Pharmacological Studies of Cernilton Cernitin GBX and Cernitin T-60

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Introduction

Mixed hormones, consisting of androgens and estrogens, are commonly used for pharmacotherapy of hypertrophy.

The drug under study here is a non-hormonic preparation developed by AB Cernelle (Sweden), called CERNILTON, which is a mixture of two components, one being the oily substance Cernitin GBX and the other the water-soluble substance Cernitin T60. The two substances, contained at a ratio of 1:20, are extracted from several kinds of pollens admixed in definite proportions, after decomposition of allergens.

The subjects covered by the present study are as follows:

1. Observation of Symptoms (mice)
2. Influence on Spontaneous Movements (mice)
3. Influence on Blood Pressure and Respiration (cats)
4. Influence on Smooth Muscles (guinea pigs)
5. Diuretic Action (rats)
6. Anti-Inflammatory Action (rats)
7. Antigen-Antibody Reactions (guinea pigs)

Materials and Methods of Experiments

1. *Observation of Symptoms*

Animals were ddN strain healthy male mice with body weight of 20—25 g, each group consisting of 10 animals. The dose given was 20 times the maximum dose in human body (60 kg) both for GBX and T60 (GBX 8 mg/kg, T60 160 mg/kg). GBX was suspended in 0.1 N NaOH and 1% Tween 80 while T60 dissolved in 0.9% NaCl. Both substances were administered intravenously to the tail at a speed of 0.5 ml/min.

Observation was made as to general symptoms for 60 minutes after administration and thereafter as to both general symptoms and death occurrences.

2. *Influence on Spontaneous Movements*

Animals, were ddN strain healthy male mice with body weight of 20—25 g, each group consisting of 10 animals. Spontaneous movements were determined by means of a revolving activity wheel with a diameter of 20 cm and a width of 5 cm, and the volume of spontaneous movements was judged by frequency of revolution. The value obtained during the 10 minutes before administration was used as the control, and the determination was carried out every 10 minutes over a period of 90 minutes after administration.

Mice with values less than 70 revolutions per minute were excluded from the experiment.

GBX was suspended in 1% Tween 80 while T60 dissolved in 0.9% NaCl to make the doses 50, 100, 200 and 500 mg/kg/10 ml. The sample drugs were given by the oral route.

3. *Influence on Blood Pressure and Respiration*

After anesthetization with Pentobarbital Na at a dose of 40 mg/kg intravenously, cats (1—3 kg) were fixed in a dorsal position and changes in blood pressure recorded on smoked paper by means of a cannula and mercury mannometer previously inserted into the carotid artery; respiration was recorded simultaneously by means of a cannula and tambour inserted into the trachea.

GBX, an acidic oily substance, was alkalized to a pH of about 8.5 with 0.1 N NaOH to make intravenous administration possible. For use of the control another 0.1 N NaOH solution with the same pH was prepared. The sample drugs were administered through a vinyl tube previously inserted into the cosal vein. ECG was taken by leading from the chest both before and after administration.

4. *Influence on Smooth Muscles*

Influence was examined by means of the Magnus method on the isolated intestine, uterus and bronchus of guinea pigs and the isolated prostate of rats.

5. *Diuretic Action*

Animals were Donryu strain healthy male rats with body weight of 120—150 g bred under controlled conditions, each group consisting of 3 animals. The animals were housed separately in metabolic cages and fasted for 18 hours, and then urination was forced by finger pressure applied to the lower abdomen just before experiment. 0.9% NaCl was administered orally at a dose of 20 ml/kg while sample drugs at doses of 50, 100 and 500 mg/kg, GBX suspended in 1% Tween 80 and T60 dissolved in 0.9% NaCl. The volume of urine voided in 3 hours after administration plus that voided compulsorily was taken as the total volume of urine.

6. *Anti-Inflammatory Action*

Studies were made by means of a method using croton oil and egg albumin as inflammation-inducing substances, combined with the filter paper-pellet method. Phenylbutazone (Irgapyrine, FB) was used as the control drug.

a. *Method using croton oil and egg albumin:* Animals were Donryu strain healthy male rats with body weight of 150—200 g bred under controlled conditions (room temperature 25 ± 1° C, humidity 55 ± 5 %), each group consisting of 6 animals. The animals were employed without anesthetization. Inflammation-inducing substances used were 1% croton oil (diluted with olive oil) and 10% egg albumin; they were given at a dose of 0.1 ml/body to the rt. heel subcutaneously to induce edema. Edema-suppressing effects were judged by the swelling rate, which was obtained from measurement of swelling with a caliper 6 times (0.5, 1, 2, 3, 5, 24 hours). The sample drugs were given orally one hour before administration of the inflammation-inducing substances.

b. *Filter-paper-pellet method:* Animals were Donryu strain healthy male rats with body weight of about 130 g bred under controlled conditions (room temperature 25 ± 1° C, humidity 55 ± 5 %), each group consisting of 6 animals. After anesthetization with ether, the hair was clipped of the lumbar area and the skin incised to a length of 1—1.5 cm slightly to the right of and along the median line. Following insertion to the back site of filter paper (Toyo Roshi No. 2 cut in roud form with weight of 3 mg) sterilized with dry heat at 120° C for 40 minutes, button suture was performed and the sample drugs administered for 4 days. The filter paper was removed 5 days after insertion and then weighed, both immediately after difference in weight was taken as the weight of the exudates and used as a guide to judgment of the degree of inflammation.

7. *Anti-gen Antibody Reaction*

a. *Sensitization by intraperitoneal administration:* Animals were divided into two groups, each consisting of 5 healthy male guinea pigs with body weight of 350—450 g bred under controlled conditions. The animals were sensitized with GBX (dissolved in 0.1 N NaOH, pH 8.5) in one group and with T60 (dissolved in 0.9% NaCl) in the other at a dose of 50 mg/body every other day 3 times in all. Three weeks after the last administration, the sample drugs were administered to a dose of 10 mg/body via the penis vein, and observation was made as to symptoms and death occurrences.

b. *Sensitization by oral administration:* As CERNILTON is given by the oral route in clinical use; sensitization was carried out through this route. Animal groups were set up in the same manner as in the case of intraperitoneal administration. Administration was carried out with T60 only, which was given at doses of 1 and 2 g/body every other day 3 times in all. Three weeks after the last administration T60 was administered via the penis vein at a dose of 30 mg/body, and observation was made as to symptoms and death occurrences.

Results of Experiments

1. *Observation of Symptoms (mice)*

a. *GBX Administration Group:* No abnormal symptoms or death occurred In the 0.1 N NaOH suspension group or in the 1% Tween 80 suspension group.

b. *T60 Administration Group:* As in the GBX administration group, abnormal symptoms or death occurred in no cases.

2. *Influence on Spontaneous Movements (mice)*

Since mice would normally show very unstable reactions in the immediate post-administration phase, only the values obtained after a lapse of 5 minutes were employed for evaluation. Value so obtained were expressed in percentage, with the control value (obtained before administration) taken as 100.

a. *GBX Administration Groups (Fig. 1):* Spontaneous movements were decreased by 25% at 15 minutes in the control group (1% Tween 80 10 ml/kg), decreasing further with lapse of time. With administration of GBX at doses of 50 and 100 mg/kg, the movements were decreased by 20—25% at 15 minutes, thereafter following a course similar to that of the control group. The 200 and 500 mg/kg groups, too, showed a similar tendency, and there was no group which registered significantly less suppression than the control group. Abnormal behavior or side-effects were not noted.

b. T60 Administration Groups (Fig. 2): Spontaneous movements were decreased by 40% at 15 minutes and by 70% at 75 minutes in the control group receiving 0.9% NaCl at a dose of 10 ml/kg. With administration of T60 at doses of 50, 200 and 500 mg/kg, the movements were decreased by 30—40 % at 15 minutes, decreasing further with lapse of time. At a dose of 100 mg/kg, the suppression was marked in the beginning as compared with the 200 and 500 mg/kg groups, but then showed a recovering tendency after 75 minutes. Generally speaking, the degree of suppression was of the same between the T60 and control groups or slightly lower in the former. No abnormal behavior or side-effects were observed.

3. *Influence on Blood Pressure and Respiration (cats)*

a. *Influence of Solvent 0.1 N NaOH (Figs. 3, 4):* With intravenous administration of 0.1 N NaOH (pH 8.5) at doses of 0.1, 0.3 and 0.5 ml/kg, the blood pressure was lowered by 1.5, 2.6 and 8.4%, respectively, after a temporary rise. The lowering, however, was also temporary. As to respiration, only slight excitement was noted in 3 of 6 cases of the 0.5 ml/kg groups. Changes in ECG were insignificant.

b. *Influence of GBX Figs. 5, 6, 7, 12):* With administration of GBX at doses of 0.1, 0.3, 0.5 and 1 mg/kg, the blood pressure was only slightly lowered (1—3%) after a temporary rise (1—3%), with no significant changes in respiration of ECG. At doses of 3, 5, 10 and 20

mg/kg, the pressure was lowered by 6, 8, 14 and 24%, respectively, after a slight temporary rise (average 0.6—2.4%), the duration being proportionate to dosage, about 5 minutes in case of the 20 mg/kg group. Influence on respiration was insignificant at doses of 3 and 5 mg/kg. At a dose of 10 mg/kg 2 of 5 cases showed a slight degree of excited respiration, while at a dose of 20 mg/kg all cases showed slight to moderate degrees of excited respiration. Electrocardiographically, only the 20 mg/kg group showed an increased heart rate (4.3%) immediately after administration. This occurred only in one of 5 cases; in the other 4 cases the rate remained unchanged or was decreased (average 5.7%).

c. *Influence of T60 (Figs. 8, 9, 10, 11, 12):* Influence was stronger with T60 than with GBX both on blood pressure and respiration. With intravenous administration in doses of 0.1, 0.3, 0.5, 1, 3, 5, 10, 20, 30 and 50 mg/kg, the blood pressure was transiently raised (0.5—5.6%), with degrees not necessarily proportionate to dosage. Subsequently, however, the pressure was lowered by 13.1, 17.5, 34.1, 43.4, 49.5, 55.3, 56.4, 62.1, 69.5 and 65.2%, showing a dose-response correlation. The lowering effect was only transient at doses of 0.1—3 mg/kg, but at higher doses (5, 10, 20, 30, 50 mg/kg) the effect was lasting, about 5 minutes at doses of 30 and 50 mg/kg, and the pressure was lowering proportionally to dosage. Effects on respiration were practically nil or extremely slight at doses of 0.1—0.5 mg/kg, but at doses of 1—20 mg/kg an excited respiration proportionate to dosage was noted. At still higher doses (30 and 50 mg/kg) the degree of excitement ranged from moderate to intense, though death due to dyspnea occurred in no cases.

Electrocardiographically, no appreciable changes were noted at doses of 5 mg/kg and below, while at doses of 10, 20, 30 and 50 mg/kg the heart rate was shown to be unsteady in the immediate post-administration phase, decreasing in some cases (2 cases, average 58%) and increasing in others (3 cases, average 19%). Changes in wave shapes were not significant.

4. *Influence on Smooth Muscles*

1) *Influence on Isolated Intestine, Uterus and Bronchus (guinea pigs)*

a. *Influence of GBX:* Though only slightly, spontaneous movements were enhanced in the smooth muscles of the intestine and uterus at concentrations of 10^{-4} g/ml and higher (final concentrations). Spastic action was not noted.

b. Influence of T60 (Figs. 13, 14, 15): At a concentration of 10^{-5} g/ml a slight degree of spasm was noted in the intestine and uterus, while at cocentrations of 10^{-4} g/l and higher a definite spastic action was noted. On the other hand, rise in tonus of the bronchial muscle was observed at a concentration of 10^{-3} g/ml.

2) *Influence on Isolated Prostate (rats)*

a. *Influence of GBX (Fig. 16):* Influence on the prostate was not revealed at all at concentrations of 10^{-3} g/ml and lower.

b. *Influence of T60 (Fig. 16):* At concentrations of 10^{-4} g/ml and lower, T60 exerted no spastic action on the prostate; but the action was noted at a concentration of 10^{-3} g/ml the degree being about the same as those observed with Ach 10^{-7}—10^{-6} g/ml and $BaCl_2$ 2 X 10^{-4}.

5. *Diuretic Action (rats)*

Sample drugs and 0.9% NaCl were given by mouth and observation was made as to the volume of urine excreted.

a. *Influence of GBX (Table 1):* With oral administration of 1% Tween 80 at a dose of 5 ml/kg (control), the urinary volume at 3 hours was 1.46 ml on the average. With GBX at doses of 50, 100 and 500 mg/kg the volume (1.43, 1.40, and 1.26 ml) was slightly lower than the control and tended to decrease as the dosage was increased. Hence, GBX exerts no diuretic action.

b. *Influence of T60 (Table 1):* With oral administration of 0.9% NaCl (control), the urinary volume at 3 hours was 1.28 on the average. At doses of T60 50, 100, and 500 mg/kg the volume was 1.15, 1.35 and 0.70 ml, with no diuretic action.

6. Anti-Inflammatory Action (rats)

1) *Effects on Croton Oil-Induced Edema*

a. *Croton oil-induced edema (Table 2):* Edema due to croton oil varied little in terms of swelling rate up to 5 hours. Thereafter, it increased with time, showing an increase of 50—55% at 24 hours.

b. Effects of GBX (Table 2): Suppressive effects on croton oil-induced edema were not observed at all from 0.5 to 2 hours at oral doses of GBX 100, 200 and 500 mg/kg. At doses of 100 and 500 mg/kg the swelling rate was increased with time, whereas at a dose of 200 mg/kg the rate was decreased by 6, 7 and 12% at 3, 5 an 24 hours over the control, with significant difference at a risk rate of 5% at 24 hours (Table 10).

c. *Effects of T60 (Table 3):* Suppressive effects on croton oil-induced edema were not noted at all with T60 at a dose of 100 mg/kg. At doses of 200 and 500 mg/kg edema was suppressed by 14 and 7% at 0.5 hour, 12 and 9% at 1 hour, 6 and 4% at 3 hours, 6 and 2% at 5 hours, and 7% at 24 hours (500 mg/kg group only) over the control, with significant difference at a risk rate of 5% between the 200 mg/kg and control groups at 0.5 and 1 hour (Table 10).

2) *Effects of Albumin-Induced Edema*

a. *Effects of GBX (Table 4):* Albumin-induced edema was suppressed by 9% at 5 hours at a dose of 100 mg/kg and by 11% at 0.5 hour at a dose of 500 mg/kg over the control, but no significant difference was noted at either dose. At a dose of 200 mg/kg suppressive effects were scarcely noted up to 5 hours. On the other hand, at 24 hours, all groups showed a suppression of 9—10% with significant difference at a risk rate of 1% against the control (Table 10).

b. *Effects of T60 (Table 5):* With administration of T60 at doses of 100, 200 and 500 mg/kg, the swelling rate tended to increase with dosage. Hence, T60 exerts no suppressive action on albumin-induced edema.

3) *Effects Observed by Means of Filter-Paper-Pellet Method*

a. *Effects of GBX (Table 6):* With oral administration of 1% Tween 80 at a dose of 5 ml/kg (control), the weight of granuloma was 163.2 mg on the average. With administration of GBX, the weight tended to decrease as the dosage was increased, the values being 83.1, 80.7 and 74.9 mg for the 100, 200 and 500 mg/kg groups, respectively, or 50.9, 49.4 and 45.9%, taking the control value as 100. No significant difference, however, was noted between these and the control groups at a risk rate of 5%.

The average weight of dry granuloma (150°C, 40 minutes) was 20.0, 10.8, 13.8 and 12.6 mg for the control, 100, 200 and 500 mg/kg groups, respectively. Expressed in percentage, the values were 100, 54.0, 69.0 and 63.0%, with the control taken as 100.

b. *Effects of T60 (Table 7):* The average weight of granuloma on oral administration of 0.9% NaCl at a dose of 5 ml/kg (control) was 139.2 mg. On the other hand, with administration of T60 at doses of 50, 100, 200 and 500 mg/kg, the weight was 73.3, 40.2, 46.3 and 73.8 mg, or 52.7, 28.9, 33.3 and 53.0%, taking the control as 100. While the difference was insignificant between the control and the 50 and 500 mg/kg groups, it was significant at a risk of 5% between the control and the 100 and 200 mg/kg groups (Table 11).

The weight of dry granuloma was 20.5 mg for the control and 11.0, 6.5, 9.1 and 12.1 mg for the 50, 100 and 500 mg/kg groups, i.e. 53.7, 31.7, 44, 4 and 59.0%, taking the control as 100. The values were relatively low in the 100 and 200 mg/kg groups.

c. *Effects of GBX + T60* (Table 8): GBX and T60 were mixed at a ratio of 1:1 and given orally. At doses of 25 mg + 25 mg, 50 mg + 50 mg, 100 mg + 100 mg, 200 mg + 200 mg and 500 mg + 500 mg, the average weight of granuloma was 105.2, 87.3, 77.3, 105.0 and 110.5 mg, while the control (1% Tween 80 5 ml/kg) was 163.2 mg. Taking the control as 100, the values were then 64.5, 53.5, 47.4 and 66.7%, with no significant difference between the control and experimental groups.

The average weight of dry granuloma was 20.0 mg for the control group and 16.0, 11.3, 11.5, 13.5 nd 15.6 mg for the experimental groups, or 80.0, 56.5, 57.5, 67.5 and 78.0%, taking the control as 100.

d. *Effects of Phenylbutazone (PB) (Table 9):* With oral administration of PB at doses of 100 and 200 mg, the average weight of granuloma was 86.2 mg (average of 5 cases) and 62.6 mg. Taking the control as 100 (163.2 mg: average of 6 cases), the values were then 52.8 and 38.4, respectively, with significant difference at a risk rate of 5% (Table 10).

The average weight of dry granuloma, on the other hand, was 20.0 mg for the control group and 17.6 and 20.3 mg for the PB 100 and 200 mg/kg groups, or 88.0 and 101.5%, respectively.

The anti-inflammatory effect was approximately of the same degree between the T60 and GBX T60 groups and the PB groups, or slightly higher in the former groups. Toxicity was higher with PB. With PB at doses of 100 and 200 mg/kg, death occurred in one out of 6 cases in each dose group, while with GBX, T60 and GBX + T60 there were no such occurrences.

e. *Weight of exudates (Table 11, Fig. 17):* The difference in weight between granuloma and dry granuloma was taken as the weight of exudate and used as a guide to judgment of anti-inflammatory effects.

With GBX at doses of 200 and 500 mg/kg, the values were lower than the control, though the difference was insignificant. With T60 at doses of 200 and 500 mg/kg and PB at a dose of 200 mg/kg, the difference was significant at a risk of 10% against the control.

7. *Antigen-Antibody Reactions (guinea pigs)*

1) *Sensitization by Intraperitoneal Route*

a. *Sensitization with GBX:* Animals were allowed to assume free positions immediately after provocative administration, but not abnormal symptoms were observed and death occurred in no cases even after 24 hours.

b. *Sensitization with T60:* One out of 5 cases died on the 18th day after commencement of the experiment, and therefore observation was made only in 4 cases. All 4 cases showed intermittent coughing 1—4 times from about one minute after provocative administration. One case developed persistent dyspnea; it gradually weakened and eventually died after 24 hours.

In case of albumin shock, death usually occurs in 2—3 minutes. Since with T60 a longer time was required, and since a sudden lowering of pressure was noted on intravenous administration of T60, it is difficult to say that the death was due to shock. Nevertheless, a slight shock symptom was clearly observed.

2) *Sensitization by Oral Route*

At doses of 1 and 2 g/body there were observed no abnormal symptoms or only slight coughing, and death occurred in no cases.

Summary

Results obtained above may be summarized as follows.

1. With intravenous administration of GBX and T60 at a dose 20 times the maximum dose in human body (60 kg), there occurred neither abnormal symptoms nor death in mice.

2. With oral administration of GBX and T60 at doses of 50, 100, 200 and 500 mg/kg, spontaneous movements, as determined in mice by means of a revolving activity wheel, showed

no suppression. Abnormal behavior was not observed, either.

3. With intravenous administration of GBX at doses of 0.1—1.0 mg/kg, the influence on blood pressure was only slight, while at doses of 3, 5, 10 and 20 mg/kg there occurred a pressure lowering proportionate to dosage (6—24%) after a transient rise (average 0.6—2.4%). The effect was lasting, about 5 minutes at a dose of 20 mg/kg. With intravenous administration of T60 at doses of 0.1, 0.3, 0.5, 1, 3, 5, 10, 20, 30 and 50 mg/kg, the blood pressure lowered, after a transient rise (average 0.5—5.6%), by 13.1, 17.5, 34.1, 43.4, 49.5, 55.3, 56.4, 62.1, 69.5 and 65.2%. There was noted a correlation between the pressure lowering and excited respiration. The effect was transient at doses of 3 mg/kg and below while lasting (proportionate to dosage) at higher doses, about 5 minutes at doses of 30 and 50 mg/kg.

4. With intravenous administration of GBX at a dose of 10 mg/kg, a slight degree of excited respiration was noted. At a dose of 20 mg/kg the degree ranged from slight to moderate.

5. With intravenous administration of T60, respiration was slightly excited at doses of 0.5 mg/kg and below. The degree, however, increased with dosage, ranging from moderate to intense at doses of 30 and 50 mg/kg, through death due to dyspnea occurred in no cases.

6. ECG changes were not marked with GBX. With T60 at doses of 10—50 mg/kg, bradycardia (58% at 50 mg/kg) or tachycardia (19% at 50 mg/kg) was noted immediately after administration. No marked changes, however, were noted in the wave shapes.

7. With GBX at a high concentration (10^{-4} g/ml), spontaneous movements of the intestine and uterus were enhanced in guinea pigs; spasms were not caused. With T60 enhanced spontaneous movements of slight spasm was noted at a concentration of 10^{-5} g/ml, and at a high concentration of 10^{-4} g/ml the tonus was definitely increased. The tonus of the bronchus was increased only at an extremely high concentration of 10^{-3} g/ml (T60). The isolated prostate of rats showed increased tonus only at a high concentration of T60 (10^{-3} g/ml).

8. At dosesof 500 mg/kg and below, oral administration of GBX and T60 exerted scarce diuretic action in rats.

9. Croton oil-induced edema was not suppressed with GBX at doses of 100, 200 and 500 mg/kg up to 5 hours after administration. Suppression was noted only at a dose of 200 mg/kg at 24 hours. T60, too, showed suppressive effects only at a dose of 200 mg/kg, with significant difference against the control at 0.5 and 1 hour.

Albumin-induced edema was suppressed at 24 hours with GBX at doses of 100, 200 and 500 mg/kg. The effect, however, was not observed with T60.

10. While by means of the filter-paper-pellet method anti-inflammatory effect was not revealed with GBX, it was noted with T60 at doses of 100 and 200 mg/kg. With GBX + T60 mixed at a ratio of 1:1, the effect was not observed.

Anti-inflammatory effect was approximately of the same degree between the T60 and GBX + T60 groups and the FB group, or slightly higher in the former groups. Toxicity was lower also in the former groups.

11. Anaphylaxis did not occur with GBX. With T60 coughing occurred in all 4 cases, one of which died of dyspnea subsequently. Sensitization by the oral route induced no specific abnormal symptoms.

Discussion

What may give rise to questions is the lowering of blood pressure and anaphylaxis seen with T60 and, possible, the action on smooth

muscles. The central depressing action was not revealed.

While diuretic action was not noted here, in another experiment the urinary volume was slightly increased after prolonged administration.

Suppressive action on croton oil-induced edema was noted with GBX and T60 at a dose of 200 mg/kg, the former at 24 hours and the latter at one hour after administration. On the other hand, suppressive action on albumin-induced edema was noted at 24 hours with GBX. By means of the filter-paper-pellet method, suppressive action was noted with T60, the degree being about the same as that with Phenylbutazone while the toxicity being much lower. This point may well be included in the mechanism of action of this drug in the treatment of prostatitis and prostatic hypertrophy since, as revealed in our earlier study on its subacute and chronic toxicity, the drug can reduce the weight of the prostate without affecting generation of sperms even at small doses.

With intravenous administration of this drug at a dose of 10 mg/kg, the blood pressure may be lowered by about 50 mm Hg, due possibly to the 1% content of potassium in T60. In practice, however, such problem would not occur since clinically the drug is administered by the oral route.

Although GBX will not induce anaphylactic shock by itself, it may cause a mild degree of anaphylaxis at a probability of 25%, if given intravenously at a dose of 10 mg/kg after sensitization with T60 subcutaneously. This danger, however, is extremely remote since the drug produces no abnormal symptoms by the oral route and furthermore it is already confirmed as having practically no antigenicity or sensitinogenicity (Kimura et al., Bacteriological Dept., Nippon Medical College, “Immuno-Serological Studies of Cernitin GBX and Cernitin T60”).

Influence on smooth muscles occurs only at high concentrations of T60 (10^{-5} g/ml and higher), which may also be due to the presence of 1% potassium in T60.

Conclusions

1. With intravenous administration of GBX and T60 at a dose of 20 times as much as the maximum dose in human body, there occurred no abnormal symptoms in mice. Neither was influence noted on spontaneous movements in mice with oral administration of GBX at a dose 63 times of T60 60 times as much as the maximum dose in human body.
2. The blood pressure was lowered proportionally to dosage after a transient rise both with GBX and T60. The degree of lowering, however, was greater with T60, by 6—24% with GBX at doses of 3—20 mg while by 13.1—69.5% with T60. The pressure was lowered for 5 minutes. Bradycardia and tachycardia were noted with T60, but ECG was not markedly changed.
3. Even on smooth muscles the influence was greater with T60. While spontaneous movements of the intestine were enhanced in guinea pigs with GBX at high concentrations, with T60 spasm occurred and the bronchial muscle and prostate increased in tonus.
4. Diuretic action was not observed in rats, though observable by prolonged administration.
5. Croton oil-induced edema was suppressed at a dose of 200 mg/kg both with GBX and T60, the former at 24 hours and the latter at one hour after administration. Suppression of albumin-induced edema was noted with GBX, while by means of the filter paper-pellet method suppressive action was noted with T60.
6. The risk of anaphylactic shock was about 25% in guinea pigs. The danger, however, is extremely remote since the drug causes no abnormal symptoms by the oral route and since immunologically it is confirmed as having practically no antigenicity or sensitinogenicty.

Table 1. Diuretic Action of GBX and T60

After Premedication with 0.9% NaCl 20 ml/kg

Sample Drugs	Body Weight	Urinary Volume 0-3 hrs.	Average
1% Tween 80 5 ml/kg P.O.	124 130 158	1.50 1.45 1.42	1.46
Cernitin GBX 50 mg/kg P.O.	130 133 113	1.40 1.45 1.45	1.43
Cernitin GBX 100 mg/kg P.O.	130 130 135	1.60 1.35 1.25	1.40
Cernitin GBX 500 mg/kg P.O.	140 121 112	1.62 1.00 1.15	1.26
0.9% NaCl 5 ml/mg P.O.	136 124 134	1.20 1.35 1.30	1.28
Cernitin T60 50 mg/kg P.O.	124 140 130	1.45 0.76 1.25	1.15
Cernitin T60 100 mg/kg P.O.	129 136 124	0.90 1.60 1.54	1.35
Cernitin T60 500 mg/kg P.O.	132 152 128	0.65 0.4 0.80	0.70

Table 2. Effects of GBX on Croton Oil – Induced Edema (rats)

Drug/Time (hr)	1% Tween 80 5 ml/kg P.O	Cernitin GBX (mg/kg P.O)		
		100	200	500
Control	4.70 ± 0.18	4.80 ± 0.18	4.79 ± 0.18	4.51 ± 0.52
0.5	6.33 ± 0.45	6.44 ± 0.42	6.43 ± 0.21	6.58 ± 0.50
1	6.33 ± 0.49	6.64 ± 0.53	6.43 ± 0.28	6.48 ± 0.71
2	6.20 ± 0.40	6.59 ± 0.56	6.45 ± 0.06	6.42 ± 0.56
3	6.48 ± 0.57	6.59 ± 0.57	6.32 ± 0.42	6.31 ± 0.37
5	6.68 ± 0.49	6.90 ± 0.60	6.47 ± 0.51	6.51 ± 0.37
24	7.29 ± 0.40	7.38 ± 0.42	6.84 ± 0.28	17.18 ± 0.41

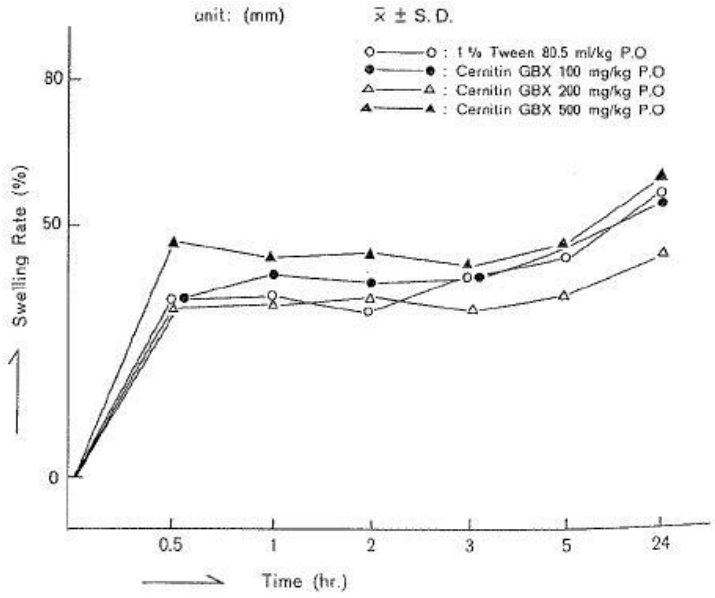


Table 3. Effects of T60 on Croton Oil – Induced Edema (rats)

Drug/Time (hr)	0.9% NaCl 5 ml/kg P.O	Cernitin T60 (mg/kg P.O)		
		100	200	500
Control	4.67 ± 0.22	4.67 ± 0.29	4.76 ± 0.15	4.60 ± 0.19
0.5	6.74 ± 0.28	6.68 ± 0.24	6.27 ± 0.47	6.33 ± 0.35
1	6.83 ± 0.36	6.74 ± 0.21	6.41 ± 0.28	6.36 ± 0.20
2	6.57 ± 0.31	6.58 ± 0.21	6.32 ± 0.27	6.20 ± 0.26
3	6.50 ± 0.29	6.48 ± 0.28	6.37 ± 0.29	6.23 ± 0.20
5	6.48 ± 0.26	6.63 ± 0.26	6.35 ± 0.34	6.34 ± 0.26
24	7.14 ± 0.49	7.10 ± 0.41	7.39 ± 0.09	6.75 ± 0.66

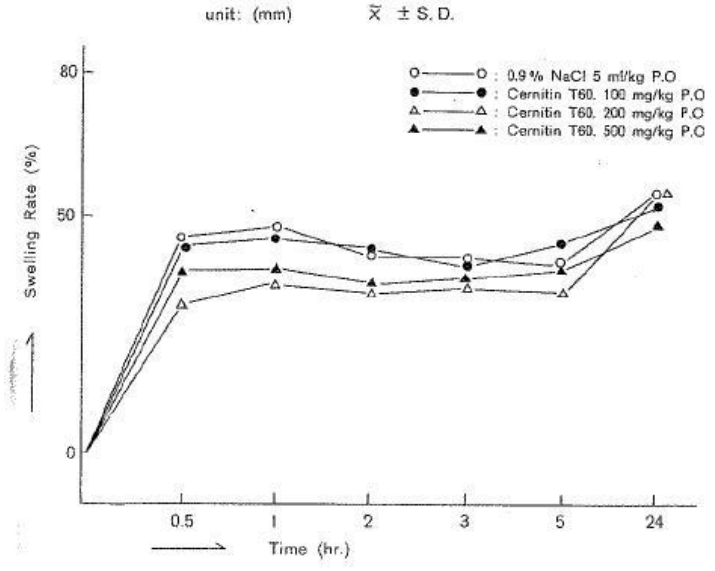


Table 4. Effects of GBX on Albumin – Induced Edema (rats)

Drug/Time (hr)	1% Tween 80 5 ml/kg P.O	Cernitin GBX (mg/kg P.O)		
		100	200	500
Control	4.55 ± 0.15	4.80 ± 0.09	4.73 ± 0.09	4.70 ± 0.28
0.5	7.21 ± 0.71	7.52 ± 0.26	7.39 ± 0.15	6.98 ± 0.89
1	7.12 ± 0.29	7.28 ± 0.34	7.27 ± 0.40	7.09 ± 0.78
2	7.08 ± 0.15	7.20 ± 0.51	7.27 ± 0.38	7.05 ± 0.55
3	7.03 ± 0.32	7.16 ± 0.39	7.25 ± 0.30	7.27 ± 0.50
5	6.96 ± 0.27	6.92 ± 0.46	7.11 ± 0.26	6.98 ± 0.61
24	5.70 ± 0.13	5.50 ± 0.25	5.46 ± 0.23	5.42 ± 0.30

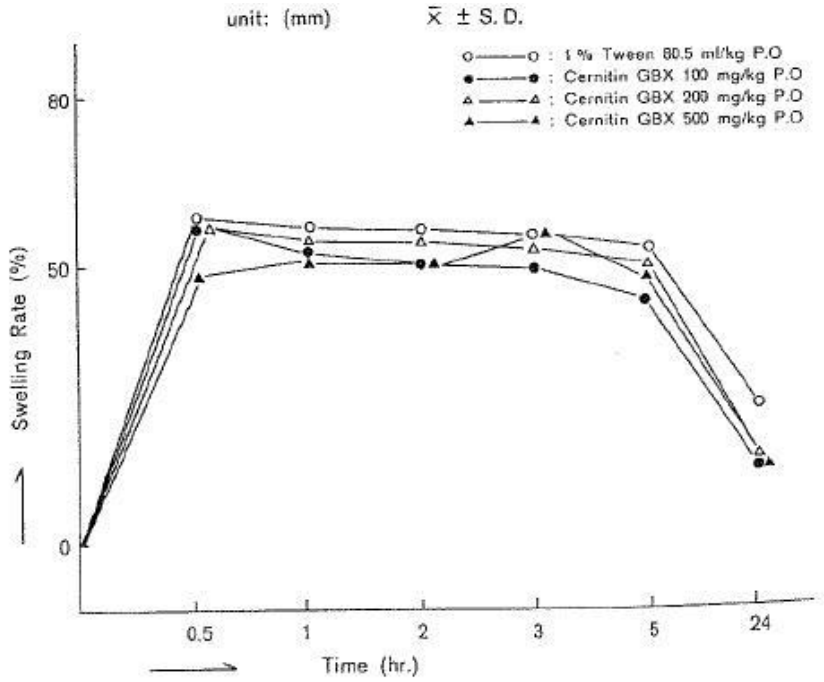


Table 5. Effects of T60 on Albumin – Induced Edema (rats)

Drug/Time (hr)	0.9% NaCl 5 ml/kg P.O	Cernitin GBX (mg/kg P.O)		
		100	200	500
Control	4.88 ± 0.26	4.74 ± 0.08	4.53 ± 0.25	4.55 ± 0.33
0.5	7.43 ± 0.52	7.24 ± 0.69	6.96 ± 0.68	7.06 ± 0.74
1	7.31 ± 0.31	7.08 ± 0.30	7.21 ± 0.43	7.26 ± 0.69
2	7.42 ± 0.34	7.07 ± 0.29	7.18 ± 0.47	7.28 ± 0.50
3	7.09 ± 0.41	7.04 ± 0.40	7.07 ± 0.55	7.18 ± 0.38
5	6.82 ± 0.32	6.76 ± 0.35	6.84 ± 0.37	6.82 ± 0.41
24	5.17 ± 0.16	5.28 ± 0.31	5.51 ± 0.42	5.71 ± 0.42

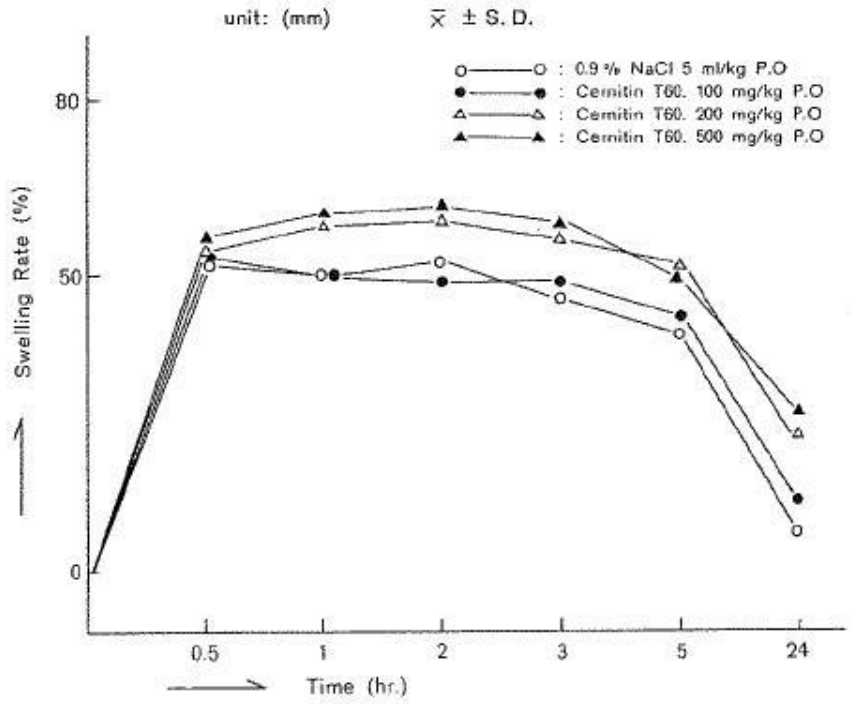


Table 6. Anti-Inflammatory Action of GBX by Means of Filter Paper-Pellet Method (rats)

NO	Granuloma				Dry Granuloma			
	1 % Tween 80 5 ml/kg P.O	Cernitin GBX (mg/kg P.O)			1% Tween 80 5 ml/kg P.O	Cernitin G BX (mg/kg P.O)		
		100	200	500		100	200	500
1	57.5	34.0	128.5	68.5	12.0	5.5	22.5	12.5
2	193.5	146.5	65.0	70.0	22.5	15.0	10.5	12.0
3	348.5	42.0	43.0	57.5	40.5	7.0	9.5	11.0
4	57.5	75.5	24.5	114.0	9.0	11.5	6.5	17.5
5	96.0	134.5	86.5	105.0	12.5	17.0	14.0	16.5
6	226.0	166.0	136.0	34.5	23.5	8.5	19.5	6.0
$\bar{X}$ ± S. E	163.2 ± 46.9	88.4 ± 19.2	80.7 ± 18.4	74.9 ± 12.2	20.0 ± 4.8	10.8 ± 1.9	13.8 ± 2.5	12.6 ± 1.7

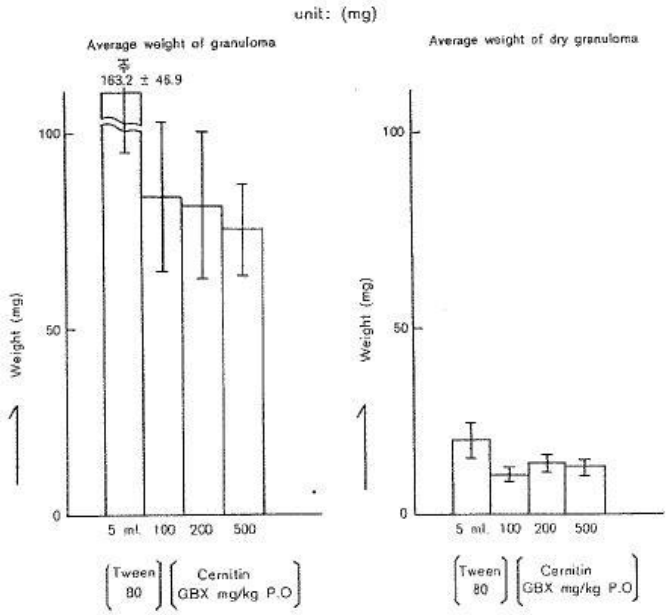


Table 7. Anti-Inflammatory Action of T60 by Means of Filter Paper-Pellet Method (rats)

NO	Granuloma					Dry Granuloma				
	0.9% NaCl 5 ml/kg P.O	Cernitin T60 (mg/kg P.O)				0.9% NaCl 5 ml/kg P.O	Cernitin T60 (mg/kg P.O)			
		50	100	200	500		50	100	200	500
1	288.5	66.0	41.0	95.5	46.0	37.0	8.5	7.0	17.0	8.5
2	77.0	63.0	31.5	31.0	73.5	11.0	11.5	5.5	7.5	12.5
3	67.0	86.0	25.0	21.0	71.0	9.5	11.5	4.5	5.0	11.5
4	299.5	73.5	44.0	51.0	63.5	47.0	11.0	6.5	10.0	11.0
5	53.5	75.0	46.0	37.5	82.0	8.5	12.0	70	7.0	12.0
6	77.5	76.5	53.5	41.5	106.5	14.5	11.5	8.5	8.0	17.0
7	111.5					16.0				
$\bar{X}$ ± S. E	139 ± 40.5	73.3 ± 3.3	40.2 ± 4.2	46.3 ± 10.7	73.8 ± 8.2	20.5 ± 5.7	11.0 ± 0.5	6.5 ± 0.6	9.1 ± 1.7	12.1 ± 1.1

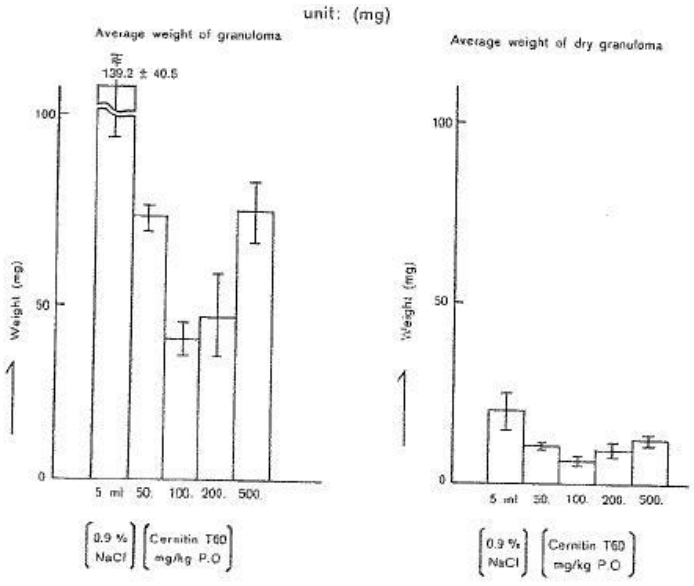
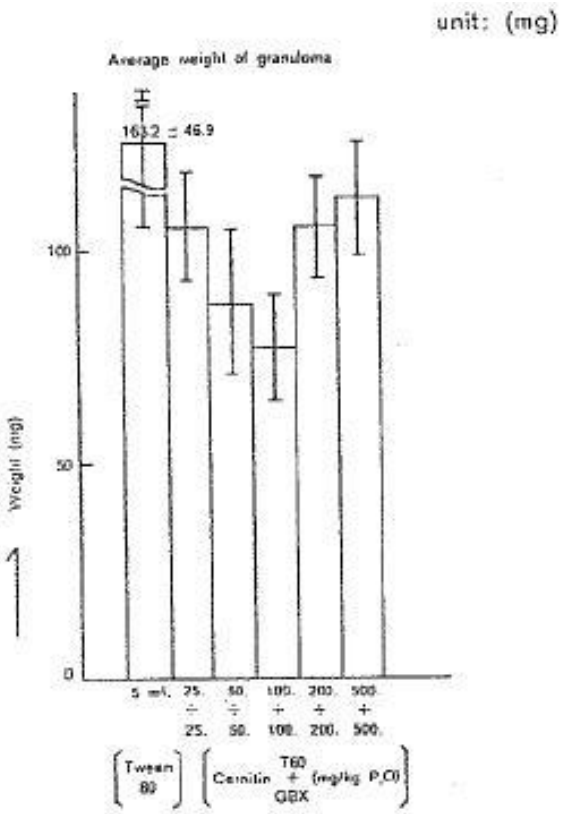


Table 8. Anti-Inflammatory Action of T60 + GBX

NO	Granuloma					
	1% Tween 80 5 ml/kg P.O	Cernitin T60 + GBX (mg/kg P.O)				
		25 + 25	50 + 50	100 + 100	200 + 200	500 + 500
1	57.5	134.0	53.0	30.0	64.0	102.0
2	193.5	64.0	57.0	64.0	76.0	49.0
3	348.5	90.0	45.5	114.5	130.5	121.0
4	57.5	111.0	139.5	97.0	116.5	106.0
5	96.0	85.0	122.5	80.5	139.0	146.0
6	226.0	147.0	106.5	77.5	104.0	139.0
$\bar{X}$ ± S. E	163.2 ± 46.9	105.2 ± 12.8	87.3 ± 16.5	77.3 ± 11.8	105.0 ± 12.2	110.5 ± 14.2



by Means of Filter Paper-Pellet Method (rats)

NO	Dry Granuloma					
	1% Tween 80 5 ml/kg P.O	Cernitin T60 + GBX (mg/kg P.O)				
		25 + 25	50 + 50	100 + 100	200 + 200	500 + 500
1	12.0	22.0	6.5	4.0	9.5	16.0
2	22.5	10.5	8.5	11.0	11.0	8.0
3	40.5	13.5	8.0	14.0	16.5	16.0
4	9.0	17.5	15.0	12.0	16.0	15.5
5	12.5	13.5	18.5	14.5	15.0	19.0
6	23.5	19.0	11.0	14.0	13.0	19.0
$\bar{X}$ ± S. E	20.0 ± 4.8	16.0 ± 1.7	11.3 ± 1.9	11.5 ± 1.6	13.5 ± 1.2	15.6 ± 1.7

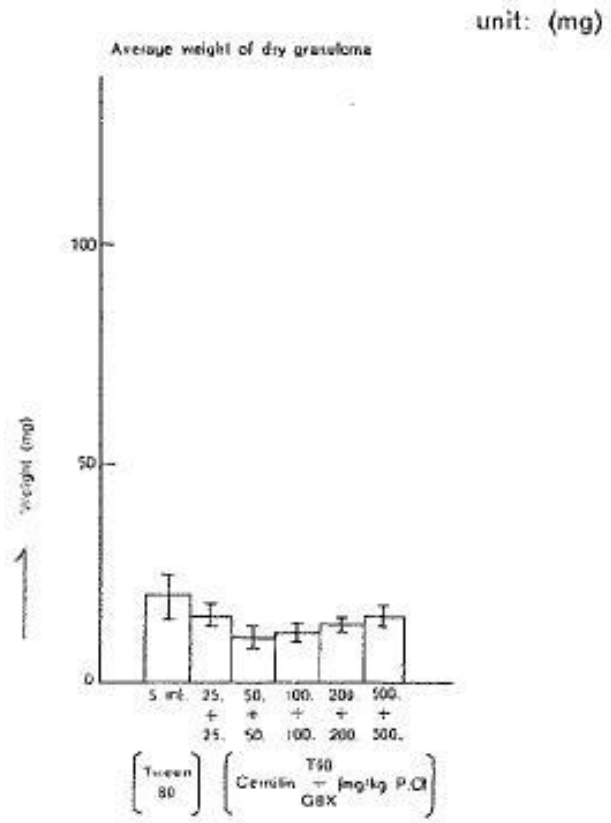


Table 9. Anti-Inflammatory Actino of Irgapyrine by Means of Filter Paper-Pellet Method (rats)

NO	Granuloma		Dry Granuloma	
	Irgapyrin mg/kg P.O		Irgapyrin mg/kg P.O	
	100	200	100	200
1	Death	64.5		13.0
2	67.0	Death	10.0	
3	160.0	59.0	34.5	16.0
4	70.0	63.0	14.5	35.0
5	85.5	72.0	14.5	17.0
6	48.5	54.5	14.5	20.5
$\bar{X}$ ± S. E	86.2 ± 19.4	62.6 ± 2.9	17.6 ± 4.3	20.3 ± 3.9

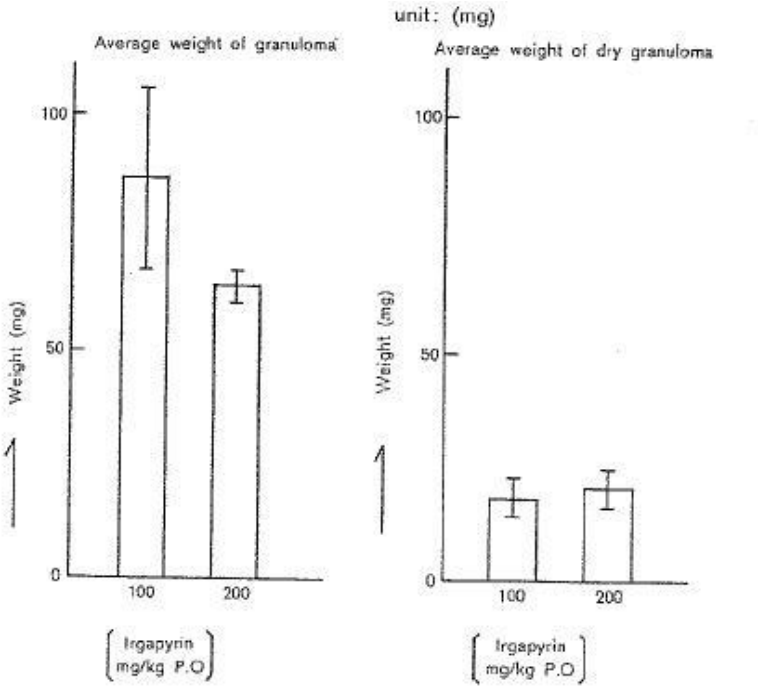


Table 10. Anti-Inflammatory Action of GBX and T60 and GBX (Summary)

Sample Drugs	Dosage (mg/kg)	Croton Oil						Egg albumin						Filter Paper-pellet method		
		0.5 h	1	2	3	5	24	0.5 h	1	2	3	5	24	5	⊟	▤
GBX	100	—	—	—	—	—	—	—	—	—	—	—	+**	—		
	200	—	—	—	—	—	+*	—	—	—	—	—	+**	—		
	500	—	—	—	—	—	—	—	—	—	—	—	+**	—		
T-60	50	—	—	—	—	—	—	—	—	—	—	—	—	—		
	100	—	—	—	—	—	—	—	—	—	—	—	—	+*		
	200	+*	+*	—	—	—	—	—	—	—	—	—	—	+*		
	500	—	—	—	—	—	—	—	—	—	—	—	—	—		
GBX + T-60	25+25													—		
	50+50													—		
	100+100													—		
	200+200													—		
	500+500													—		
Irgapyrine	100													—		
	200													+*		

— ineffective + effective +* : with significant difference against control at 0.05>p
+**: with significant difference against control at 0.01>p

Table 11. Weight of Exudate
(Granuloma minus Dry-granuloma)

Sample / Dose	Control	50 mg/kg (P.O.)	100 mg/kg (P.O.)	200 mg/kg (P.O.)	500 mg/kg (P.O.)	
GBX	143.2 ± 42.2 (100)		88.9 ± 69.5 (62.1)	66.8 ± 16.0 (46.6)	64.0 ± 8.3 (44.7)	
T-60	118.2 ± 34.9 (100)	62.3 ± 3.1 (52.5)	33.7 ± 3.7* (28.4)	37.2 ± 9.0* (31.3)	61.7 ± 7.1 (52.0)	
Irgapyrine	143.2 ± 42.2 (100)		68.6 ± 15.4 (47.9)	42.3 ± 5.1* (29.5)		
Sample / Dose	Control	25 + 25	50 + 50	100 + 100	200 + 200	500 + 500
GBX + T-60	143.2 ± 42.2 (100)	89.2 ± 11.2 (62.3)	76.1 ± 15.0 (53.1)	65.7 ± 10.5 (45.9)	91.5 ± 11.1 (64.0)	95.0 ± 12.7 (66.3)

Control: GBX, Irgapyrine, GBX + T-60 } 1 % Tween 80

T-60 : 0.9 % NaCl

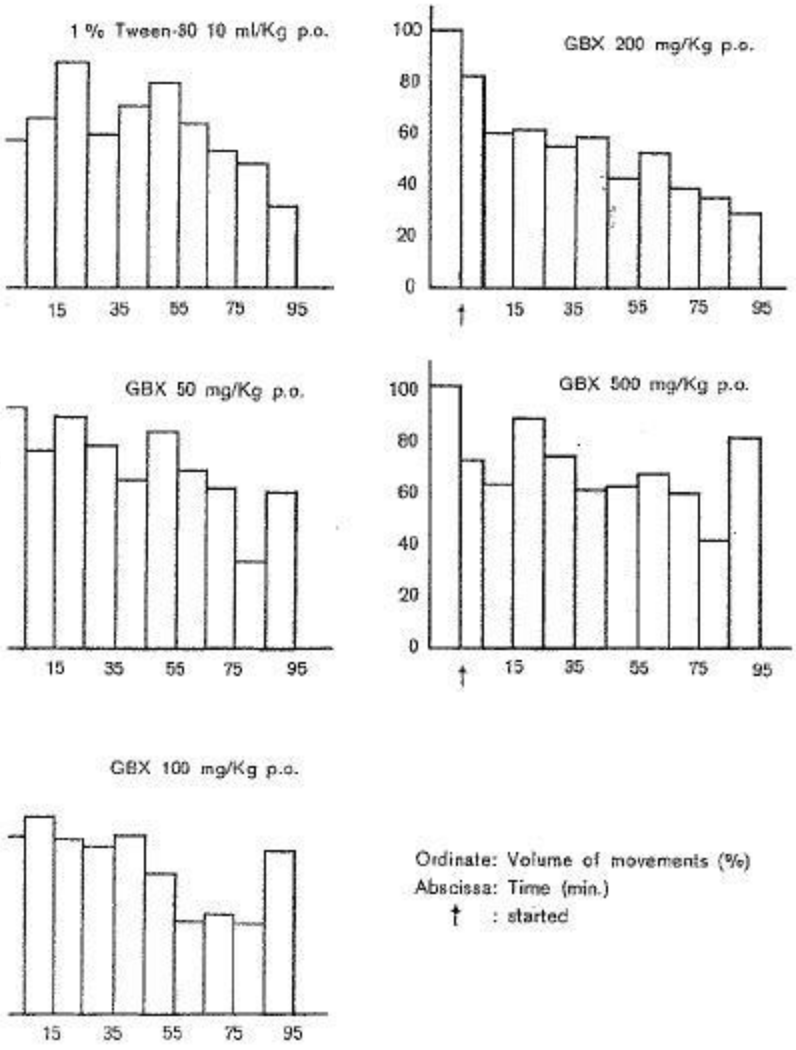
( ) : %

* : 0.1 > P (Effective)

Mean ± S.E.

Volume of Spontaneous Movements After Administration of GBX (mice)

(Before Administration = 100)



Ordinate: Volume of movements (%)
Abscissa: Time (min.)
† : started

Fig. 2. Volume of Spontaneous Movements after Administration of T60 (mice)

(Before Administration = 100)

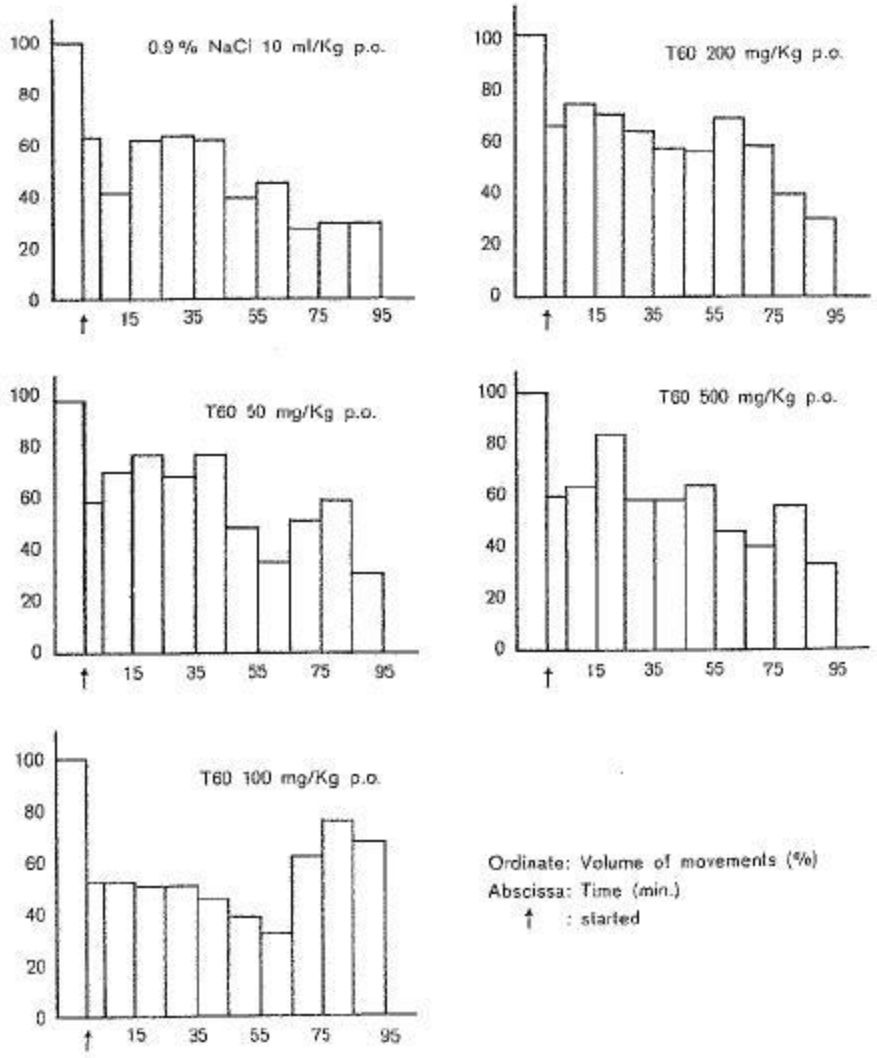


Fig. 3. Influence of Cernilton on Blood Pressure and Respiration (cat).

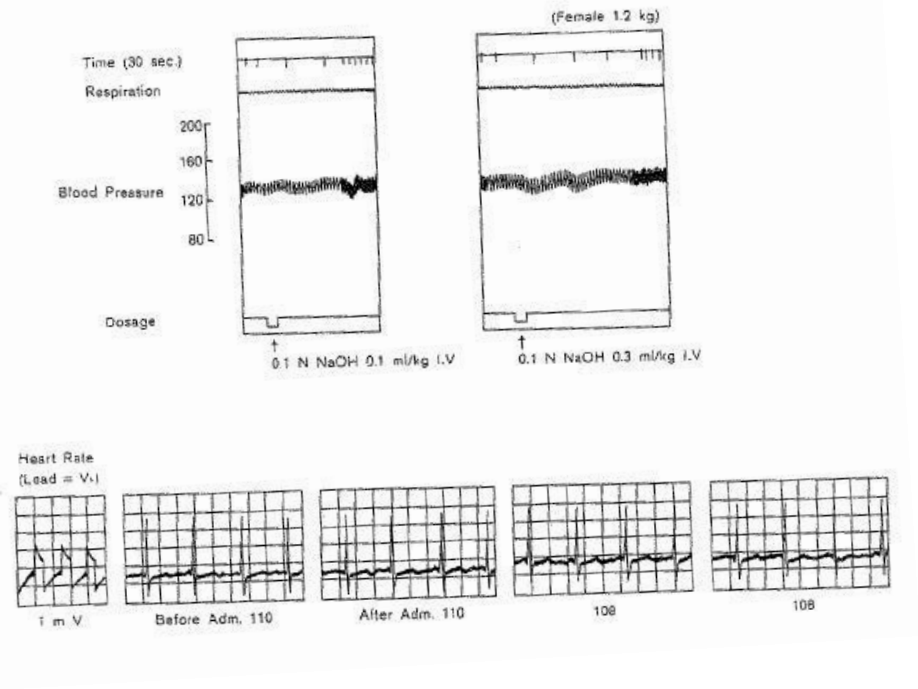


Fig. 4. Influence of Cernilton on Blood Pressure and Respiration (cat).

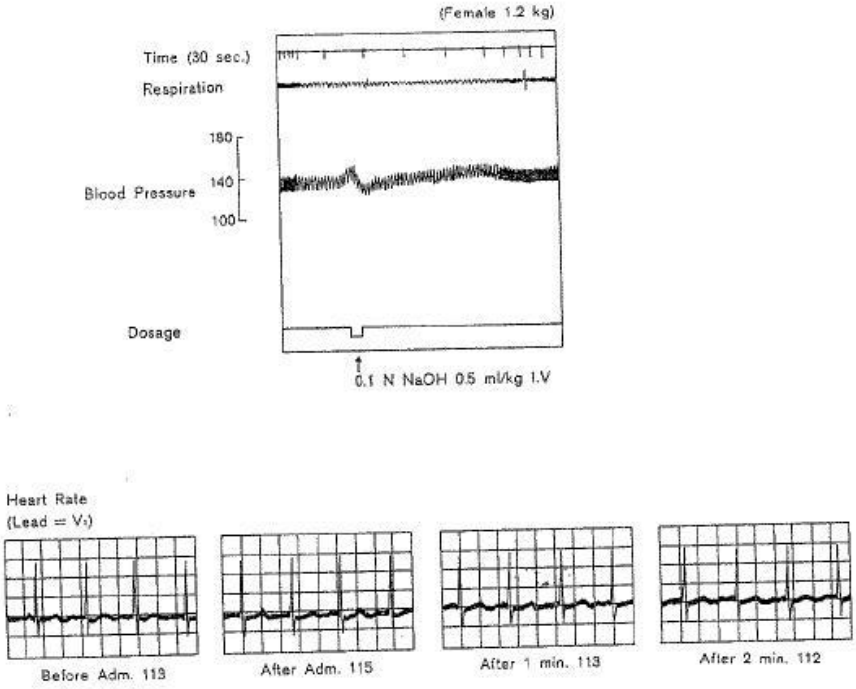
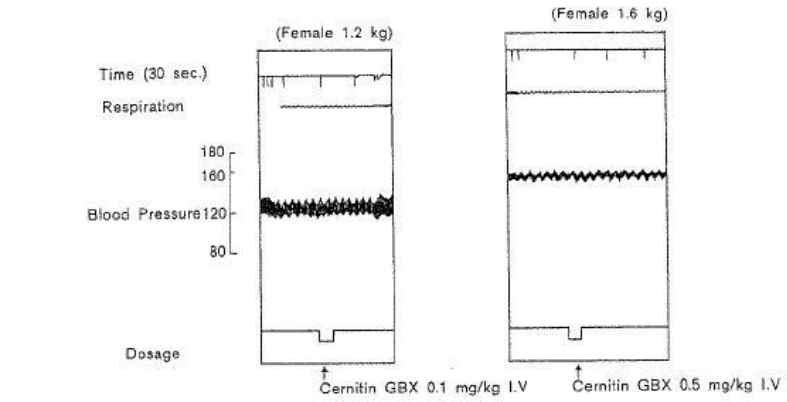


Fig. 5. Influence of Cernilton on Blood Pressure and Respiration (cat).



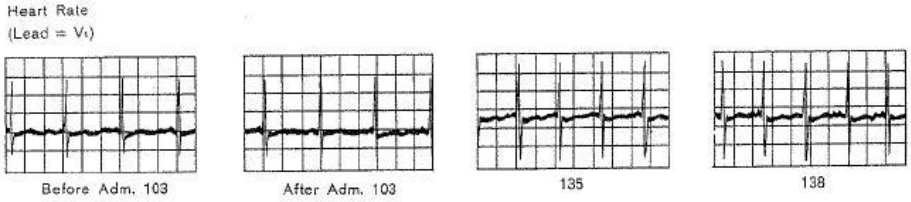
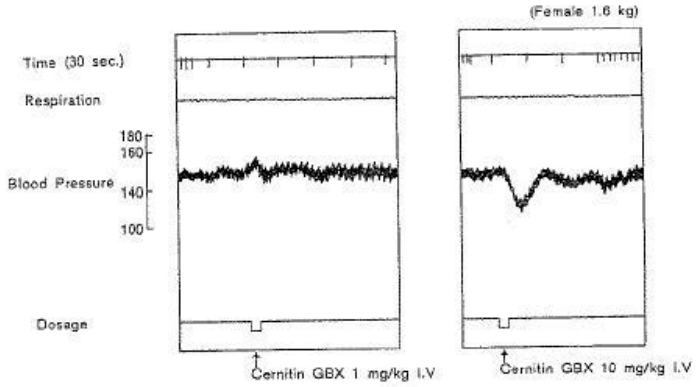


Fig. 6. Influence of Cernilton on Blood Pressure and Respiration (cat).



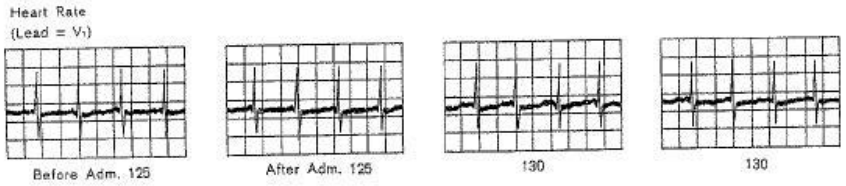


Fig. 7. Influence of Cernilton on Blood Pressure and Respiration (cat).

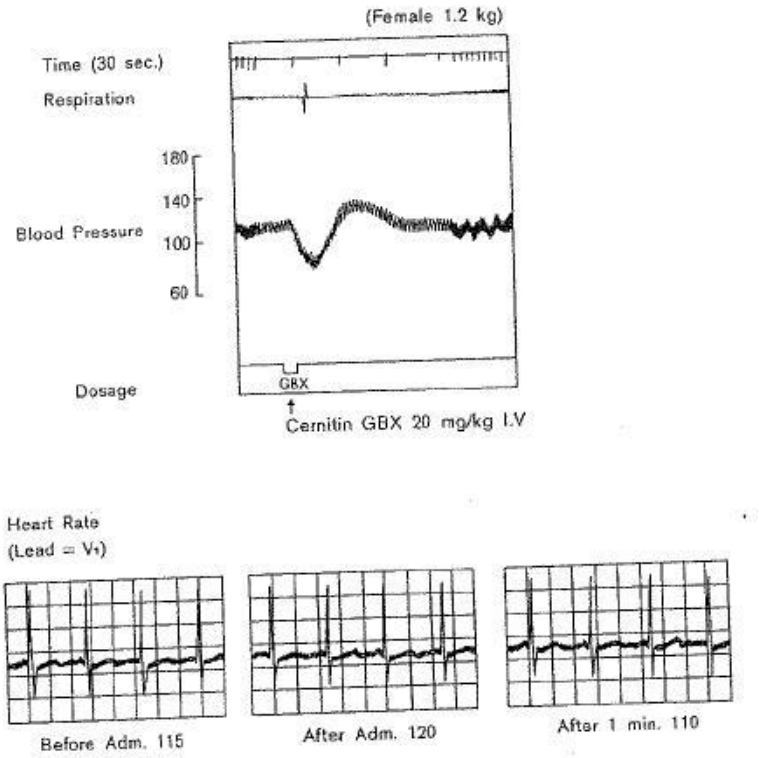


Fig. 8. Influence of Cernilton on Blood Pressure and Respiration (cat).

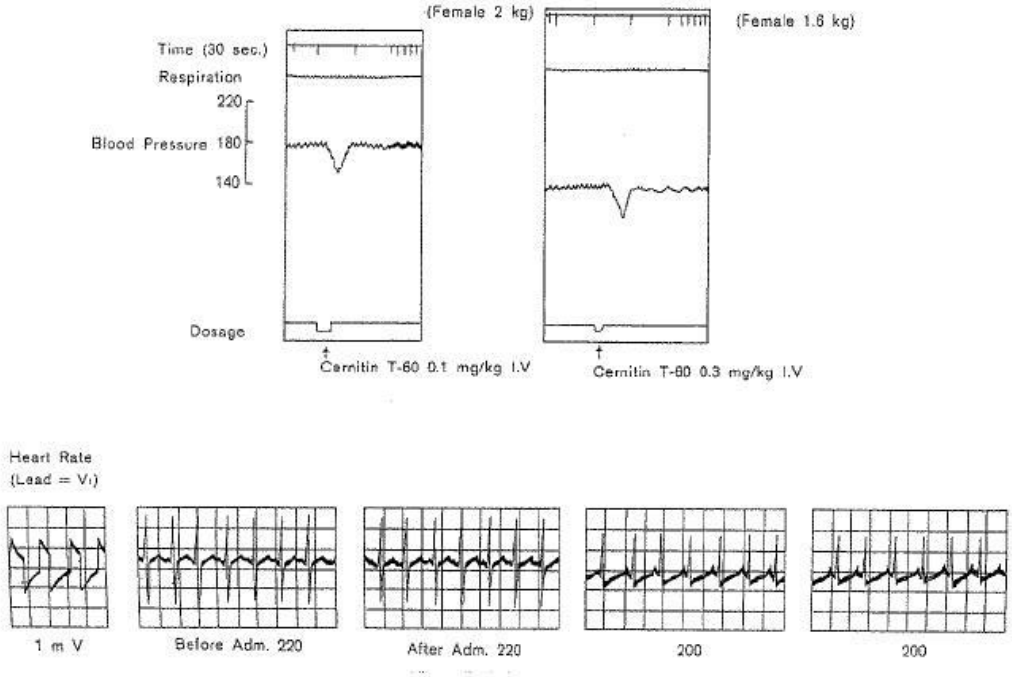


Fig. 9. Influence of Cernilton on Blood Pressure and Respiration (cat).

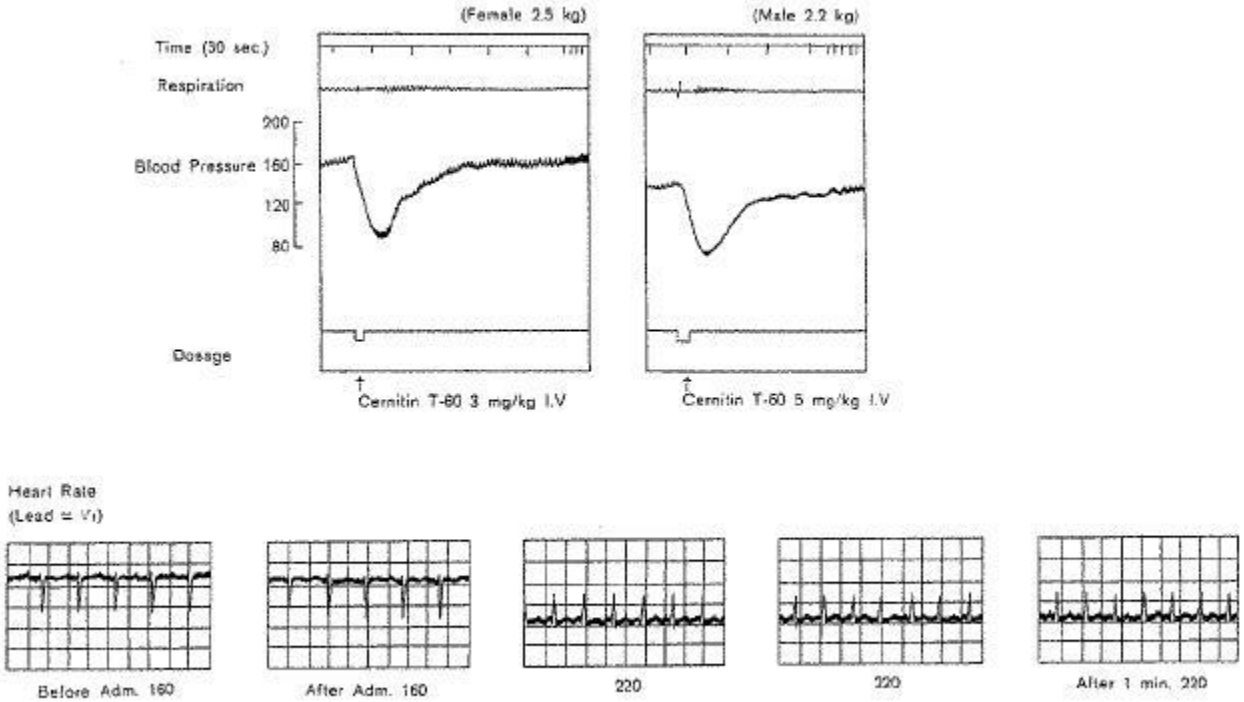


Fig. 10. Influence of Cernilton on Blood Pressure and Respiration (cat).

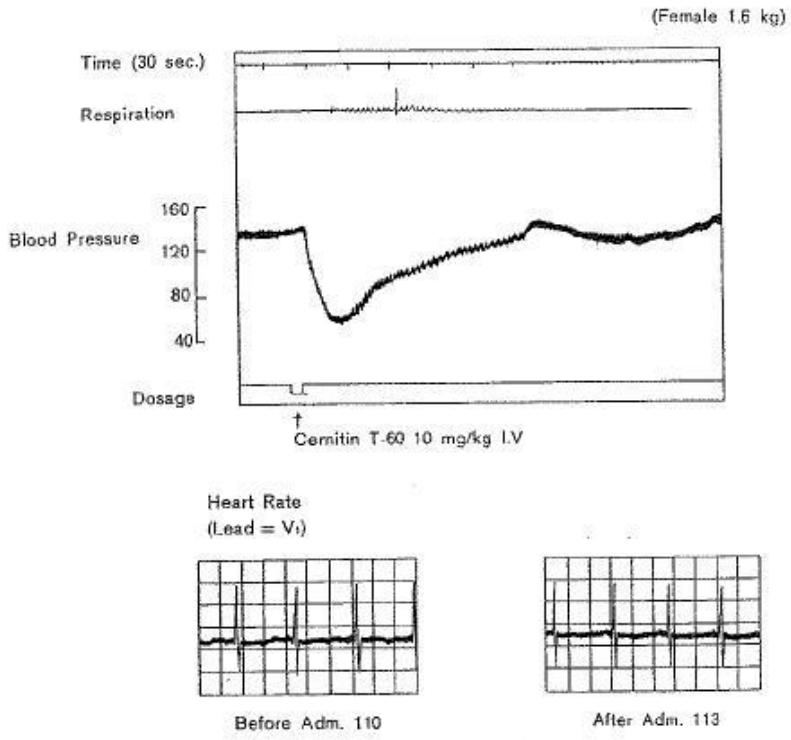


Fig. 11. Influence of Cernilton on Blood Pressure and Respiration (cat).

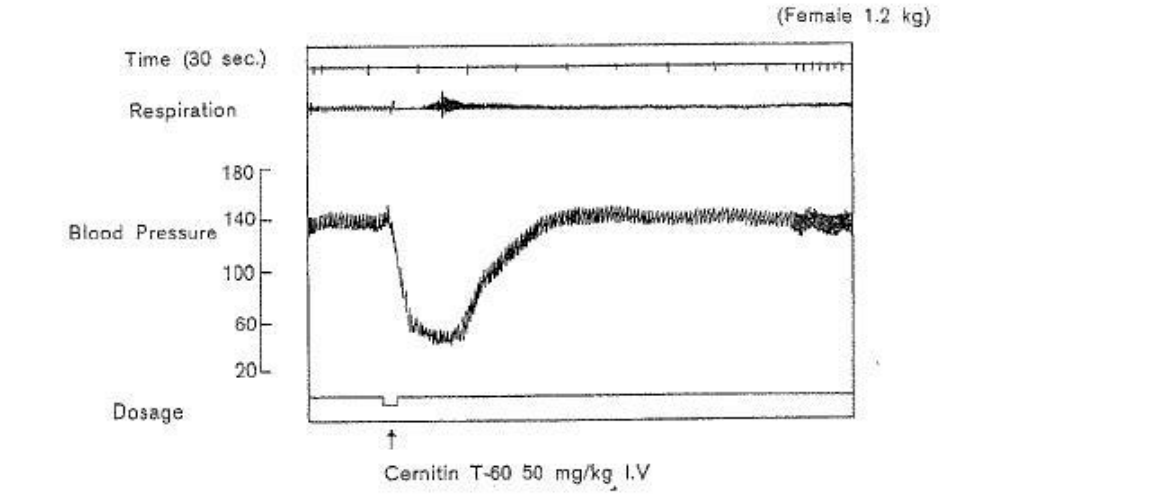


Fig. 12. Pressure Lowering Action of Cernilton

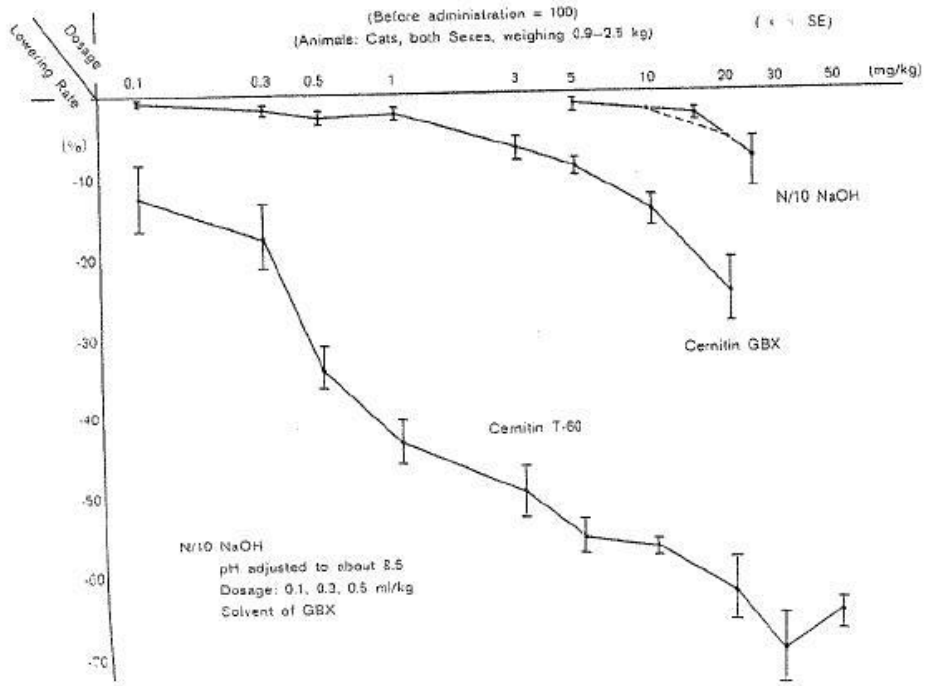


Fig. 13. Influence of T60 on Isolated Intestine (Guinea pigs)

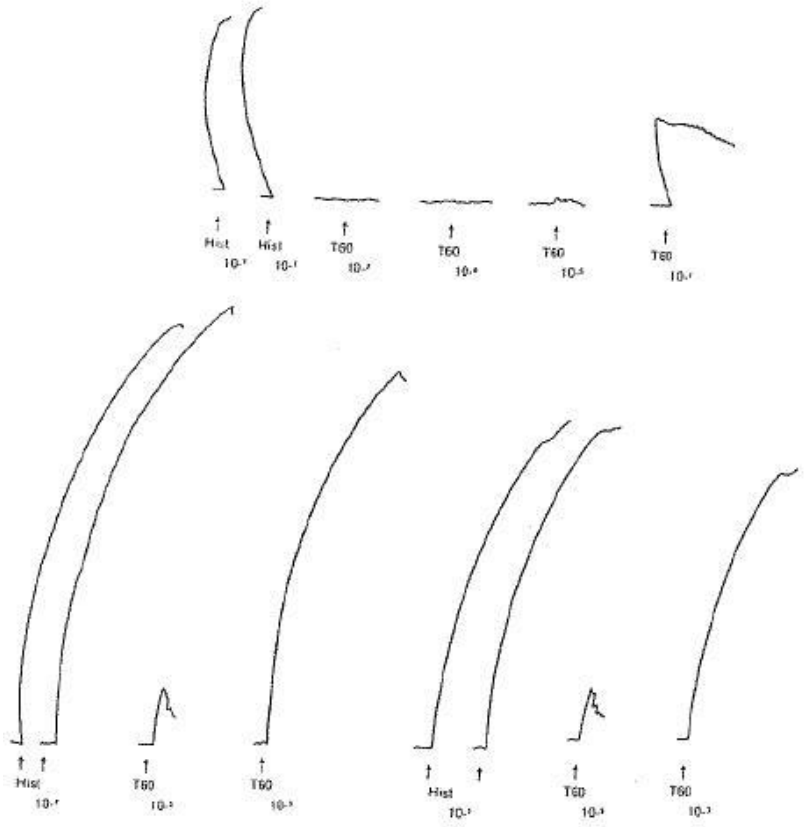


Fig. 14. Influence of T60 on Isolated Uterus (Guinea pigs)

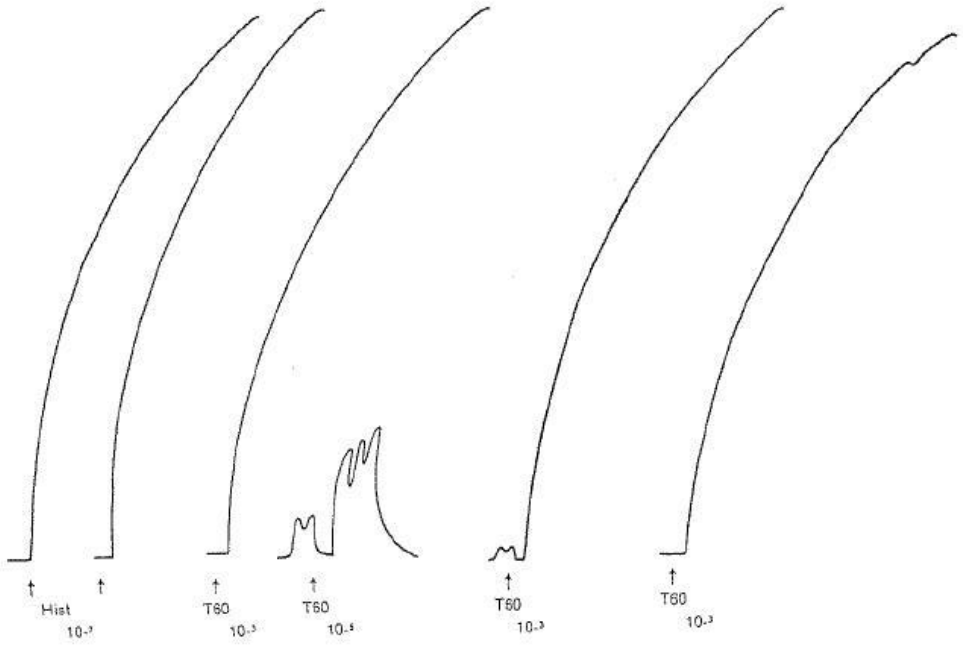


Fig. 15. Influence of T60 Isolated Bronchus (guinea pigs)

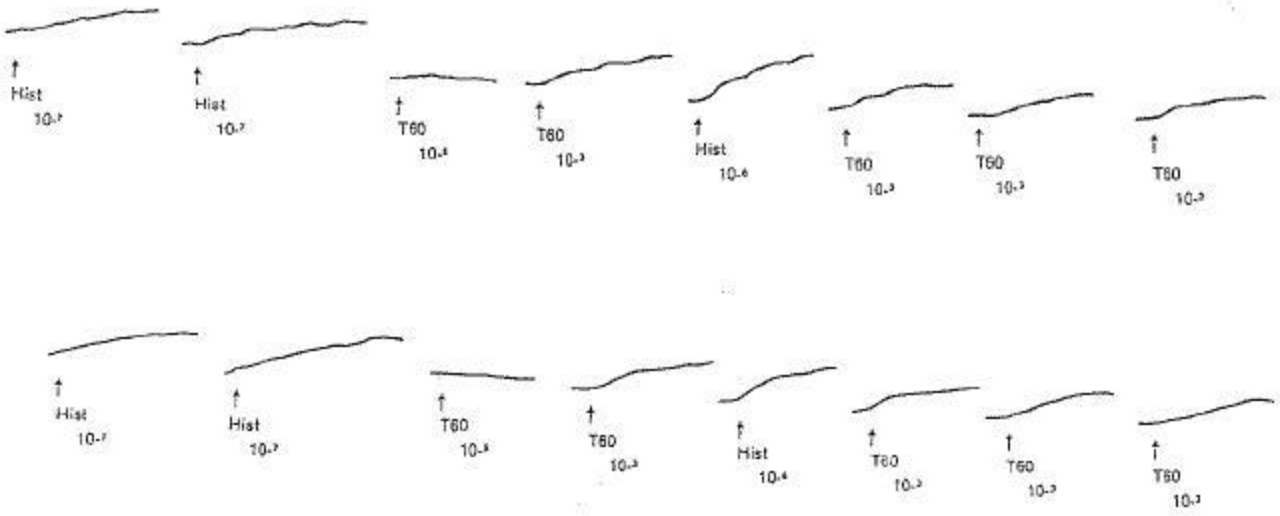


Fig. 16. Influence of GBX and T60 Isolated Prostate (rats)

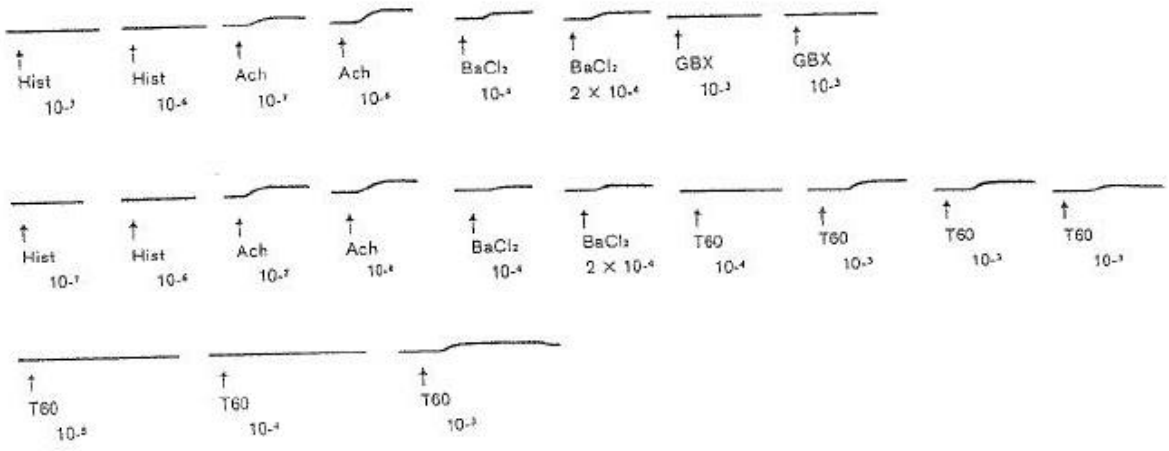


Fig. 17. Weight of Exudate