $24 \pm 1°$ C and observed continuously for the next two hours. Mortality in different groups over a 24 h period was noted and LD_{50} calculated.

Effect on cardiovascular system, respiration and nictitating membrane of cat

Cats of either sex weighing 2–3 kg were anaesthetized with pentobarbitone sodium (30·0 mg/kg i.p.). Arterial blood pressure was recorded by means of a mercury manometer attached to a cannula in the carotid artery and respiration from the cannulated trachea through a Marey's tambour. Contractions of the nictitating membrane due to electrical stimulation of the preganglionic sympathetic fibres (4–5 volts, 1 ms, 10 Hz for 5 s) were recorded by means of a frontal writing lever. Responses were recorded on a kymograph. A polythene tube was inserted into a femoral vein for injection of drugs. The effect of Centchroman on the pressor response to i.v. adrenaline and depressor responses to histamine and acetylcholine was also studied.

Effect on isolated guinea-pig ileum

The action of Centchroman was examined on the response of the guinea-pig ileum suspended in 20 ml oxygenated Tyrode solution at 37° C to acetylcholine (0·01 μg/ml), histamine (0·03 μg/ml), nicotine (0·6 μg/ml) and 5-hydroxytryptamine (0·6 μg/ml).

Results

Anti-inflammatory activity

Centchroman was effective in a dose range of 20·0 to 160·0 mg/kg in all the models of inflammation and arthritis in which it was tested.

Carrageenin-induced oedema in mice. Centchroman inhibited the oedema in mice. The ED_{50} in this test was found to be 96·0 mg/kg compared with 78·0 mg/kg for cortisone as shown in Table 1.

TABLE 1. *Effects of Centchroman and cortisone on carrageenin-induced oedema in mice*

Pretreatment	Dose mg/kg	Oedema (mg) ±S.E.	% Inhibition	ED_{50} mg/kg
Nil	—	52·8±2·53	Control	
Centchroman	20	55·6±1·35	—	
	40	44·5±1·41	15·6	
	80	25·2±1·22	52·2	96·0
	160	15·6±0·85	70·3	
Nil	—	53·4±4·03	Control	
Cortisone	25	35·6±4·21	33·3	
	50	31·8±4·15	40·4	
	100	22·4±4·50	58·0	78·0

TABLE 2. *Effects of Centchroman, cortisone and oestrone on carrageenin-induced oedema in rats*

Pretreatment	Dose mg/kg	Oedema (ml) +S.E.	% Inhibition	ED_{50} mg/kg
Nil	—	1·08±0·03	Control	
Centchroman	20	0·79±0·05	26·8	
	40	0·43±0·02	60·1	
	80	0·13±0·02	87·9	36·0
Nil	—	1·15±0·05	Control	
Cortisone	20	0·73±0·08	36·5	
	40	0·61±0·08	47·0	
	80	0·40±0·01	65·2	45·0
Nil	—	0·50±0·04	Control	
Oestrone	20	0·53±0·06	—	
	40	0·45±0·07	9·3	
	80	0·46±0·08	8·0	

Carrageenin-induced oedema in rats. The compound inhibited oedema in rats in a dose range of 20·0 to 80·0 mg/kg (ED_{50} 36·0 mg/kg), while cortisone was somewhat less active (ED_{50} 45·0 mg/kg). The results in Table 2 also show that oestrone had a very weak anti-inflammatory activity.

Formaldehyde arthritis in rats. Centchroman significantly inhibited formaldehyde-induced arthritis in rats (see Fig. 2 and Table 3). At 40·0 mg/kg Centchroman and phenylbutazone produced approximately equal inhibition.

Cotton pellet test in rats. The compound inhibited granuloma formation in all the doses in which it was administered (Table 4). Phenylbutazone also inhibited granuloma formation at a dose of 20·0 mg/kg.

Adjuvant arthritis in rats. Centchroman, 20·0 (mg/kg) per day, inhibited the acute as well as the chronic phase of adjuvant arthritis as shown in Figure 3. The experiment was continued for 31 days after which time the oedema even in the control animals started decreasing.

Effect of local injection of compound on inflammation. Both Centchroman (1·5 mg) and phenylbutazone (3 mg) were ineffective, when administered locally,

TABLE 3. *Effects of Centchroman and phenylbutazone on formaldehyde-induced arthritis in rats*

Pretreatment	Dose mg/kg	Initial volume (ml) ±S.E.	Mean 10 day volume (ml) ±S.E.	Oedema (ml)	% inhibition
Nil	—	1·51±0·09	2·84±0·13	1·33	Control
Centchroman	20	1·85±0·06	2·47±0·13	0·62	53·3
	40	1·99±0·06	2·48±0·12	0·49	63·1
	80	2·00±0·05	2·31±0·22	0·31	76·6
Phenylbutazone	40	1·92±0·10	2·41±0·10	0·49	63·1

TABLE 4. *Effects of Centchroman and phenylbutazone on granuloma induced by cotton pellets in rats*

Pretreatment	Dose mg/kg	Granuloma dry weight (g) ±S.E.	% inhibition
Nil	—	0·27±0·02	Control
Centchroman	20	0·26±0·05	4
	40	0·20±0·02	23·7
	80	0·18±0·01	34·6
Phenylbutazone	20	0·24±0·03	8·9

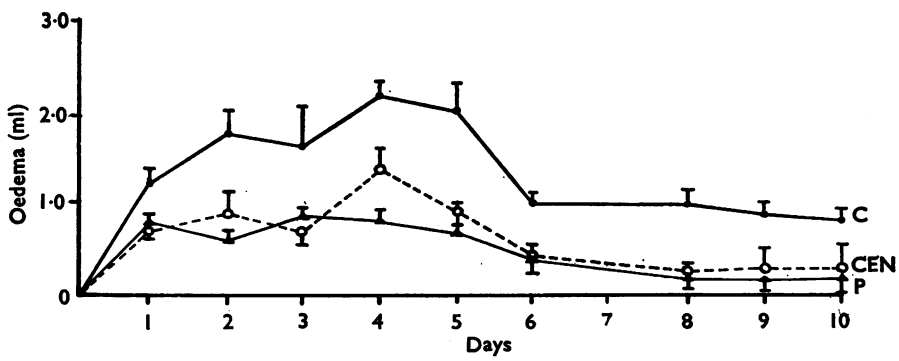

FIG. 2. Graph showing the mean oedema of left hind paw in groups of 5 rats with standard errors of mean (vertical bars). Arthritis was induced by injecting 0·1 ml of 2·0% formaldehyde solution in the left plantar aponeurosis region on days 1 and 3 of the experiment. Note reduction of the oedema by Centchroman, 40·0 mg/kg (CEN) and phenylbutazone, 40·0 mg/kg (P) as compared to the control group (C).

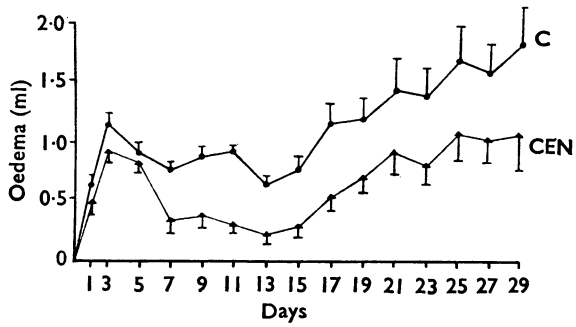

FIG. 3. Graph depicting the mean oedema developed in the left hind paw of groups of 5 rats from day 1 to day 31 of the experiment. The arthritis was induced by injection of 0·5 mg of killed *Mycobactrium tuberculosis* suspension in 0·1 ml liquid paraffin into the left planter aponeurosis on day 0. Note the significant difference in the oedema of the control group (C) and Centchroman (20·0 mg/kg) (CEN) treated groups. Standard error of the mean is shown by the vertical bars.

TABLE 5. *Effects of Centchroman on adrenal cholesterol and ascorbic acid and liver ATPase*

Pretreatment	Ascorbic acid (mg/100 g) ±S.E.	Cholesterol (g/100 g) ±S.E.	Liver, μM Pi liberated per hour	ATPase % increase
Nil	284±5·5	3·8±0·4	8·99	Control
Centchroman 40 mg/kg	283±20·0	4·3±0·3	10·40	15·7
Phenylbutazone 20 mg/kg	329±10·4	4·2±0·4	11·06	23·0

in inhibiting kaolin-induced oedema whereas carrageenin-induced oedema was reduced by 22% and 38% respectively.

Effect on adrenal function. In adrenalectomized rats Centchroman produced approximately the same degree of inhibition as in normal rats. It did not produce any depletion in adrenal cholesterol or ascorbic acid levels (see Table 5). However, the liver ATPase activity was stimulated by 15·7%. Phenylbutazone produced similar effects.

Effect on serum transaminases and white blood corpuscles. Centchroman (40 mg/kg) like phenylbutazone prevented the inflammation-induced increase in SGOT and SGPT levels. The total leucocyte count was found to increase in animals with inflammation while it was lowered by phenylbutazone. Centchroman had no significant effect on the total leucocyte count. Similarly, it had no

TABLE 6. *Effect of Centchroman and phenylbutazone on serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and white blood corpuscle (T.L.C.)*

Pretreatment	SGOT ±S.E.	SGPT +S.E.	T.L.C. per mm³ ±S.E.	Absolute eosinophilic count per mm³ ±S.E.	Absolute lymphocytic count per mm³ ±S.E.
Nil (without inflammation)	7·3±1·7	3·0±1·0	9500 ±500	158±40	7012±601
Nil (with inflammation)	19·8±0·9[a]	17·4±1·1[a]	10710 ±607	312±36	7242±659
Centchroman 40 mg/kg	7·8±0·8[b]	4·8±1·2[b]	8052 ±660	198±59	5899±454
Phenylbutazone 20 mg/kg	4·2±0·8[b]	7·2±0·9[b]	6310[a,b] ±212	100±27[b]	4275±154[a,b]

a – $P = <0·01$ compared with normal rats; b – $P = <0·01$ compared with rats with inflammation.

significant effect on the absolute eosinophilic and lymphocyte counts unlike phenylbutazone which significantly lowered both (Table 6).

Ulcerogenic index in rats. Centchroman (40 mg/kg) had a lower ulcerogenic index than phenylbutazone (20 mg/kg) (Table 7).

TABLE 7. *Effects of Centchroman and phenylbutazone on ulcerogenic index in rats*

Pretreatment	Dose (mg/kg)	Degree of single ulceration per rat 1	2	3	4	5	ADU	% RU	UI
Nil	Control	0	0	0	0·5	0·5	0·2	40	0·08
Centchroman	40	0·5	1	1	0·5	1	0·8	100	0·80
Phenylbutazone	20	1·5	3	1	1·5	1·5	1·7	100	1·70

Average degree of single ulceration (ADU) was determined by adding together the degree of single ulceration divided by the number of animals in the batch. On the basis of the per-cent of rats with ulcerations (% RU), the ulceration index (UI) was calculated (see **Methods**).

Antipyretic effect in rats

Centchroman (80 mg/kg i.p.) produced mild respiratory stimulation and pyretic rats whereas phenylbutazone (20 mg/kg) showed significant antpyretic activity.

Antagonism of phenylquinone writhing in mice

Centchroman prevented phenylquinone-induced writhing in mice and the ED_{50} for this activity was found to be 40·0 mg/kg. It did not exhibit any analgesic activity in the Haffner's technique up to a dose of 80 mg/kg.

Antagonism of bradykinin-induced bronchospasm in guinea-pigs

Centchroman (1·0 mg/kg i.v.) caused 80% reduction in the bradykinin-induced bronchospasm. Histamine-induced bronchospasm, however, remained unaffected.

Effect on gross behaviour and acute toxicity

Centchroman (80 mg/kg i.p.) produced mild respiratory stimulation and piloerection in mice. The LD_{50} by the i.p. route was 400 mg/kg. The maximum dose which could be administered orally was 1,600 mg/kg and this produced only 20% mortality in both mice and rats. The LD_{50} by the i.p. route was not determined in rats.

Effect on cardiovascular system, respiration and nictitating membrane of cat

The compound produced a dose-dependent hypotension of short duration. A dose of 1·0 mg/kg i.v. lowered the blood pressure by 20% for 5 min whereas 5·0 mg/kg i.v. lowered it by 50% for about an hour. There was transient respiratory stimulation. The adrenaline pressor response was reduced by 35% but this effect was not dose-dependent. The nictitating membrane response was inhibited by 5–10%. There was no significant change in acetylcholine and histamine depressor responses.

Effect on isolated guinea-pig ileum

The compound did not induce contraction in the isolated ileum up to a concentration of 5 μg/ml. It blocked the spasm induced by acetylcholine, histamine, nicotine and 5-hydroxytryptamine to approximately the same degree (Table 8).

TABLE 8. *Effects of Centchroman on guinea-pig isolated ileum*

Concentration of Centchroman (μg/ml)	% Inhibition of contraction induced by Acetylcholine	Histamine	Nicotine	5-Hydroxytryptamine
0·5	9	16	16	—
1·25	33	43	37	21
2·5	52	75	69	42
5·0	76	86	80	60

Discussion

Centchroman has been found to possess significant anti-inflammatory activity in acute as well as chronic models of inflammation. The potency as an anti-inflammatory agent varies in different test systems. In the carrageenin-induced oedema test, cortisone is slightly more potent than Centchroman in mice, whereas the order is reversed in rats. We have previously shown cortisone and phenylbutazone to be almost equiactive in this model in rats (Srimal & Dhawan, 1972). In chronic tests like the formaldehyde-induced arthritis and the cotton pellet test phenylbutazone and Centchroman are almost equiactive. The anti-inflammatory activity of Centchroman is further proved by the fact that it prevents inflammation-induced increase in SGOT and SGPT levels. Liver ATPase is increased by Centchroman as well as phenylbutazone. Uncoupling of oxidative phosphorylation is known to occur with many anti-inflammatory drugs (Falcone & Madison, 1959; Whitehouse & Haslam, 1962). The specific inhibition by Centchroman of bradykinin-induced spasm of the guinea-pig bronchial tree is similar to that reported with other anti-inflammatory drugs like phenylbutazone and acetylsalicylic acid (Collier & Shorley, 1960).

The compound possesses a lower ulcerogenic index (0·8) than phenylbutazone (1·7) and is, therefore, likely to cause less gastric irritation. Moreover it has a higher intraperitoneal (400 mg/kg) and oral (>1,600 mg/kg) LD_{50} than phenylbutazone (367·0 and 417·5 mg/kg respectively; Srimal, Sharma, Tangri & Dhawan, 1973). It does not, on the other hand, produce leucopaenia and eosinopaenia like that produced by phenylbutazone (Table 6).

The anti-inflammatory activity of Centchroman does not appear to be mediated via the pituitary-adrenal axis since it is not significantly altered by adrenalectomy. This is further supported by lack of effect of Centchroman on adrenal ascorbic acid and cholesterol levels and eosinophil count in the blood. The weak oestrogenic activity is also unlikely to be responsible for this action because oestrone, which has twice the oestrogenic activity of Centchroman, possesses negligible anti-inflammatory activity (Table 2). It is probable that like phenylbutazone the anti-inflammatory effect is exerted directly on the inflamed tissues. This is supported by the demonstrable anti-inflammatory effect of the compound on local administration (see above). The compound did not produce any lowering in temperature of pyretic rats but it antagonized the phenylquinone-induced writhing syndrome in mice. It thus appears to have some analgesic activity. The only other effect of Centchroman found in the present study was a non-specific inhibitory activity on the smooth muscle of the ileum. A relaxant action on the vascular smooth muscle may be responsible for the observed cardiovascular actions of the compound.

Centchroman has already undergone three months toxicity study in rats and monkeys and found to be non-toxic. Preliminary clinical pharmacological studies in human volunteers indicate that the compound is well tolerated up to a dose of 120 mg per day. Further studies are in progress.

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