Pharmacological Studies on Cernilton, a New Remedy for Prostatitis and Prostatomegaly (2)

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I. Introduction

CERNILTON contains two major ingredients, Cernitin T-60 (T-60) and Cernitin GBX (GBX) mixed at a ratio of 20:1. The drug is clinically effective in the treatment of prostatomegaly.

In the present study acute toxicity test was carried out with CERNILTON (Cer) with a view to determine the LD_{50} in rats and mice and observe symptoms produced at a large dose. Subacute toxicity test was carried out with Cer, T-60 and GBX in rats and chronic toxicity test with Cer. Observations were made on physical development and general toxic symptoms. Liver function test, pathohistological examinations and other tests were also carried out.

II. Methods of Tests

1. *Acute Toxicity Tests of T-60, GBX and Cer*

Method: Animals used were Donryu strain rats (bodyweight 150-180 g) and ddN strain mice (bodyweight 20-25 g) of both sexes at 6-8 weeks of age, each group consisting of 5-10 animals. The sample drugs were given by three routes: oral, subcutaneous (only males), and intraperitoneal (only males). Following medication, animals were observed hourly for 6 hours and then only daily 7 days as to acute toxic symptoms and presence of death. Dead animals were subjected to laparotomy and the thoracic and abdominal organs were macroscopically observed. The LD_{50} was calculated on the basis of the total number of deaths in 7 days by means of the Probit method. T-60 and Cer were dissolved or suspended in 1% CMC and GBX was suspended in olive oil. The volume was so adjusted that the animals would receive 20 ml/ kg by oral route, 10 ml/ kg by the subcutaneous route, and 5 ml/ kg by the intraperitoneal route.

Dose (Table 1): According to the report by Tor Magnusson, the LD_{50} of T-60 is 5g/ kg by intraperitoneal route in mice. The dose levels were set up on the basis of this report.

2. *Subacute Toxicity Tests of T-60, GBX and Cer*

Donryu strain rats of both sexes weighing about 80g were used. The animals were raised under controlled conditions (room temperature 25±1°C, humidity 55±5%) and a total of 12 groups was set up, each group consisting of 10 males and 10 females. Four groups of animals were used for each sample drug. Sample drugs were given by the oral route with the aid of a gastric sound, once daily over a period of 35 days.

The dose levels were determined on the basis of the data obtained by Tor Magnusson as well as the results obtained in our laboratory concerning the LD_{50} of T-60, GBX and Cer. It was also taken into consideration that chronic toxicity test was to be carried out.

T-60 and Cer were suspended in 1% CMC solution. Concentrations were so adjusted that the maximum daily volume would be 4 ml/100g for T-60 and 2 ml/100g for Cer. Original solution (specific gravity 0.94) was used for GBX.

Observations were made on the following items: a) body weight: Determined daily until the end of administration. b) Food consumption: Determined for 10 days after the 25th day of administration. c) Observation of toxic symptoms: Daily until the end of administration. d) Blood test: Blood was collected from 10 animals (5 males, 5 females) in each group before administration and on the 30th day of administration. RBC count, hemoglobin value (cyanmethemoglobin method), WBC count, and WBC percentage were determined. e) Urine: Urine was collected from 10 animals (5 males, 5 females) in each group before administration and at the end of administration. Sugar, protein and urobilinogen were examined with Uristix (Ames Co.). The volume and color of urine were also examined. f) Liver function test: BSP excretion test was performed by Gaebler's method prior to autopsy at the end of administration. Hepatosulphalein was injected in doses of 10 mg/ kg into the vein of a hind limb and the amount of excreted dye was measured after 5 min. In addition, serum GOT and GPT levels were measured using ESGOT testing agent. g) Total cholesterol, total protein, blood sugar: Total protein was measured simultaneously with liver function test. Blood sugar was measured by Somogyi-Nelson's method and cholesterol level by Zak-Henly's method. h) Autopsy: 1) Macroscopic observation of organs: Major organs. 2) Weight: Hypophysis, thymus, heart, liver, kidneys (bilateral), adrenal glands (bilateral), spleen, prostate, testis (unilateral), epididymus (unilateral) and spermatocyst. 3) Pathohistological examinations: Brain, lungs, heart, liver, spleen, kidneys, stomach, intestine, pancreas, thymus, adrenal, spermatocyst and bone marrow. Each organ was fixed with 10% formalin solution and embedded in paraffin. It was then sliced and stained with hematoxylin eosin for microscopic observations.

3. *Chronic Toxicity test of Cer*

The conditions of animal breeding and method of administration were the same as in the subacute toxicity test. The drug was given for 180 days. Five dose groups were set up on the basis of the results obtained in the subacute toxicity test. T-60 was first dissolved in 1% CMC solution, after which GBX was added and mixed well with a homogenizer. The drug was so adjusted in concentration that the maximum volume would be 1ml/100g.

Items of observation were as follows. a) Body weight. b) Food consumption. c) Toxic symptoms. d) Blood test. e) Urine test. f) Liver function test. g) Total cholesterol, total protein, blood sugar. h) Autopsy.

III. Results

1. Acute Toxicity Tests of T-60, GBX and Cer
A. Observation of toxic symptoms and death
a. Rats

1) *T-60 administration groups*

a) Oral route: Though varying in intensity, symptoms were similar in all groups, regardless of sex. Animals crouched immediately after administration. Piloerection and decreased activity ensued. None, however, died and all the animals returned to normal after 6-24 hours.

b) Subcutaneous route: Symptoms were essentially the same as those observed in a) in all five groups. However, animals screamed for 5-10 seconds as if complaining of pain when injected subcutaneously. Subcutaneous induration was present at the site of injection for three days and then disappeared. Death occurred in no cases even by this route.

c) Intraperiotoneal route: Crouching, piloerection, decreased activity, and apprehension were noted. In survivals these symptoms disappeared after 6-12 hours, but in dead cases the symptoms did not disappear and the animals died after 24 hours in the state of natural death.

2) *GBX administration groups*

a) Oral route: Crouching, piloerection and decreased activity were seen from about 3 min after administration, but no specific symptoms

were shown. All animals recovered after 6 hours and survived.

b) Subcutaneous route: Death occurred in no cases, and symptoms were same as those seen in a). The site of injection presented swelling and induration as in the case of T-60, but they disappeared in about 5 days.

c) Intraperitoneal route: Death occurred by the third day. Symptoms noted were crouching, decreased activity and slight tremor. In the 1.95 and 2.34g/ kg dose groups, where all animals survived, the symptoms disappeared after 24 hours.

3) *Cer administration groups*

a) Oral route: Crouching, piloerection, decreased activity, and apprehension occurred from about 2 min after administration, but no specific toxic symptoms were seen. All animals recovered and returned to normal after 24 hours. Death was not noted.

b) Subcutaneous route: Symptoms were similar to those observed in a) and no specific toxic symptoms were disclosed. Some cases developed slight inflammation at the site of injection which lasted 3 days. Screaming lasting several seconds was noted at the time of injection, but death occurred in no cases.

c) Intraperitoneal route: Besides the symptoms observed in a) and b), slight general tremor was noted. In survivals the animals recovered after 12-24 hours, while in dead cases the symptoms persisted and the animals died within 24 hours. No further deaths, however, occurred during the 7 days of observation.

b. Mice

1) *T-60 administration groups*

a) Oral route: Difference due to sex was not noted. Intensity of symptoms varied somewhat with doses, but generally symptoms were same as those observed in rats. In other words, piloerection, decreased activity and tremor were noted, but they disappeared after 24 hours. In dead cases these symptoms were marked and animals died within 24 hours.

b) Subcutaneous route: For a few seconds after administration the animals ran about within the cage as if complaining of pain. Activity decreased somewhat more by this route than by the oral route, though it returned to normal after 6 hours. Death occurred between the 24th and 72nd hours of administration. Inflammation manifested at the site of injection in all cases as in rats, but likewise it disappeared in about 72 hours.

c) Intraperitoneal route: In dead cases in the large dose group, the animals developed piloerection, crouching, tremor of extremities and eventually died. In survivals symptoms as piloerection, decreased activity, and slight tremor were noted, but they disappeared after 6 hours.

2) *GBX administration groups*

a) Oral route: As with T-60 administration, symptoms were same in both sexes and slightly varied in intensity with doses. Namely, piloerection and decreased activity occurred immediately after administration, and in the large dose group slight general tremor was noted. Some cases developed diarrhea which lasted about 48 hours. Dead cases were all noted within 24 hours.

b) Subcutaneous route: In the large dose group the animals jumped around in the cage immediately after administration, showed piloerection, crouching, and staggering after one minute and died after 24-72 hours. In the small dose group these symptoms appeared too, but they disappeared after 48 hours. Inflammation at the site of injection was more marked than with T-60 administration but disappeared after 4 days.

c) Intraperitoneal route: Piloerection, decreased activity, and slight tremor appeared immediately after administration. Death occurred in some cases after 24-120 hours. Recovery

was slow in survivals but they returned to normal by the 6th day.

3) *Cer administration groups*

a) Oral route: Symptoms were similar to those observed with T-60. Piloerection, decreased activity, and tremor were seen. Death occurred within 24 hours. These symptoms also occurred in survivals but they disappeared after 6 hours.

b) Subcutaneous route: As with oral administration, piloerection, decreased activity and tremor appeared. However, they were of slighter degree than with subcutaneous injection of T-60. Death occurred within 48 hours.

c) Intraperitoneal route: In the large dose group, the same tremor as seen with T-60 administration appeared and death ensued. In survivals in the small and medium dose groups, piloerection, decreased activity, and slight tremor all appeared, but they disappeared within 10 hours.

B. LD_{50} (Table 2)

The LD_{50} in rats was not obtainable by the oral and subcutaneous routes because all animals survived even at the maximum permissible dose. However, by the intraperitoneal route, the toxicity of the sample drugs was considerably high. The LD_{50} was 7.58g/ kg for T-60, 3.31 for GBX, and 6.66 for Cer. GBX was the most toxic while T-60 and Cer were approximately of the same degree.

In mice the LD_{50} was successfully obtained by routes. As a result, it was found that GBX was the least toxic by the subcutaneous route. On the other hand, by the intraperitoneal route, GBX was the most toxic of all sample drugs. Of the three routes, the intraperitoneal route was the most toxic while the oral and subcutaneous routes were approximately of the same degree.

2. Subacute Toxicity Tests of T-60, GBX and Cer

A. Body weight and death (Figs. 1,2,3)

a. T-60 administration groups

1) Control group: The control group receiving 1% CMC solution at a dose of 40ml/ kg showed normal weight increases both in males and females.

2) T-60 6.0g/kg group: The males showed normal weight increases at the control group and registered a greater weight increase than the control group throughout the period, with a difference of about 14 g at the end of administration. In females weight decrease was noted around the 25th day of administration, but as a whole they showed the same weight increase tendency as the control group. Death occurred in one male and one female, on the 23rd and 24th day, respectively.

3) T-60 12.0g/kg group: Weight increase in males slowed down transiently from about the 20th day, but generally the tendency was the same as that of the control group. Females showed a greater weight increase than the control throughout the period, with a difference of about 5g on the 30th day of administration. The difference, however, was not statistically significant. Death occurred in one male and one female on the 33rd and 21st day respectively.

4) T-60 24.0g/kg group: Males showed a greater weight increase than the control group up to the 20th day as in the previous two groups, but from about the 25th day weight increase slowed down markedly, with practically no weight increase shown up to the end of administration. The body weight on the 30th day was about 30g less than that of the control group, thus with a significant difference between the two groups. In females weight increase slowed down from about the 20th day, the body weight being lower than that of the control group by about 5g on the 30th day. Death occurred in 2 males (9, 32nd day) and 3 females (26, 20, 32nd day).

b. GBX administration groups

1) Control group: The control group receiving 1% CMC solution at a dose of 10 ml/kg showed normal weight increases both in males and females.

2) GBX 5.0g kg group: Both males and females showed normal weight increases, and the body weight at the end of administration was about the same as that of the control group. Death occurred in one male on the 33rd day.

3) GBX 10g/kg group: In males weight increase slowed down from about the 20th day, and the body weight was about 30g lower than that of the control group in the 30th day, with significant difference. Females showed normal weight increases. The bodyweight at the end of administration was little different from that of the control group.

4) GBX 20g/kg group: In males weight increase slackened more than in the previous 3 groups, and although it subsequently recovered, the body weight on the 30th day was about 40g lower than that of the control group. The difference was of significance. In females, contrarily, weight increase progressed quite favourably and the body weight was about 12g higher than of the control group on the 30th day. Death occurred in one male (14th day) and 3 females (6, 9, 32nd day).

c. *Cer administration groups*

1) Control group: The control group, which was given 20ml/kg of 1% CMC solution, showed normal weight increases in both sexes as the control groups of T-60 and GBX. Death occurred in one male, but this was due to an error in administration.

2) Cer 6.3g/ kg group: Both males and females showed exactly the same weight increase as the control group up to the 20th day, but then it was slightly inhibited. Difference in body weight, however, was noted at the end of administration in comparison with the control group. One male died on the 34th day.

3) Cer 12.6g/kg group: Males had a slightly greater weight increase than the control group from about the 15th day, with a difference of about 5g on the 30th day of administration. Females showed exactly the same weight increase as the control group, with no difference in body weight at the end of administration. Death occurred in 2 males (22, 34th day) and one female (31st day).

4) Cer 25.2g/kg group: Weight increase slowed down from about the 5th day in both sexes lasting until the end of administration. In males, although there was no weight decrease, the difference with the control group reached as much as 20g on the 30th day. In females, a transient weight decrease was noted on the 30th day, and the difference with the control group at the end of administration was about 16g, with significant difference. Death occurred in no cases.

B. *Food consumption (Table 3)*

Food consumption tended to decrease in both males and females with all sample drugs as compared with that in the control group. This tendency was particularly marked in the T-60 24.0g/kg, GBX 20.0g/kg, and Cer 25.2g/kg groups where the food consumption was only about half that of the control group.

C. *Observation of toxic symptom*

a. *T-60 administration groups*: In the 12.0g/kg and 24.0g/kg groups, piloerection, soft feces and depression appeared from about the 15th day. In addition, mild tremor of forelimbs, salivation, face-washing and coughing appeared every day 5-10 min after administration, disappearing gradually after 10 min with animals coughing repeatedly. Toward the end of administration, tremor of forelimbs intensified with a longer recovery time required, and in all cases loss of appetite, emaciation and piloerection were noted persistently.

b. *GBX administration groups*: From about the 15th day, in addition to the aforementioned symptoms of piloerection and soft feces, forward opening of forelimbs was noted in the 10.0g/kg and 20.0g/kg groups, beginning from immediately to 5 min after administration. From about 5-10 min after administration salivation, face-washing, tremor of forelimbs, searching behaviour, and coughing appeared, and

animals, after repeating face-washing and severe tremor of forelimbs, moved toward recovery after 30-40 min of administration. In the 20.0g/kg group there was found mixed in soft feces something whose colour was indicative of the sample drug. Loss of appetite, emaciation and depression were markedly observed in the latter period of administration.

c. *Cer administration groups*: In the 12.6g/kg and 25.2g/kg groups about two-thirds of the animals showed salivation, face-washing, chronic tremor of forelimbs and coughing about 10 min after administration from about the 20th day. These symptoms continued for about 20 min and then gradually improved. In the 25.2g/kg group several animals developed searching behaviour. It gradually improved after the animals repeated coughing and face-washing. Furthermore, loss of appetite, emaciation, piloerection, and soft feces were persistently observed until the end of administration.

D. Blood tests (Table 4, 5, 6)
a. T-60 administration groups

1) RBC: RBC was slightly decreased in the experimental groups as compared to that in the control group. Significant difference was noted between the T-60 24.0g/kg and control groups in both sexes.

2) Hemoglobin: Hemoglobin was decreased in the experimental groups as compared to that in the control group. The males of all groups and the females of the 12.0g/kg and 24.0g/kg groups showed significant difference from the control group.

3) WBC: WBC showed no marked changes in males. In females it increased more in the experimental groups than in the control group, with significant difference noted in the cases of 12.0g/kg and 24.0g/kg groups.

4) WBC percentage: Neutrophiles, basophiles, acidophiles and lymphocyte were all within normal limits in distribution, and no morphologically abnormal cells were disclosed.

b. *GBX administration groups*

1) RBC: RBC showed no marked changes in the experimental groups as compared to that in the control group on the 30th day.

2) Hemoglobin: Hemoglobin decreased in the females of the 10.0g/kg group as compared to that in the control group, with significant difference. No marked changes were noted in the other groups.

3) WBC: In males all experimental groups showed a decreasing tendency in comparison to the control group, with significant difference in the case of the 20.0g/kg group. In females no such tendency was revealed.

4) WBC percentage: WBC percentage showed no specific abnormalities, and no morphologically abnormal cells were disclosed.

c. *Cer administration groups*

RBC, hemoglobin, WBC and WBC percentage as determined on the 30th day show no marked changes in the experimental groups as compared to those in the control group. Morphologically abnormal cells were not revealed.

E. *Urine tests*

Toward the end of administration sugar was detected in the Cer 25.2g/kg group and protein and urobilinogen in the GBX 20.0g/kg group, in 3-4 cases each, but the levels were not significantly high. Results obtained both before and after administration showed no abnormalities. Comparison between the experimental and control groups also showed no abnormalities. The urinary volume tended to increase in the experimental groups. The color tone was normal.

F. Liver function test (Tables 7, 8, 9)
a. T-60 administration groups

1) BSP excretion test: In males BSP excretion was delayed in the experimental groups in comparison to that in the control group, with

significant difference noted in the cases of the 6.0g/kg and 12.0g/kg groups. No such delay was seen in females.

2) Transaminase: In males GOT and GPT were lower in the experimental groups than in the control group, with significant difference in the cases of the 6.0g/kg and 24.0g/kg groups, the former both in GOT and GPT and the latter in GOT. In females both GOT and GPT showed no significant difference between the experimental and the control group.

b. GBX administration groups

1) BSP excretion test: The experimental groups showed no tendency of delayed excretion in both sexes in comparison to the control group. Rather, excretion was somewhat delayed in the control group.

2) Transaminase: GOT showed no difference between the experimental and control groups in both sexes. GPT was slightly raised in the experimental groups in both sexes, with significant difference from the control in the cases of the females of the 5.0g/kg group.

c. Cer administration groups

1) BSP excretion test: In males excretion tended to delay in all experimental groups with increase in dosage, but no significant difference was noted from the control. In females, delayed excretion was not revealed at all.

2) Transaminase: In males both GOT and GPT were lower in the experimental groups than in the control group, with significant difference in the cases of the 12.6g/kg and 25.2g/kg groups, the former in GPT and the latter in both GOT and GPT. In females both GOT and GPT were lower in the 6.3g/kg and 25.2g/kg groups. In the 12.6g/kg group GOT and GPT tended to rise, but the difference was not significant from the control.

G. Total cholesterol, total protein, blood sugar (Tables 7, 8, 9)

a. *T-60 administration groups*: Total cholesterol was raised in the males of the 12.0g/kg and 24.0g/kg groups compared to that in the control group. It was found lowered in the females of the 24.0g/kg group, with significant difference from the control. Blood sugar tended to rise with increase in dosage in females and total protein registered a significantly high value in the males of the 24.0g/kg group. Otherwise, there was no difference from the control.

b. *GBX administration groups*: Total cholesterol was lowered in the females of the 10.0g/kg group, with significant difference from the control. Otherwise, there was no difference between the experimental and control groups in both sexes. Blood sugar was higher in the experimental groups in both sexes, with significant difference in the cases of 5.0g/kg group (males), 10.0g/kg group (females), and 20.0g/kg group (both sexes) and 10.0g/kg (females) groups than in the control group, with significant difference.

c. *Cer administration groups*: Total cholesterol tended to increase in the experimental groups in both sexes as compared to that in the control group. The difference was significant in the case of the 25.2g/kg group (males). Blood sugar was lower in the 6.3g/kg and 12.6g/kg groups in both sexes than the control but was higher in the 25.2g/ kg group in both sexes. The difference, however, was not significant. Total protein in the experimental groups showed no difference from the control in both sexes.

H. Autopsy

a. *Macroscopic observation of organs*: No specific changes were disclosed in the thoracic, abdominal, and endocrinological organs. Smear specimens of the seminal vesicle, epididymus and testis showed no abnormalities. It is unlikely that the testicular function was impaired.

b. Organ weight (Tables 10, 11, 12, Fig. 4)
1) T-60 administration groups

a) Hypophysis: The weight showed no marked variations between the experimental and

control groups. A higher weight than the control with significant difference, however, was noted in the males of the 12.0g/kg group and the females of the 24.0g/kg group.

b) Thymus: The weight was lower in the males of the 24.0g/ kg group and the females of the 12.0g/kg and 24.0g/kg groups than the control, each with significant difference.

c) Heart: The weight tended to be decreased in the high-dose groups in both sexes. In the 24.0g/kg group it was decreased in both sexes, with significant difference from the control.

d) Liver: There was no difference between the experimental and control groups in both sexes.

e) Kidney: Here, too, the weight tended to be decreased in the high-dose groups in both sexes, with significant difference bilaterally between the 24.0g/kg and control groups in males.

f) Adrenal gland: In males the weighy tended to increase in the experimental groups as compared to that in the control group, with significant difference in the cases of the 12.0g/kg group (bilateral) and the 24.0g/kg group (left). In females the weight was little affected except that it was slightly increased in the 6.0g/kg group.

g) Spleen: Except that the weight was decreased in the 24.0g/kg group in both sexes, there was no marked difference between the experimental and control groups.

h) Prostate: The weight decreased as the dosage was increased, and significant difference was noted between the control and the 12.0gkg and 24.0g/kg groups.

i) Testis, epididymus: None of the experimental groups showed difference with the control group.

j) Seminal vesicle: The weight was higher in the 6.0g/kg group than the control, but was lower in the 24.0g/kg group with significant difference.

2) *GBX administration groups*

a) Hypophysis: The weight was increased in the males of the 10.0g/kg group, with significant difference from the control. Otherwise, no difference was revealed between the experimental and control groups in both sexes.

b) Thymus: In males, the weight tended to decrease as the dose was increased, while in females the decreasing tendency was evident.

c) Heart: In males the weight was lower in the 20.0g/kg group, with significant difference from the control, but in females it was rather increased in all experimental groups.

d) Liver: The weight tended to be increased in the experimental groups in both sexes, but none of the groups showed any significant difference with the control group.

e) Kidneys: In males the weight tended to decrease as the dosage was increased, and significant difference was noted between the 20.0g/kg and the control groups (left). In females, contrarily, the weight increased with dosage, with significant difference from the control in the case of the 20.0g/k group (left).

f) Adrenal glands: The weight increased with dosage in experimental groups in both sexes. In the 20.0g/kg group significant difference was noted from the control in the left kidney in both sexes. In the right kidney, the males of all experimental groups showed significant difference from the control.

g) Spleen: In males the weight tended to decrease with increase in dosage. In females an increasing tendency was noted, but neither showed significant difference from the control.

h) Prostate: The weight was lower in the experimental groups than the control, but not so evidently as in the T-60 administration groups. The relation to dosage was not clear.

i) Testis, epididymus: The weight of the testes in the 20.0g/kg group and that of the epididymus in the 5.0g/ kg and 10.0g/kg groups were lower than the control, with significant difference.

j) Seminal vesicle: the weight was lower in all the experimental groups, with significant difference from the control in the case of the 20.0g/kg group.

3) *Cer administration groups*

a) Hypophysis: The weight was increased in the males of the 12.6g/kg and 25.2g/kg groups in the females of the 6.3g/kg group, with significant difference from the control.

b) Thymus: It weighed less in the females of the 25.2 g/kg group than in the control group, with significant difference. Otherwise, there was no difference between the experimental and control groups in both sexes.

c) Heart: None of the experimental groups showed great difference from the control group in both sexes.

d) Liver: The weight tended to increase with dosage in the experimental groups in both sexes. The difference between the 25.2g/kg group and the control was significance in both sexes.

e) Kidneys: As in the liver, the weight tended to increase with dosage in both sexes in the experimental groups, and the 25.2g/kg showed significant difference from the control.

f) Adrenal glands: The weight was increased in experimental groups in both sexes except in the 6.3g/kg group. Significant difference was noted from the control in the 6.3g/kg (right, both sexes), 12.6g/kg (bilateral, both sexes) and 25.2g/ kg (bilateral, both sexes) groups.

g) Spleen: Except that the weight was lower in the females of the 25.2g/kg group, with significant difference from the control, no great difference was noted between the experimental and control groups.

h) Prostate: The weight was higher in the experimental groups but with no significant difference. A correlation between dosage and weight was not revealed.

i) Testis: the weight increased as the dosage was increased in the experimental groups, with significant difference in the cases of the 12.6g/kg and 25.2g/kg groups.

j) Epididymus: No difference was shown between the experimental and control groups.

k) Seminal vesicle: All experimental groups showed a higher weight, but with no significant difference.

4) *Weight of prostate per 100g of body weight (Fig. 4)*

a) T-60 administration groups: As the dosage was increased, the weight tended to decrease in the experimental groups, with significant difference from the control in the cases of the 12.0g/kg and 24.0g/kg groups.

b) GBX administration groups: With increase in dosage, a slight weight-increasing tendency was noted in the experimental groups, but the difference was of no significance.

c) Cer administration groups: The weight was slightly higher in the experimental groups, but with no significant difference.

c. Pathohistological observations
1) T-60 administration groups

a) Control group

(1) Prostate: The parenchyma of the prostate was composed of glandular ducts. The ducts had no clear-cut basal membranes and the interior surface was covered with glandular epithelium. The width of the ducts varied greatly depending on the height of the ducts. For convenience' sake the letters X and Y will be used here, the former indicating narrow glandular ducts and the altter wide ducts. There appearded three types of glandularducts: X ducts, dilated X ducts, and Y ducts. Seminal

ductules with abundant polynucleic gigantic cells, which seemed to be Sertoli's cells, were found in some areas. Otherwise, there were no abnormal findings.

(2) Liver: Venous congestion and slight deposit of fat droplets were seen. No other abnormal findings were obtained.

No abnormalities were found in the other organs.

b) T-60 6.0g/kg group

(1) Prostate: Findings varied from case to case, some consisting of only Y ducts and others X and dilated X ducts.

(2) Testis: Marked degeneration of seminal ductules and atrophic seminal ductules rich in gigantic cells were seen in a small number of cases.

(3) Liver: Fat deposit, diffuse cellular atrophy, and cellular dissociation were noted. They were severe in degree.

(4) Hypophysis: Congestion was disclosed in a small number of cases.

c) T-60 12.0g/kg group

(1) Prostate: Similar findings were obtained in all cases. It consisted mainly of X ducts, mixed with dilated X ducts.

(2) Testis: No specific findings were obtained.

(3) Liver: The changes were particularly evident in females and atrophied liver cells and pimelosis were markedly seen. In a small number of cases pimelosis was quite marked, and moreover intrasinusoidal congestion was associated.

(4) Kidney: Congestion and urinary cast were noted in about half the cases.

(5) Hypophysis: Marked congestion and congestive edema was seen in a small number of cases.

d) T-60 24.0g/kg group

(1) Prostate: Slight degeneration of ducts was noted in about half the cases. Findings, however, were not uniform, some cases consisting of X and Y ducts and others dilated X or Y ducts.

(2) Testis: Hypoplasia of seminal ductules and necrosis due to coagulation of sperms were noted in a small area in all cases.

(3) Liver: Atrophy of cells and scattered fatty droplets were shown in about half the cases. Marked congestion was noted in a few cases.

(4) Kidneys: A moderate degree of congestion was shown in all cases. Otherwise, there were no specific findings.

(5) Pancreas: Localized vacuolation or pimelosis of the acinus was observed in a small number of cases.

(6) Hypophysis: All cases showed slight congestion.

(7) Thyroid: In many cases colloid was thin (some cases devoid of it) and the epithelium was vacuolated.

2) GBX administration groups

a) Control group

(1) Prostate: In all cases it consisted of X ducts and dilated X ducts. In a small number of cases Y ducts were also noted.

(2) Testis: No abnormal findings.

(3) Liver: Very slight cellular dissociation, congestion and scattered fatty droplets were shown in a small number of cases.

No changes were disclosed in the other organs.

b) GBX 5.0g/kg group

(1) Prostate: In many cases Y ducts were somewhat abundantly noted, but generally the findings were similar to those in the T-60 6.0g/kg group.

(2) Testis: No specific findings.

(3) Liver: Diffuse cellular atrophy and fatty droplets were observed.

(4) Kidney: No specific findings.

(5) Hypophysis: Congestion was seen in all cases.

No specific findings were obtained in the other organs.

c) GBX 10.0g/kg group

(1) Prostate: Findings were similar in all cases. The prostate consisted mainly of X ducts, and dilated X ducts were few.

(2) Testis: No specific findings.

(3) Liver: Atrophy of cells and deposit of fat droplets were noted as in the T-60 12.0g/kg group. They were less marked than those in the T-60 administration groups but more marked than those in the Cer administration groups.

(4) Hypophysis: Congestion or congestive edema was seen in about half the cases.

d) GBX 20.0g/kg group

(1) Prostate: Some cases consisted of X and dilated X ducts while others consisted mainly of Y ducts.

(2) Testis: No specific findings.

(3) Liver: Cellular dissociation and localized cellular atrophy were noted in many cases, but fat scarcely appeared.

(4) Kidney: No specific findings.

(5) Hypophysis: Somewhat marked congestion was disclosed in about half the cases.

(6) Thyroid: The colloid was thin and the epithelium was vacuolated.

3) Cer administration groups

a) Control group

(1) Prostate: The prostate consisted of X ducts in about half the cases. In the other half it consisted of X ducts and dilated X ducts.

(2) Testis: No specific findings.

(3) Liver: Slight congestion and deposit of fatty droplets were noted in all cases.

(4) No specific findings.

(5) Hypophysis: Acidophilic cells were slightly increased in a small number of cases.

No specific changes were shown in the other organs.

b) Cer 6.3g/kg group

(1) Prostate: In many cases it consisted of X ducts and dilated X ducts, while in some cases Y ducts were markedly observed.

(2) Testis: Slight hypoplasia of sperms was shown in a small number of cases.

(3) Liver: In about half the cases deposit of fatty droplets and diffuse cellular atrophy were slightly noted.

No specific changes were seen in other organs.

c) Cer 12.6g/kg group

(1) Prostate: It consisted of X ducts in all cases, mixed with Y ducts in a few cases. Slight degeneration or disappearance of glandular ducts was noted in most cases, but the degree was slight. The stroma showed no abnormalities.

(2) Testis: Seminal ductules suggestive of hypoplasia of sperms were locally noted immediately below the capsule.

(3) Liver: Slight cellular atrophy, dissociation of cell cords, and deposit of fatty droplets were seen.

(4) Kidney: Slight congestion of the cortico-medullary border zone and glomeruli and urinary casts occurred in about half the cases.

(5) Hypophysis: Increased acidophilic cells were seen in a small number of cases.

d) Cer 25.2g/kg group

(1) Prostate: In most cases X ducts were dilated, some with degeneration or disappearance of the glandular epithelium. The degree of change varied with cases. Y ducts were generally scarce.

(2) Testis: Seminal ductules suggestive of hypoplasia of sperms were disclosed in a small number of cases, but degeneration or necrosis was not observed. Generally findings were scarce.

(3) Liver: Slight cellular atrophy and deposit of scattered fatty droplets occurred in about half the cases.

(4) Kidney: Only slight congestion of glomeruli was noted in about half the cases, and the renal tubules showed no changes at all.

(5) Hypophysis: Only acidophilic cells were slightly increased.

(6) Thyroid: The colloid was thin and sometimes absent, and the epithelium was vacuolated in many cases.

3. Chronic Toxicity Test of Cer
A. Body weight and death (Fig. 5)

a. Control group: The control group, which received 10ml/kg of CMC solution, showed normal weight increases in both sexes.

b. Cer 1.6g/kg group: In males the body weight was greater than the control, by about 20g on the 30th day and by about 35g on the 105th day. Thereafter, it decreased transiently. After the 135th day increase and decrease occurred alternately and the difference with the control at the end of administration was about 12g. In females weight increase became somewhat unsteady after the 135th day and the body weight slightly decreased after the 165th day. However, the tendency was the same as that of the control as a whole. Death occurred in one male and one female, on the 134 and 166th day respectively.

c. Cer 3.2g/kg group: The body weight in males showed no decrease up to the 165th day and was about 20g greater than the control. After the 165th day it slightly decreased so that the difference with the control was only about 5g at the end of administration. In females, the body weight ceased increasing after the 105th day and then slightly decreased after the 165th day. At the end of administration it was about 10g smaller than the control. One male died on the 117th day.

d. Cer 6.3g/kg group: As in the previous two groups, the males showed normal weight increase up to the 165th day without weight decrease, and the body weight was about 20g greater than the control. However, toward the end of administration the body weight decreased slightly so that the difference with the control ended up with about 14g. In females, the weight increase slowed down after the 105th day and the body weight at the end of administration was about 1.5g smaller than the control. One male died on the 165th day.

e. Cer 12.6g/kg group: The males showed a slower weight increase than the control and the three previous groups. This tendency was intensified after the 105th day and the body weight at the end of administration was about 40g smaller than the control with significant difference. In females, the body weight ceased increasing after the 120th day. On the contrary, it tended to decrease and the difference with the control at the end of administration was about 16g, though with no significant difference. Death occurred in 2 males, on the 124th and 164th day.

B. Food consumption (Table 13)

The food consumption decreased from about the second month in the 12.6g/kg group (both sexes) and from about the fourth month in the 6.3g/kg group (both sexes). The other groups showed no great difference with the control, but

generally the consumption tended to decrease as the dosage was increased.

C. Observation of toxic symptoms

The toxic symptoms were nearly the same as those observed in the subacute toxicity test. In the 1.6 and 3.2g/kg groups, beginning from about the 90th day, there occurred immediately after administration face-washing, coughing and slight general tremor in about one-third of the cases lasting about 15 min. At the time of recovery the animals were slightly in sedation. The intensity of symptoms did not change until the end of administration. Piloerection was also noted. Generally speaking, the intensity of symptoms was stronger in males. In the 6.3g/kg and 12.6g/kg groups the same symptoms as seen above appeared from about the 70th day and tremor of forelimbs, searching behavior, coughing, face-washing and salivation from about the 100th day. The symptoms lasted about 15 min and then gradually moved toward recovery. Toward the end of administration loss of appetite, emaciation and piloerection occurred in all cases and alopecia in one male.

D. Blood tests (Tables 14, 15)

a. WBC: No marked difference was noted between the experimental and control groups when comparison was made before administration, on the 90th day and 180th day. Nevertheless, the number was found to be smaller than the control in the males of the 6.3g/kg group on the 180th day and in both sexes of the 12.6g/kg group, all with significant difference.

b. Hemoglobin: Except that the males of the 6.3g/kg group were smaller in number than the control with significant difference on the 90th day, there was no difference between the experimental and the control groups in both sexes at all time periods.

c. WBC: Some difference was noted between the experimental and the control groups before administration and on the 90th day in both sexes, but the difference was not significant. On the 180th day, however, the males of all experimental groups showed a smaller value than the control, with significant difference in the cases of the 1.6g/kg, 3.2g/kg and 12.6g/kg groups. In females, WBC was slightly increased in all experimental groups, but no significant difference was noted.

e. WBC percentage: As in the subacute toxicity test, the results showed no marked difference in cell distribution between the experimental and control groups at all time periods. No morphologically abnormal cells appeared.

E. Urine tests

No abnormal findings were obtained in sugar, protein, and urobilinogen. The urinary volume, however, tended to increase in the experimental groups.

F. Liver function test (Table 16)

a. BSP excretion test: No tendency of excretion delay was noted in the experimental groups in both sexes as compared to the control. In the females of the 3.2g/kg group the excretion was even faster than the control.

b. Transaminase: GOT and GPT were not increase with dosage in the experimental groups in both sexes, but GPT in the males of the 3.2g/kg group showed a higher value than the control and GOT in the females of the 12.6g/kg group a lower value, each with significant difference was noted.

G. Total cholesterol, total protein, blood sugar (Table 16)

a. Total cholesterol: There was no definite tendency noted. In males the 3.2g/kg and 6.3g/kg groups showed the same value as the control, the 1.6g/kg group a lower than the control, and the 12.6g/kg group showed a slightly higher value than the control, but no significant difference was noted.

b. Total protein: The males of the 1.6g/kg and 3.2g/kg groups showed a lower value than the

control, with significant difference. In females, there was no difference between the experimental and the control groups.

c. Blood sugar: Males generally showed a higher value than the control in all groups, but the correlation to dosage was not evidenced. However, in females, the value tended to increase with dosage and significant difference from the control was noted in the 3.2, 6.3, and 12.6g/kg groups.

H. Autopsy

a. Macroscopic observation of organs: In a few cases in the 6.3g/kg and 12.6g/kg groups there was noted a chronic inflammatory picture in the lung. Otherwise, no macroscopic changes were noted in the thoracic, abdominal or endocrine organs. Smear specimens of the seminal vesicle, epididymus and testis showed no abnormalities. It is unlikely that the function of the testis was impaired.

b. Organ weight (Table 17, Fig. 6)

1) Hypophysis: The weight was lower in the males of the 12.6g/kg group and in the females of all experimental groups than the control, with significant difference.

2) Thymus: The weight tended to decrease with increase in dosage in all experimental groups in both sexes, with significant difference from the control in the cases of the 6.3g/kg (males) and 12.6g/kg (both sexes) groups.

3) Heart: Except that the females of the 1.6g/kg group were greater than the control, there was no difference between the experimental and control groups.

4) Liver: Males showed no difference between the experimental and control groups, but the females of the experimental groups were generally greater than the control, with significant difference.

5) Kidney: It weighed less in the males of the 12.6g/kg group in the bilateral kidneys than the control, with significant difference. Otherwise, there was no difference between the experimental and control groups.

6) Adrenal gland: Generally, the weight tended to increase in the experimental groups in both sexes, with significant difference from the control in the cases of the 1.6g/kg (females), 3.2g/kg (males) and 6.3g/kg (males) groups.

7) Spleen: In males, the weight was decreased in the 12.6g/kg group, with significant difference from the control. The other three groups showed a slightly higher weight than the control. In females, all experimental groups showed a higher weight than the control, and the weight tended to increase, with dosage. The difference with the control was significant in the cases of the 3.2g/kg, 6.3g/kg and 12.6g/kg groups.

8) Prostate: It weighed less in all the experimental groups, and furthermore the weight tended to decrease as the dosage was increased. The difference with the control was significant in the cases of the 3.2g/kg, 6.3g/kg, and 12.6g/kg groups. Even in terms of weight per 100g of body weight, the tendency was of the same and significant difference was noted in the 3.2g/kg, 6.3g/kg, and 12.6g/kg groups.

c. Pathohistological findings
1. Control group

a) Prostate: The glandular ducts folded and were covered with relatively high epithelium which was slightly protruding like papilloma. They were composed of two types of ducts, one type of being a narrow lumen (hereinafter abbreviated as X) and the other being a wide lumen with low epithelium (hereinafter abbreviated as Y). Marked widening of lumen was seen in some Y ducts and the epithelium underwent squamous metaplasis in response to the widening. However, falling-off or disappearance of the epithelium was not observed.

b) Testis: The pictures of seminal ductules varied slightly with cases depending on the stage of spermatogenesis, such as delayed

spermatogenesis (hereinafter abbreviated as C), homogenous coagulation (D), numerous Sertoli's cells (E), and transitory impairment of maturation of sperms (F). Degeneration of spermatoblasts (G) was not found at all.

c) Liver: Slight dissociation and partial atrophy of liver cells with fatty droplets were disclosed in a small number of cases. But generally lesions were scarce. Congestion occurred in no cases.

d) Kidneys: Deposit of basophilic crystals in the area between the cortex and medulla, slight congestion of stroma or urinary casts were observed in a small number of cases.

e) Spleen: The pulp slightly coarse, and the blood volume varied with cases.

f) Thymus: The medulla was wide and the cortex developed well, but the cellular density was coarse in many cases.

g) Adrenal gland: In general, the cortex was of uniformly light staining and in some cases distinction of different layers was rather difficult. Severe congestion was also seen in the area between the cortex and medulla in some cases.

h) Thyroid: Colloid was thin. The epithelium was entirely within normal limits.

i) Hypophysis: Acidophiles increased in number in many cases. The organ was slightly edematous, and hemorrhage was seen in a few cases.

No specific findings were obtained in the heart, lungs, brain, pancreas, digestive tracts, bone marrow and ovary.

2) Cer 1.6g/kg group

a) Prostate: The epithelium of X ducts was vacuolated and fell off at time. On the other hand, Y ducts showed marked squamous metaplasia in a few cases, but the difference with the control was not significant.

b) Testis: The findings C, D, E and F were seen sporadically in the normal seminal ductules.

c) Liver: Slight but wide-spread atrophy of liver cells, associated with irregularly-sized nuclei, was seen in a small number of cases. Congestion and fatty droplets were noted sporadically.

d) Kidney: Mild congestion and urinary casts were found in the greater majority of the cases in both sexes. In females, concentric round or irregularly-shaped basophilic crystals, which had been seen in the control group, were found in the parenchyma in the cortico-medullary border area.

e) Spleen: The pulp was congestive and coarse and was deficient in cells in general.

f) Pancreas: Partial atrophy and vacuolation of the acinus and mild edema of stroma were seen in a small number of cases.

g) Thymus: The parenchyma was coarse and the cortical cells also under-developed in a small number of cases. Cellular necrosis was found in one case.

h) Adrenal gland: In the greater majority of cases the fat was deficient and the cortex was uniformly of light staining. In a few cases there occurred severe congestion in the zonal cortex.

i) Thyroid: In most cases it lacked colloid and the epithelium underwent squamous metaplasia at times.

j) Hypophysis: In males acidophiles increased, but in females it consisted chiefly of main cells.

k) There was no great difference between the control and experimental groups in regard to other organs; brain, heart, lungs, digestive tracts, ovary and bone marrow.

3) Cer 3.2g/kg group

a) Prostate: Falling-off or degeneration of epithelium of X ducts was seen in many cases. In general, X ducts were slightly enlarged and Y ducts rather decreased.

b) Testis: The findings C, E, F were seen more frequently in this group than in the previous two groups. However, they were not so severe as to cause dysfunction.

c) Liver: Findings were similar to those seen in the 1.6g/kg group. Atrophy of liver cells, congestion, and deposit of fatty droplets were seen in a few cases.

d) Kidney: The basophilic crystals seen in the 1.6g/kg group were noted in all females, but none in males. Mild congestion and swelling of main tubules were found in nearly all cases. Granule-form deposit was present in Bowman's capsule in a few cases.

e) Spleen: The lymphatic tissue was decreased. The pulp was coarse and deficient in cells. Congestive edema was seen in a few cases.

f) Heart: Histocytes somewhat increased in the pericardium forming small cell foci. In one case they penetrated into the myocardium and in another case they formed round cellular infiltration in the myocardium.

g) Thymus: Both cortex and medulla were coarse and lacked cellular density.

h) Adrenal gland: Congestion was generally noted in females. In males congestion was not evident, and the cortex was of light staining and lacked fat.

i) Thyroid: Scanty colloid. The epithelium was somewhat atrophied and vacuolated in many cases.

j) Hypophysis: As in the 1.6g/kg group, acidophilic cells were increased in males while in females it consisted chiefly of main cells.

No specific findings were obtained in other organs as lungs, digestive tracts, pancreas, ovary and bone marrow.

4) Cer 6.3g/kg group

a) Prostate: Y ducts were fewer than X ducts. In X ducts, some cases were with necrosis of hyaline degeneration of the epithelium, others with stenotic ducts or higher epithelium, while still others with vacuolation or falling-off of epithelium. Y ducts under marked squamous metaplasia. An intermediate type between X ducts and Y ducts was also seen. The stroma was not affected very much, although serious infiltration was seen in some.

b) Testis: All the findings C, D, E and F were observed in mature seminal tubules corresponding to the stages of spermatogenesis. In most cases the tubules were normal and completely free from disturbances interfering with spermatogenesis.

c) Liver: Mild diffuse atrophy of liver cells was seen in a few cases, and the degree was somewhat stronger than that in the 3.2g/kg group. The severity of congestion varied with cases, but was not necessarily stronger than that in the previous three groups.

d) Kidney: Deposit of basophilic crystals was noted in the corticomedullary border area in females. In males this was not noted at all. Turbid swelling, degeneration or necrosis of the epithelium of the renal tubules occurred in no cases.

e) Spleen: The pulp was enlarged and in some cases it became coarse due to congestive edema. Reticulum cells, hematopoietic cells or giant cells were sparse, and deposit of hemosiderin was not evident.

f) Heart: Histocytes and round cells infiltrated beneath the pericardium and then through the stroma to the superficial layer of the myocardium. This was found in one case.

g) Pancreas: Sporadic small acinous atrophy and vacuolation or fatty degeneration of the epithelium was seen in a few cases.

h) Thymus: Serous infiltration of the cortex and medulla was seen over a wide area in a few males. In females the parenchyma was dense in all cases and hemorrhage occurred in no cases.

i) Adrenal gland: Severe congestion and dissociation of cortical cells were found in a few cases. Generally fat was scanty.

j) Thyroid: In general colloid was scanty. Vacuolar swelling and falling-off of the epithelium were seen in some cases.

No specific changes were seen in the brain, hypophysis, lungs, digestive tracts, ovary and bone marrow.

5) Cer 12.6g/kg group

a) Prostate: The findings were nearly the same as those in the 6.3g/kg group. In some cases the epithelium of X ducts completely degenerated and disappeared retaining only the basal membrane, in some other cases vacuolar degeneration and atrophy of the epithelium was noted, and in still other cases Y ducts underwent squamous metaplasia of the epithelium.

b) Testis: Degenerative cells of findings C, D, E, F and G of seminal tubules appeared somewhat more in this group than in the 6.3g/kg group. Of these, seminal tubules with coagulative necrosis and slight calcification (D) and those which consisted of only Seltori's cells (E) were relatively abundantly noted. Nevertheless, most seminal tubules were normal and such marked degeneration as indicating loss of testicular function occurred in no cases.

c) Liver: Diffuse liver atrophy occurred somewhat more frequently in this group than in the 6.3g/kg group. Irregularly-sized nuclei, congestion, and deposit of fatty droplets were extensively noted. Moreover, they were severe in degree.

d) Kidneys: Congestion was considerably severe in both sexes. Turbid swelling of main renal tubules was noted in some cases, and in females basophilic crystals were noted in all cases.

e) Spleen: Lymphatic follicles were slightly atrophied and the pulp was edematous. Giant cells and hemosiderin were not particularly evident.

f) Pancreas: Partial vacuolation of the acinus and fatty droplets occurred in a few cases, the degree being about the same as that in the 6.3g/kg group.

g) Adrenal gland: In males the blood and fat were scarce and the cortex was of light staining. Contrarily, in females, the organ was congestive and contained abundant fat.

h) Hypophysis: Main cells comprised the greater part of the parenchyma and acidophilic cells were sparse. Cells were generally full and congestion was only sporadically seen. In one case a large follicle consisting of mucoid epithelium was noted.

No specific changes were noted in other organs as brain, heart, lungs, digestive tracts, thyroid, ovary, and bone marrow.

IV. Summary

Acute, subacute and chronic toxicity tests were carried out with T-60, GBX and Cernilton using rats and mice, and the following results were obtained.

1. Acute toxicity test

The LD_{50} as determined in Donryu stain rats was high with each sample drug, and there was no difference between sexes. The lethal dose was the smallest by the intraperitoneal route with all sample drugs, whereas the LD_{50} was unobtainable by the oral and subcutaneous routes. In ddN strain mice the results were the same. As in rats, the lethal dose was the smallest by intraperitoneal route, although the sensitivity was somewhat higher than in rats. By

the oral and subcutaneous routes, GBX showed the lowest toxicity both in rats and mice. The toxicity was of the same degree both with T-60 and Cer and symptoms manifested at an early period. By the subcutaneous route, GBX exhibited the strongest toxicity both in rats and mice. The toxic symptoms seen with T-60 and Cer at a large dose were piloerection and depression occurring from immediately after to 10 min. after administration and tremor and gait disturbance after 10-30 min. In death cases these symptoms lasted 1-3 hours. Such symptoms also occurred in survivals, but they were rather slight in degree and the animals recovered in about 24 hours. With GBX no specific symptoms occurred and only piloerection, depression, emaciation, local swelling and enduration were noted by the oral and subcutaneous routes. By the intraperitoneal route slight tremor was noted additionally. In death cases food consumption and body weight decreased and animals died in 2-6 days after showing emaciation.

2. Subacute toxicity test

With T-60 suppression of weight increase appeared at a dose of 24.0g/kg in both sexes, with significant difference noted between the males and the control. Death also occurred in a few cases at this dose. With GBX suppression of weight increase occurred evidently in the males of the 10.0g/kg and 20.0g/kg groups, with significant difference from the control. Death occurred in a few cases in each of the 10.0g/kg and 20.0g/kg group. With Cer there was no marked influence noted in either sex, but in the 25.2g/kg group suppression of weight increase occurred in both sexes, with significant difference from the control in the case of females. Death occurred in 2-3 cases in each group.

Toxic symptoms were the same with all sample drugs.

General toxic symptoms appeared from the 15-20th day. Salivation, face-washing, coughing, tremor of forelimbs, and searching behavior occurred 5-20 min after administration lasting 10-20 min, and then from about 30-40 min after administration the animals gradually moved toward recovery after repeating the symptoms of coughing, face-washing, and slight tremor of forelimbs. Toward the end of administration loss of appetite, emaciation, piloerection and depression were markedly observed in all large-dose groups.

RBC, hemoglobin and WBC counts showed significant difference between experimental and control groups at times with T-60 and GBX, but not with Cer.

Results of BSP excretion test were similar with both T-60 and Cer. Namely, excretion was delayed only in males. With GBX delayed excretion was not revealed. GOT and GPT were not raised with any of the sample drugs. Total cholesterol in the T-60 administration groups and blood sugar and total protein in the GBX administration groups were higher than the control with significant difference. In the Cer administration groups total cholesterol slightly increased. Blood sugar was not found related to dosage.

In organ weight some changes were noted in the hypophysis in the Cer administration groups. The weight of the thymus tended to decrease in the experimental groups with all sample drugs. At a large dose the difference with the control was considerably great. The weight of the liver tended to increase with dosage in the GBX and Cer administration groups. It decreased, however, in the T-60 administration groups. The kidney weight increased with dosage in both sexes of the T-60 administration groups and the males of the GBX administration groups. Contrarily, it decreased in the females of the GBX administration groups and both sexes of the Cer administration groups. With regard to the adrenal gland, the weight increased with dosage in both sexes in all administration groups except in the females of the T-60 administration groups. The spleen showed no definite tendency. At a large dose, however, all administration groups showed a lower weight than the control in both sexes except in the

females of the GBX administration groups. The prostate decreased in weight as the dosage was increased in the T-60 and GBX administration groups. In the Cer administration groups the weight was greater than the control. In terms of weight per 100g of body weight, a decreasing tendency was noted in the T-60 administration groups and an increasing tendency in the GBX administration groups. In the Cer administration groups it increased slightly more than the control, but with no significant difference. The weight of the testis showed a decreasing tendency in the GBX administration groups and an increasing tendency in the Cer administration groups. The weight of the spermatocyst was decreased in the T-60 and GBX administration groups and increased in the Cer administration groups.

Pathohistological findings at a small dose were as follows. The prostate showed no great difference among the various groups receiving T-60, GBX, and Cer. The difference with the control was not great either, and there was no tendency of the development of the glandular ducts being suppressed. The testis and liver showed pathological changes in the T-60 and Cer administration groups, but not in the GBX administration groups. The kidney showed no changes with any of the sample drugs.

At a medium dose, Cer produced some changes in the glandular ducts of the prostate. With T-60 and GBX no specific changes were produced. The glandular ducts consisted mostly of high epithelium in all administration groups receiving T-60, GBX and Cer. The testes showed no changes with T-60 and GBX. However, spermatogenesis was slightly suppressed with Cer. In the liver Cer produced the least changes, followed by GBX and T-60 in that order. The kidney was not changed with GBX. The changes were approximately of the same degree and same frequency with Cer an T-60. In the hypophysis acidophilic cells were increased with Cer.

At a large dose, the prostate was composed of high epithelium in many cases in the Cer administration groups. In the T-60 and GBX administration groups dilated ducts were found mixed. Degeneration of X ducts was seen with all sample drugs. In the Cer administration groups, in particular, it occurred in nearly all cases, although the degree was about the same as that in the T-60 and GBX administration groups.

The testis was affected more in the T-60 administration groups than in the other administration groups. In the GBX administration groups it was not affected at all. The findings of the liver varied greatly among the three administration groups, the Cer administration groups showing less findings than the T-60 administration groups and the GBX administration groups showing no difference with the control group. The kidney, too, was little affected by the administration of GBX. However, congestion was seen with Cer and T-60, which occurred far more frequently with the latter. In the hypophysis congestion did not occur with Cer, but instead acidophilic cells were increased. With administration of T-60 and GBX, congestion was noted.

It can be said from the foregoing that hyperplasia of the prostate was suppressed somewhat more strongly with Cer than with T-60 and GBX, but the liver, kidney and other organs were less affected with Cer than with T-60.

3. Chronic toxicity test

In the large-dose group receiving 12.6g/kg, the animals showed normal weight increases as the control group in both sexes except that the weight increase was suppressed after the 100th day of administration. Death occurred in 1-2 cases in each group but no animals died due to drug poisoning. With administration of Cer, face-washing, coughing and general tremor of light degree were noted in the 1.6g/kg and 3.2g/kg groups from immediately after to 15 min after administration beginning from about the 90th day. In the 6.3g/kg and 12.6g/kg groups the foregoing symptoms manifested from about the 70th day; from about the 100th day tremor of forelimbs, searching behavior, coughing, face-

washing, and salivation occurred. Toward the end of administration loss of appetite, emaciation and piloerection were markedly noted in the 12.6g/kg group in both sexes. RBC and WBC counts, hemoglobin value, and WBC percentage all showed no marked alterations except that RBC and WBC counts were found increased or decreased around the 180th day in the males of the 1.6g/kg, 3.2g/kg, and 12.6g/kg groups. The urinary volume was somewhat increased, but otherwise no abnormalities were noted in urine in the experimental groups. BSP excretion and transaminase activity, too, showed no great difference between the experimental and control groups. Blood sugar was increased with dosage and significant difference was noted from the control in the case of females in the 3.2g/kg, 6.3g/kg, and 12.6g/kg groups. Total cholesterol was increased with significant difference from the control in the males of the 12.6g/kg group. Total protein was decreased in the males of the 1.6g/kg and 3.2g/kg groups, with significant difference from the control. Macroscopically, no specific changes were found in the organs. The weights of the hypophysis, prostate, thymus, adrenal gland, and spleen were decreased, especially that of the prostate which was only about 2/3 of the control.

Pathohistologically, specific findings were obtained in the prostate and testis. In the case of the prostate, when continuous administration was carried out at a small dose (1.6g/kg), the epithelium of the glandular ducts vacuolated or fell off. At a medium or large dose (3.2-12.6g/kg) degeneration, necrosis or atrophy of the epithelium occurred in many cases. Changes in the seminal ductules in the testis varied considerably. At a medium dose (3.2g/kg) thre occurred delayed spermatogenesis, increased Sertoli's cells, and suppression of maturation of spermatid. In the 6.3g/kg group, in addition to these, degeneration of spermatid was abundantly seen. In the 12.6g/kg group calcification as well as coagulative necrosis of seminal ductules was noted in many cases. In all groups, however, the seminal ductules were not all degenerated; rather, a great majority of ductules presented normal pictures. Smear specimens of the seminal vesicle, testis and epididymus showed no abnormalities, and it was unlikely that the function of the testis was impaired.

The liver showed congestion, deposit of fatty droplets and cellular atrophy in a few cases in the 1.6g/kg group. These changes intensified slightly with dosage and occurred in many cases in the 12.6g/kg group. In the kidney deposit of basophilic crystals was noted in the cortico-medullary border zone in many cases, including the control group. The signification of this manifestation is not clear. Congestion was noted in the control groups as well as in the experimental groups. In the experimental groups, however, it increased in intensity and frequency as the dosage was increased, and in the 12.6g/kg group turbid swelling of renal tubules was disclosed in some cases. Changes were also noted in other organs as the heart, lung, thymus, pancreas, and thyroid, but they were not considered due to administration of the drug since they were also noted in the control group and since the dose-response correlation was not established.

V. Discussion and Concluding Remarks

The LD_{50} of Cer, T-60 and GBX as determined in rats and mice was very large by the oral route. Moreover, toxic symptoms disappeared within a short period of time. The influence on symptoms, development, blood, and organ weight varied little with the sample drugs. However, considering that sugar was detected in the urine in the Cer 25.2g/kg group and that the blood sugar level was raised in the T-60 24.0g/kg and Cer 25.2g/kg groups, continuous administration at a large dose may cause disturbance in the metabolism of sugar. Pathohistological findings at a large dose were degeneration of the epithelium of glandular ducts, hypoplasia of sperms in the testis, fat deposit in the liver, atrophy and congestion of liver cells, and congestion and urinary casts in the kidney. Taking all findings into consideration, the influence on the prostate was the strongest

with Cer and other organs were the strongest with T-60. In chronic toxicity test where rats were used, the findings were nearly the same as those seen in the subacute toxicity test. Deposit of basophilic crystals was noted in the kidney and turbid swelling in the epithelium of the renal tubules. In the pancreas partial vacuolation, pimelosis, and atrophy of the acinus were shown, but as they were also seen in the control group, they may bear some connections with the aforementioned rise in blood sugar level.

As seen above, prolonged and missive administration of Cer may cause specific disturbances in the prostate, testis, liver, and kidney and as functional disturbance rise in blood sugar level. Yet, it must be remembered that disturbances would occur only when the drug is administered at the high dosage of 6.3g/kg or 12.6g/kg, which is about 800-1,200 times the normal human dose. On the other hand, the maximum safety dose in rats is about 3.2g/kg, or about 400 times as much as the normal human dose. From all these it is concluded that the toxic symptoms will not likely to manifest in the form of side-effects on clinical levels.

Tab. 1 Dosegs of acute toxicity.

Animal	Route	Sex	T 60	GBX	Cer
			Dose (g/kg)	Dose (g/kg)	Dose (g/kg)
Donryu rats	p.o.	♂	17.92—34.40	20.74—43.00	18.84—27.09
		♀	17.02—34.40	20.74—43.00	18.84—27.09
	s.c.	♂	7.20—14.95	12.00—21.74	10.89—15.69
		♀	——	——	——
	i.p.	♂	5.50—11.40	1.95— 6.99	3.65—13.07
		♀	——	——	——
ddN mice	p.o.	♂	25.05—39.70	39.70—79.50	31.50—46.12
		♀	17.90—44.60	33.71—83.97	18.84—39.00
	s.c.	♂	4.15—21.50	13.50—48.58	9.98—17.68
		♀	——	——	——
	i.p.	♂	3.98—20.10	1.13— 2.81	4.00—14.54
		♀	——	——	——

Tab. 2 LD 50 of T-60, GBX and Cer

( ) shows fiducial limits

animal	route	sex	Cernitin T 60	Cernitin GBX	Cernilton"
Donryu rats	p.o.	♂	34.40g/kg <	43.00g/kg <	27.01g/kg <
		♀	34.40g/kg <	43.00g/kg <	27.01g/kg <
	s.c.	♂	14.95g/kg <	20.74g/kg <	15.69g/kg <
		♀	——	——	——
	i.p.	♂	7.58g/kg (7.14— 8.04)	3.31g/kg (2.99— 3.66)	6.66g/kg (6.02— 7.35)
		♀	——	——	——
ddN mice	p.o.	♂	31.90g/kg (30.38—33.50)	55.45g/kg (49.30—62.25)	37.78g/kg (36.35—39.27)
		♀	27.75g/kg (26.66—28.89)	52.25g/kg (50.21—54.38)	27.61g/kg (26.54—28.71)
	s.c.	♂	9.47g/kg (8.78—10.21)	26.13g/kg (24.47—27.92)	13.06g/kg (12.51—13.63)
		♀	——	——	——
	i.p.	♂	8.31g/kg (7.63— 9.07)	1.72g/kg (1.64— 1.81)	6.94g/kg (6.33— 7.61)
		♀	——	——	——

Tab. 3 Food consumption during T-60, GBX, and Cernilton administration (35 days).

sample	T-60				GBX				Cernilton			
	control	6.3 g/kg	12.6 g/kg	25.2 g/kg	control	6.0 g/kg	12.0 g/kg	24.0 g/kg	control	5.0 g/kg	10.0 g/kg	20.0 g/kg
dose	18.35	14.80	14.31	8.00	17.80	14.19	17.80	7.69	19.67	16.97	14.81	12.67
	15.72	12.95	12.25	7.8	14.90	14.00	16.00	7.94	15.42	13.10	14.13	13.44

(g/animal/day)

Tab. 4 Changes in blood picture during 35 days of Cernitin T-60 administration (p.o.).
(average of five cases)

Sex	Test	Dose	Red $10^6/mm^3$	Hemoglobin g/dl	White $10^3/mm^3$	Baso-phile %	Acido-phile %	Neutrophile % staff	Neutrophile % segment	Lympho-cytes %	Mono-cytes %	Others %
♂	before	control	6.29±0.37	9.8±0.43	7.86±0.73	0.2	1.0	1.0	22.4	73.8	1.6	0
		6 g/kg	4.86±0.29	10.0±0.60	7.18±0.62	0	2.2	1.6	27.2	65.6	3.4	0
		12 g/kg	5.62±0.60	9.1±0.36	6.84±0.66	0.2	2.0	1.4	20.0	72.8	3.6	0
		24 g/kg	5.42±0.32	10.6±0.47	7.96±0.46	0.4	2.2	1.6	25.2	67.6	3.0	0
	after 35 days	control	7.13±0.47	13.8±0.25	9.7 ±0.31	0.2	0.8	1.4	26.4	69.0	2.2	0
		6 g/kg	6.11±0.26	12.4±0.45*	8.80±0.33	0.2	0.4	2.0	23.6	70.8	2.8	0.2
		12 g/kg	6.55±0.27	12.2±0.41*	10.12±0.48	0.2	1.2	1.4	22.0	73.2	2.0	0
		24 g/kg	5.80±0.23*	12.7±0.23*	8.80±0.33	0	3.0	1.4	29.0	64.4	2.2	0
♀	before	control	5.49±0.27	9.6±0.35	7.16±0.66	0.2	0.6	1.4	27.0	68.8	2.0	0
		6 g/kg	5.35±0.33	10.1±0.24	7.22±0.90	0.2	1.0	1.2	23.6	71.0	3.0	0
		12 g/kg	5.25±0.26	9.1±0.36	6.86±0.50	0.2	2.0	1.2	18.8	75.6	2.2	0.2
		24 g/kg	4.87±0.33	9.0±0.37	7.04±0.41	0	0.4	0.8	31.0	62.8	4.8	0
	after 35 days	control	6.96±0.28	13.8±0.26	7.64±0.71	0	0.8	1.4	24.8	69.2	3.8	0
		6 g/kg	6.92±0.40	13.8±0.61	9.18±0.48	0	2.8	1.2	25.4	70.0	2.6	0
		12 g/kg	6.17±0.27	12.6±0.39*	10.76±0.85*	0	1.8	1.0	26.8	67.8	2.6	0
		24 g/kg	5.95±0.34*	12.5±0.39*	9.19±0.47*	0	1.4	1.8	22.2	72.0	2.6	0

± : standard error * : $P < 0.05$ significant

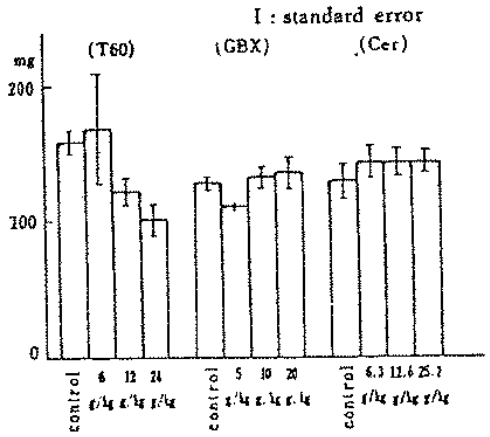

Fig. 4 Average prostate weight after 35 days T-60, GBX and CERNILTON administration (p. o.)
(Prostate weight/100 g body weight)

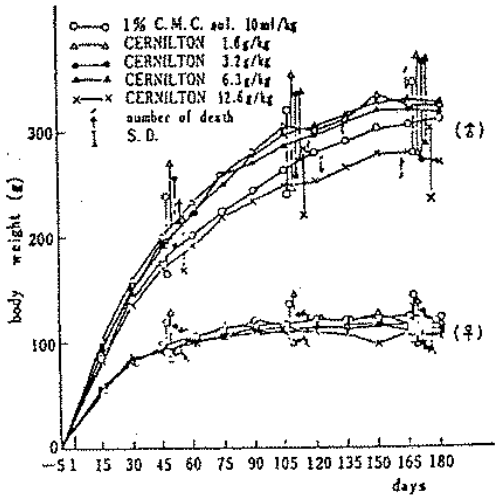

Fig. 5 Body weight increase in rats receiving CERNILTON daily for 180 days (p. o.)

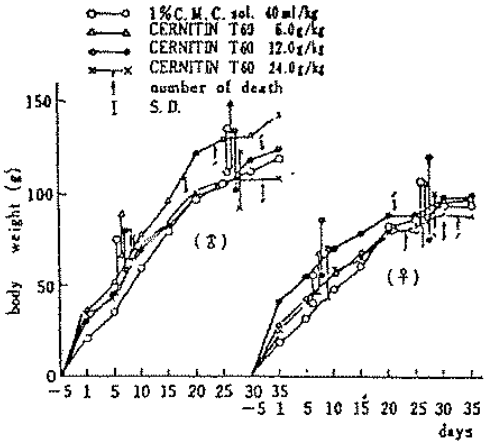

Fig. 1 Body weight increase in Rats receiving T-60 daily during 35 days. (p.o.)

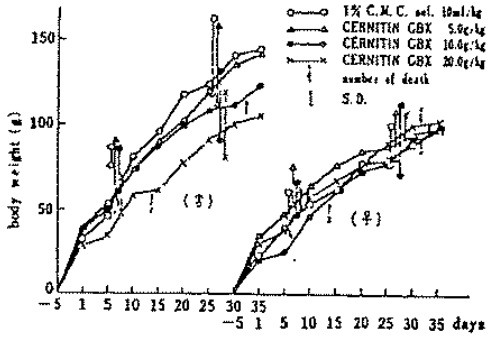

Fig. 2 Body weight increase in rats receiving GBX daily during 35 days (p. o.)

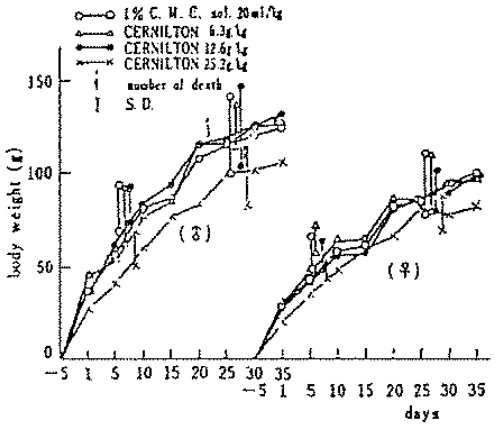

Fig. 3 Body weight increase in rats receiving CERNILTON daily during 35 days (p. o.)

Tab. 5 Changes in blood picture during 35 days of Cernitin GBX administration (p.o.)
(average of five cases)

Sex	Test	Dose	Red 10^6/mm³	Hemoglobin g/dl	White 10^3/mm³	Baso-phile %	Acido-phile %	Neutrophile % staff	Neutrophile % seg-ment	Lympho-cytes %	Mono-cytes %	Others %
♂	before	control	5.44±0.23	8.6±0.48	7.44±0.65	0.2	1.4	1.4	29.6	63.2	4.0	0.2
		5 g/kg	5.09±0.10	7.9±0.30	7.54±0.49	0	1.4	1 2	23.4	68.4	3.6	0
		10 g/kg	4.52±0.14	9.4±0.68	7.80±0.31	0.2	1.0	1.4	23.6	70.6	3.2	0
		20 k/kg	5.22±0.18	9.8±0.33	7.82±0.57	0.2	0.8	0.8	31.6	62.0	4.4	0.2
	after 35 days	control	6.01±0.20	12.5±0.30	10.38±0.25	0	1.8	1.0	27.2	66.6	2.8	0
		5 g/kg	6.07±0.40	12.5±0.26	9.98±0.94	0	2.4	1.6	22.2	72.0	1.6	0.2
		10 g/kg	5.80±0.20	12.5±0.30	9.62±0.67	0	0.8	2.4	24.0	70.4	2.4	0
		20 g/kg	5.89±0.56	12.8±0.34	8.72±0.56*	0.2	0.8	1.2	28.6	67.2	2.0	0
♀	before	control	5.95±0.16	9.5±0.41	6.40±0.25	0.4	2.0	1.6	21.4	70.0	3.4	0.2
		5 g/kg	5.32±0.45	8.0±0.27	7.64±0.48	0	1.4	0.6	14.4	70.4	5.0	0.2
		10 g/kg	6.08±0.34	9.2±0.42	6.24±0.50	0	1.6	1.0	23.8	70.4	3.2	0
		20 g/kg	5.04±0.22	10.4±0.41	7.56±0.38	0.2	0.6	2.6	27.6	64.0	4.8	0.2
	after 35 days	control	5.95±0.19	13.4±0.39	10.94±0.79	0	1.2	1.4	21.4	74.0	2.0	0
		5 g/kg	6.07±0.26	13.0±0.14	11.20±0.52	0.2	1.0	1.4	21.0	74.6	1.8	0
		10 g/kg	6.23±0.26	11.8±0.27*	8.94±0.51	0	2.0	1.6	26.4	67.6	2.2	0.2
		20 g/kg	6.27±0.25	12.5±0.43	9.42±0.53	0	1.0	0.6	21.0	75.6	1.8	0

± : standard error * : $P < 0.05$ significant

Tab. 6 Changes in blood picture during 35 days Cernilton administration (p.o.)
(average of five cases)

Sex	Test	Dose	Red 10^6/mm³	Hemoglobin g/dl	White 10^3/mm³	Baso-phile %	Acido-phile %	Neutrophile % staff	Neutrophile % seg-ment	Lympho-cytes %	Mono-cytes %	Others %
♂	before	control	5.57±0.16	10.3±0.57	9.92±0.79	0	0.8	1.2	30.6	65.6	3.8	0
		6.3 g/kg	5.70±0.28	9.2±0.30	8.18±0.23	0	0.8	1.6	24.0	70.0	3.6	0
		12.6 g/kg	5.61±0.47	10.3±0.46	8.68±0.43	0	0.8	1.4	24.6	69.2	3.8	0
		25.2 g/kg	5.04±0.22	10.4±0.41	8.56±0.38	0	0.8	0.8	23.4	74.8	1.8	0
	after 35 days	control	6.56±0.34	13.0±0.14	10.10±0.56	0	1.6	1.2	28.4	66.6	2.2	0
		6.3 g/kg	6.10±0.34	13.0±0.14	9.86±0.50	0	2.0	1.4	25.4	68.8	2.4	0
		12.6 g/kg	6.06±0.21	12.6±0.19	9.98±0.51	0.2	1.4	2.0	28.6	65.8	2.0	0
		25.2 g/kg	7.13±0.47	13.8±0.25	9.70±0.31	0	0.6	1.6	26.6	67.8	3.4	0
♀	before	control	5.65±0.20	10.0±0.53	8.56±0.88	0	0.6	1.2	25.8	68.8	3.6	0
		6.3 g/kg	6.11±0.52	9.0±0.15	8.00±0.54	0.2	0.8	1.4	26.4	69.0	2.2	0
		12.6 g/kg	5.96±0.14	10.7±0.83	7.42±0.44	0	0.8	1.6	27.0	67.0	3.6	0
		25.2 g/kg	5.19±0.25	9.7±0.44	7.18±0.21	0	1.2	1.4	26.2	68.2	3.0	0
	after 35 days	control	6.18±0.22	13.2±0.14	8.76±0.47	2.0	0	0.8	28.4	66.6	2.2	0
		6.3 g/kg	6.67±0.24	13.5±0.26	9.88±0.70	0	1.2	1.0	25.6	69.6	2.6	0
		12.6 g/kg	5.86±0.14	13.2±0.32	9.18±0.53	0	1.6	0.4	20.4	74.8	2.8	0
		25.2 g/kg	6.96±0.28	13.8±0.26	7.64±0.71	0	2.0	0.8	22.4	72.2	2.6	0

± : standard error

Tab. 7 Hepatic function on 35 th day of T-60 administration (p.o.)

Sex	Dose	BSP (%)	Transaminase (Karmen unit)		Cholesterol (mg/dl)	Blood suger (mg/dl)	Total protein (g/dl)
			GOT	GPT			
♂	control	10.4±3.8	91.8±6.0	31.3±2.2	49.7±3.4	95.6±32.5	7.1±0.3
	6 g/kg	20.5±3.0*	70.5±2.2*	24.0±1.4*	55.9±2.9	72.7± 3.8	7.4±0.2
	12 g/kg	20.5±1.7*	78.3±2.7	28.0±1.3	70.6±4.6*	69.8±26.1	7.6±0.2
	24 g/kg	13.1±2.8	68.3±0.6*	25.0±3.6	64.0±2.7*	112.8± 3.4	8.0±0.1*
♀	control	12.1±1.4	71.2±2.4	25.6±0.6	58.3±4.9	88.5±16.5	7.6±0.2
	6 g/kg	12.6±1.5	70.3±3.5	24.3±1.5	56.0±3.6	55.7± 0.6	7.4±0.1
	12 g/kg	8.2±2.0	67.0±4.4	25.5±1.8	51.1±1.9	133.4±12.6	7.8±0.1
	24 g/kg	8.8±2.8	71.0±5.6	31.3±7.1	40.0±3.2*	100.4±36.6	7.6±2.5

± : standard error * : P <0.05 significant

Tab. 8 Hepatic function on 35 th day of GBX administration (p.o.)

Sex	Dose	BSP (%)	Transaminase (Karmen unit)		Cholesterol (mg/dl)	Blood suger (mg/dl)	Total protein (g/dl)
			GOT	GPT			
♂	control	23.7±2.7	85.4±3.3	28.4±1.9	54.2±4.1	72.8± 1.8	7.4±0.2
	5 g/kg	9.2±2.1*	92.0±3.0	31.3±4.3	56.5±1.6	141.4±14.5*	7.8±0.1*
	10 g/kg	14.1±3.1*	84.8±6.0	29.0±1.8	60.5±1.9	124.9±17.9	7.5±0.1
	20 g/kg	7.8±5.0*	78.8±0.8	29.0±1.5	57.1±3.1	136.9±12.9*	7.2±0.2
♀	control	8.0±1.3	73.8±4.1	22.8±0.8	50.2±3.6	76.8± 6.0	7.0±0.2
	5 g/kg	5.6±1.0	74.5±7.3	29.5±1.0*	49.2±2.6	100.9±12.1	7.7±0.2*
	10 g/kg	4.2±1.0*	66.0±2.6	25.7±1.5	36.6±3.8*	129.0± 8.1*	7.7±0.2*
	20 g/kg	6.0±1.5	70.3±3.8	26.5±3.0	40.4±1.8	133.4± 9.1*	7.5±0.2

± : standard error * : P <0.05 significant

Tab. 9 Hepatic function on 35 th day of Cernilton administration (p.o.).

Sex	Dose	BSP (%)	Transaminase (Karmen unit)		Cholesterol (mg/dl)	Blood suger (mg/dl)	Total protein (g/dl)
			GOT	GPT			
♂	control	10.4±3.8	91.8±6.0	31.3±2.2	49.7±3.4	95.6±32.5	7.1±0.3
	6.3 g/kg	15.1±3.8	78.8±3.3	28.8±1.9	51.5±2.8	56.2±11.4	7.8±0.1
	12.6 g/kg	14.0±4.4	78.5±2.9	24.0±0.8*	55.3±5.5	59.4± 6.8	7.5±0.2
	25.2 g/kg	18.0±6.6	71.0±1.2*	26.8±1.5*	60.8±6.0	111.8± 5.8	7.6±0.1
♀	control	12.1±1.4	71.2±2.4	25.6±0.6	58.3±4.9	88.5±16.5	7.5±0.5
	6.3 g/kg	7.6±1.1*	68.4±4.2	23.2±1.2	66.2±3.6	76.5±14.6	7.6±0.1
	12.6 g/kg	7.5±1.9	77.3±4.6	26.3±1.6	63.7±6.4	72.8±14.1	7.7±0.1
	25.2 g/kg	9.4±2.2	63.8±3.8	21.3±1.3	74.8±4.6*	117.1± 7.3	7.4±0.4

± : standard error * : P <0.05 significant

Tab. 10 Average organ weight after 35 days

Sex	Dose	Hypophisis (mg)	Thymus (mg)	Heart (mg)	Liver (g)	Kidney (mg)	
						L.	R.
♂	control (8)	9.0±0.3	323.9±32.5	818.3±39.0	6.90±0.41	808.5±39.0	817.4±34.7
	6 g/kg (9)	9.4±0.8	374.0±42.4	924.0±30.1*	7.74±0.39	922.9±27.2*	879.7±31.7
	12 g/kg (9)	10.9±0.4*	335.1±19.1	878.9±23.8	6.90±0.25	795.7±24.5	807.9±19.3
	24 g/kg (7)	8.7±0.4	218.6±20.6*	715.9±23.3*	6.58±0.21	678.1±18.4*	674.6±21.6*
♀	control (10)	12.0±0.5	437.7±33.0	754.1±19.3	6.59±0.32	730.3±26.2	738.4±25.4
	6 g/kg (9)	10.7±1.8	440.4±26.4	812.7±28.8	6.38±0.16	778.0±31.1	752.3±37.5
	12 g/kg (9)	11.2±0.8	327.3±23.8*	713.3±24.5	6.58±0.24	744.9±35.8	733.4±37.6
	24 g/kg (7)	18.1±1.9*	347.0± 9.1*	655.9±28.1*	6.03±0.44	686.3±30.6	659.4±30 3

± : standard error * : P<0.05 significant ( ) : number of cases

Tab. 11 Average organ weight after 35 days

Sex	Dose	Hypophisis (mg)	Thymus (mg)	Heart (mg)	Liver (g)	Kidney (mg)	
						L.	R.
♂	control (10)	8.5±0.5	359.3±18.4	904.7±29.0	7.22±0.30	901.2±27.1	860.5±24.8
	5 g/kg (9)	7.8±0.5	338.8±28.6	901.2±30.0	8.21±0.25*	885.1±33.1	863.2±29.6
	10 g/kg (8)	9.8±0.4*	347.9±14.3	878.9±34.7	8.29±0.26*	877.0±31.3	860.3±36.6
	20 g/kg (9)	8.6±0.9	298.4±26.1	794.7±30.9*	8.83±0.29*	828.9±21.5*	806.2±26.7
♀	control (10)	12.4±2.7	408.6±10.0	765.0±24.5	6.61±0.39	756.8±34.6	737.4±16.4
	5 g/kg (9)	12.8±1.0	383.2±33.3	794.6±26.2	7.74±0.35*	731.2±30.3	716.9±34.2
	10 g/kg (7)	12.7±1.1	388.9±24.6	782.6±27.2	8.10±0.24*	838.1±32.6	816.9±26.9
	20 g/kg (7)	12.6±0.5	384.1±21.1	837.0±39.2	8.81±0.51*	917.3±41.0*	837.0±33.3

± : standard eror * : P<0.05 significant ( ) : number of cases

Tab. 12 Average organ weight after 35 days

Sex	Dose	Hypophisis (mg)	Thymus (mg)	Heart (mg)	Liver (g)	Kidney (mg)	
						L.	R.
♂	control (9)	9.0±0.3	323.9±32.5	818.3±39.0	6.90±0.41	808.5±39.0	817.4±34.7
	6.3 g/kg (9)	9.7±0.4	343.0±13.2	886.2±20.1	7.07±0.12	858.8±20.6	836.2±17.0
	12.6 g/kg (8)	10.1±0.4*	328.6±18.3	834.8±31.1	7.43±0.30	888.1±25.9	849.5±35.7
	25.2 g/kg (10)	11.2±0.4*	353.5±17.7	851.5±29.5	10.40±0.31*	1043.4±32.6	1035.7±30.0*
♀	control (10)	12.1±0.8	403.6±19.5	735.3±22.9	6.59±0.27	730.3±23.1	738.4±38.2
	6.3 g/kg (9)	15.6±1.1*	419.1±50.1	788.7±28.8	6.79±0.23	752.0±22.6	761.3±34.4
	12.6 g/kg (9)	14.4±1.3	385.1±24.0	789.0±29.3	6.76±0.22	763.0±26.7	751.0±26.4
	25.2 g/kg (10)	10.9±0.4	280.1±14.0*	709.0±15.8	9.24±0.30*	814.2±29.3	760.8± 8.4

± : standard error * : P<0.05 significant ( ) : number of cases

Cernitin T-60 administration (p.o.).

Adrenal (mg)		Spleen (mg)	Prostate (mg)	Testis (mg)	Epididymis (mg)	Seminal vecicle (mg)
L.	R.					
15.9±1.4	16.8±1.0	492.8±43.7	390.7±38.0	1029.4±29.6	434.3±28.3	695.6±101.2
20.3±1.9	17.2±2.0	588.2±20.2	405.6±40.1	1014.5±54.1	429.9±18.2	842.2±132.8
21.2±1.6*	20.6±1.0*	485.3±25.5	273.0±16.9*	1009.7±75.0	463.1± 9.9	649.4± 20.2
20.1±0.9*	19.1±0.9	419.0± 5.7	209.4±28.2*	1014.0±45.0	411.6±51.6	467.9± 29.1*
33.2±0.5	32.5±1.2	426.5±50.7				
38.8±1.6*	39.3±1.8*	467.6±25.9				
31.9±2.4	31.0±2.3	494.0±41.4				
32.1±1.6	28.3±3.0	338.9±36.6				

Cernitin GBX administration (p.o.).

Adrenal (mg)		Spleen (mg)	Prostate (mg)	Testis (mg)	Epididymis (mg)	Seminal vecicle (mg)
L.	R.					
13.7±1.2	11.5±1.1	553.5±28.2	306.7±18.1	1072.7±26.7	504.2± 9.6	741.2±74.0
16.0±0.5	14.2±0.5*	491.0±19.3	271.9±14.5	1068.6±45.4	404.8±12.4*	618.6±55.1
16.5±0.7	15.0±0.8*	477.1±27.6	300.0±24.7	1049.0±37.3	428.8±10.3*	645.8±45.3
19.1±0.8*	17.0±0.7*	432.1±77.4	280.0±28.3	1002.0±20.3*	501.8±51.4	408.1±17.8*
34.8±1.8	34.9±1.4	504.6±29.2				
38.2±1.4	39.7±3.8	532.7±40.0				
34.9±2.3	37.7±3.1	507.0±31.6				
42.6±3.0*	38.4±2.1	543.0±21.6				

Cernilton administration (p.o.).

Adrenal (mg)		Spleen (mg)	Prostate (mg)	Testis (mg)	Epididymis (mg)	Seminal vecicle (mg)
L.	R.					
15.9±1.4	16.8±1.0	492.8±43.7	290.7±38.0	1029.4±29.6	434.3±28.3	695.6±101.2
14.8±0.8	14.1±0.8*	488.4±13.8	322.6±22.1	1053.4±23.3	433.9± 8.0	715.3± 50.2
19.8±0.5*	19.5±0.5*	533.1±31.2	322.8±23.6	1115.6±24.3*	481.6± 9.7	894.3± 55.3
27.7±0.4*	27.1±0.3*	474.0±23.6	320.2±19.7	1150.4±30.2*	460.2±14.6	864.6± 56.8
34.2±1.6	28.8±1.7	474.0±29.1				
33.3±2.4	34.6±1.7*	537.7±49.7				
40.7±2.2*	38.7±1.0*	451.6±21.9				
39.3±1.2*	41.3±1.5*	392.3±15.6*				

Tab. 13 Food consumption during Cernilton administration (180 days). (g/animal/day)

Sex	Months / Dose	before	1	2	3	4	5	6
♂	control	14.92	16.01	20.00	18.90	20.40	20.03	19.81
	1.6 g/kg	16.58	20.20	22.09	20.94	21.03	21.43	19.10
	3.2 g/kg	15.82	19.32	19.64	21.95	20.65	18.57	18.19
	6.3 g/kg	16.54	18.84	19.04	19.56	17.76	17.79	14.40
	12.6 g/kg	15.38	15.85	14.85	16.03	14.00	15.10	14.19
♀	control	13.92	14.22	13.68	12.66	13.13	10.39	12.41
	1.6 g/kg	13.66	14.06	13.57	12.62	12.76	13.50	12.87
	3.2 g/kg	13.68	14.39	12.38	11.85	11.76	12.17	12.14
	6.3 g/kg	14.40	14.79	12.19	11.48	11.27	10.99	11.41
	12.6 g/kg	14.14	13.24	10.23	10.16	10.14	9.70	8.91

Tab. 14 Changes in blood picture during 180 days Cernilton administration (p.o.). (male rats)

Test	Dose	Red 10^6/mm³	Hemoglobin g/dl	White 10^3/mm³	Baso-phile %	Acido-phile %	Neutrophile staff	Neutrophile segment	Lympho-cytes %	Mono-cytes %	Others %
before	control	6.53±0.44	10.6±0.28	12.80±0.10	0	1.2	1.0	31.2	62.8	3.8	0
	1.6 g/kg	7.35±0.27	10.3±0.35	11.84±0.82	0	2.2	1.0	30.0	65.4	3.2	0.2
	3.2 g/kg	6.87±0.37	10.6±0.26	10.84±0.98	0	1.4	1.2	28.8	66.4	2.2	0
	6.3 g/kg	6.80±0.84	10.1±0.38	10.28±1.03	0	1.2	1.6	31.0	62.2	3.8	0.2
	12.6 g/kg	7.03±0.34	9.8±0.24	10.36±0.71	0	1.0	0.8	25.2	70.0	3.0	0
after 90 days	control	7.57±0.24	13.4±0.31	10.42±1.35	0.2	3.6	0.8	27.6	66.4	1.4	0
	1.6 g/kg	7.40±0.26	13.3±0.25	10.36±0.71	0.2	2.0	0.8	25.0	71.6	0.4	0
	3.2 g/kg	8.01±0.36	13.1±0.08	9.36±1.24	0.2	2.8	1.0	22.8	71.0	2.2	0
	6.3 g/kg	7.89±0.23	12.3±0.23*	8.86±0.60	0	1.6	2.2	28.2	77.6	2.4	0
	12.6 g/kg	8.23±0.28	13.2±0.17	12.3 ±0.90	0.2	3.2	0.6	28.0	65.8	3.0	0.2
after 180 days	control	7.11±0.52	11.6±0.78	12.00±0.55	0	1.8	1.6	30.2	64.8	1.6	0
	1.6 g/kg	6.91±0.52	12.6±0.96	7.98±0.28*	0	1.6	1.0	30.4	65.8	1.2	0
	3.2 g/kg	7.07±0.24	12.6±0.44	9.30±0.70*	0	1.8	1.0	25.6	69.6	1.8	0.2
	6.3 g/kg	6.55±0.41	12.8±0.32	10.64±1.06	0	1.2	0.4	29.8	66.2	2.4	0
	12.6 g/kg	6.54±0.49	12.5±0.69	9.52±0.76*	0.2	1.2	0.8	22.6	72.6	2.6	0

± : standard error * : $P < 0.05$ significant

Tab. 15 Changes in blood picture during 180 days Cernilton administration (p.o).
(female rats)

Test	Dose	Red 10^6/mm³	Hemoglobin g/dl	White 10^3/mm³	Baso-phile %	Acido-phlie %	Neutrophile %		Lympho-cytes %	Mono-cytes %	Others %
							staff	seg-ment			
before	control	7.35±0.43	11.6±0.35	12.00±0.99	0	2.4	1.0	25.2	67.2	4.0	0.2
	1.6 g/kg	7.35±0.52	11.8±0.36	10.44±0.87	0	2.6	1.2	32.4	59.2	4.6	0
	3.2 g/kg	6.94±0.44	11.2±0.36	8.28±0.38	0	0.8	1.2	24.8	69.8	3.2	0.2
	6.3 g/kg	6.84±0.34	10.9±0.13	10.44±0.53	0	2.2	1.2	20.0	73.4	2.8	0.4
	12.6 g/kg	6.64±0.48	10.9±0.42	12.08±1.25	0.4	2.6	1.0	25.4	67.0	3.6	0
after 90 days	control	7.12±0.55	12.0±0.40	11.00±1.39	0	2.2	0.6	26.0	68.0	4.0	0
	1.6 g/kg	7.95±0.27	11.2±0.23	10.46±0.36	0	3.4	0.2	29.0	65.4	2.0	0
	3.2 g/kg	8.39±0.25	12.3±0.55	10.84±0.60	0.6	2.2	0.4	28.2	65.8	2.8	0
	6.3 g/kg	8.09±0.35	12.3±0.34	8.70±0.67	0	1.6	0.2	22.2	72.0	4.0	0
	12.6 g/kg	7.53±0.23	12.1±0.35	11.20±0.81	0	4.6	0	28.2	65.2	2.0	0
afte 180 days	control	7.19±0.15	13.2±0.50	8.24±0.62	0	0.8	1.4	38.0	58.6	1.0	0.2
	1.6 g/kg	7.37±0.35	14.5±0.57	9.44±0.38	0	1.2	0.6	24.0	72.6	1.6	0
	3.2 g/kg	7.42±0.42	12.4±0.28	9.48±0.73	0	2.2	0.6	28.2	68.0	1.0	0
	6.3 g/kg	6.57±0.33	12.7±0.26	10.16±0.77	0	1.6	1.6	31.4	63.2	2.2	0
	12.6 g/kg	6.03±0.32*	12.6±0.34	9.50±0.64	0.2	4.8	1.8	28.0	65.6	2.0	0

± : standard error * : $P < 0.05$ signficant

Tab. 16 Hepatic function on 180 th day Cernilton administration (p.o.)

Sex	Dose	BSP (%)	Transaminase (Karmen unit)		Cholesterol (mg/dl)	Blood surger (mg/dl)	Total protein (g/dl)
			GOT	GPT			
♂	control	46.7±4.3	70.2± 7.2	41.4±6.3	96.4± 4.6	84.4±19.2	8.6±0.2
	1.6 g/kg	55.9±3.8	75.8± 2.1	31.5±1.0	68.1± 3.4*	108.5± 7.6	7.9±0.1*
	3.2 g/kg	50.7±8.0	64.8± 1.8	28.5±1.3	80.5±10.0	95.1± 6.4	7.8±0.2*
	6.3 g/kg	46.5±4.3	85.8± 6.5	33.8±5.3	93.7±10.7	97.8± 6.3	8.4±0.1
	12.6 g/kg	47.6±4.4	67.8± 4.2	34.0±4.1	144.2±20.2*	113.5± 8.9	8.9±0.3
♀	control	50.4±7.2	80.6± 5.7	29.2±0.8	120.1±10.6	88.3± 3.8	8.9±0.3
	1.6 g/kg	44.5±7.0	74.8± 4.1	28.0±1.1	106.8± 8.6	80.6± 8.4	9.1±2.8
	3.2 g/kg	29.7±1.9*	87.2±13.6	40.0±7.7*	106.2± 8.8	100.0± 2.3*	8.8±2.1
	6.3 g/kg	41.5±4.4	66.8± 5.5	21.8±5.7	99.7± 7.9	163.0±13.4*	8.3±0.2
	12.6 g/kg	50.2±4.2	64.6± 2.5*	35.0±3.8	140.0± 8.3	169.8±12.0*	9.0±0.1

± : standard error * : $P < 0.05$ significant

Tab. 17 Average organ weight after 180 days Cernilton administration (p.o.)

Sex	Dose	Hypophisis (mg)	Thymus (mg)	Heart (mg)	Liver (g)	Kidney (mg)		Adrenal (mg)		Spleen (mg)	Prostate (mg)
						L.	R.	L.	R.		
♂	control (10)	11.5 ±0.5	302.2 ±13.6	1333.5 ±66.9	11.09 ±0.47	1488.9 ±69.3	1427.2 ±61.9	21.9 ±1.0	22.6 ±0.9	755.1 ±56.4	753.8 ±69.0
	1.6 g/kg (9)	12.6 ±0.6	277.5 ±14.1	1419.3 ±38.7	11.66 ±0.49	1396.8 ±65.1	1336.9 ±59.8	23.6 ±0.9	24.0 ±1.1	774.4 ±29.9	659.9 ±75.5
	3.2 g/kg (9)	10.3 ±1.1	250.4 ±22.3	1457.4 ±28.2	11.42 ±0.37	1468.1 ±42.7	1410.9 ±40.2	26.9 ±1.8*	28.1 ±1.1*	794.6 ±52.7	500.8 ±37.3*
	6.3 g/kg (9)	11.0 ±0.4	237.9 ±25.3*	1469.6 ±25.3	11.67 ±0.31	1543.1 ±32.9	1491.8 ±21.8	25.6 ±1.1*	23.1 ±1.1	797.8 ±32.8	491.4 ±45.1*
	12.6 g/kg (8)	9.6 ±0.5*	189.5 ±16.8*	1264.3 ±32.5	10.37 ±0.35	1303.3 ±53.1*	1211.6 ±39.3*	23.4 ±1.4	22.9 ±1.8	562.4 ±16.5*	403.8 ±35.3*
♀	control (10)	14.4 ±1.4	196.8 ±12.1	824.1 ±15.7	6.71 ±0.34	784.7 ±31.0	784.9 ±27.8	27.9 ±0.7	28.5 ±1.2	460.0 ±21.0	
	1.6 g/kg (9)	11.1 ±0.4*	200.7 ± 3.8	907.4 ±35.2*	7.09 ±0.28	811.5 ±23.6	852.1 ±70.8	32.4 ±1.4*	33.2 ±1.3*	504.0 ±15.8	
	3.2 g/kg (10)	11.2 ±0.4*	195.8 ±10.4	888.3 ±28.2	9.27 ±0.26*	870.4 ±30.6	837.8 ±21.4	29.2 ±1.1	27.4 ±1.3	543.4 ±22.2*	
	6.3 g/kg (10)	11.2 ±0.4*	179.0 ± 9.2	822.9 ±12.3	7.01 ±0.13	769.4 ±16.2	763.2 ±19.9	29.7 ±0.9	30.3 ±0.8	542.2 ±21.4*	
	12.6 g/kg (10)	10.4 ±0.3*	163.3 ± 8.5*	863.1 ±21.3	7.19 ±0.20	770.5 ±25.2	771.5 ±18.7	28.2 ±0.5	25.5 ±1.1	607.0 ±45.0*	

±: stadard error *: $P < 0.05$ significant ( ): number of cases

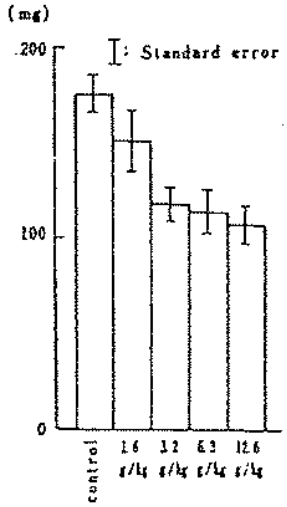

Fig. 6 Average prostate weight after 180 th day CERNILTON administration (p. o.) (prostate weight/100 g body weight)

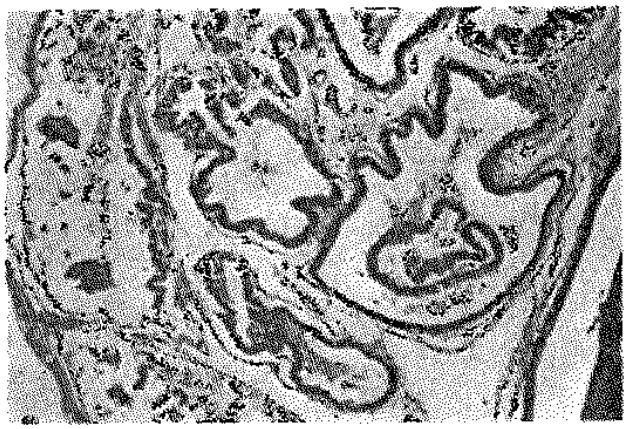

Photo 1. Prostate. Cernilton 12.6g/kg group, male (survival). Glandular ducts A (signifying Undilated glandular ducts with relatively thick epithelium which creased and protruded in the ducts like papilloma) were slightly dilated and the epithelium partially fell off and disappeared.

Photo 2: Prostate. Cernilton 12.6g/kg group, male (dead, 124th day). Glandular ducts B (signifying markedly dilated glandular ducts whose epithelium underwent squamous metaplasia) were partially but markedly atrophied in the epithelium.

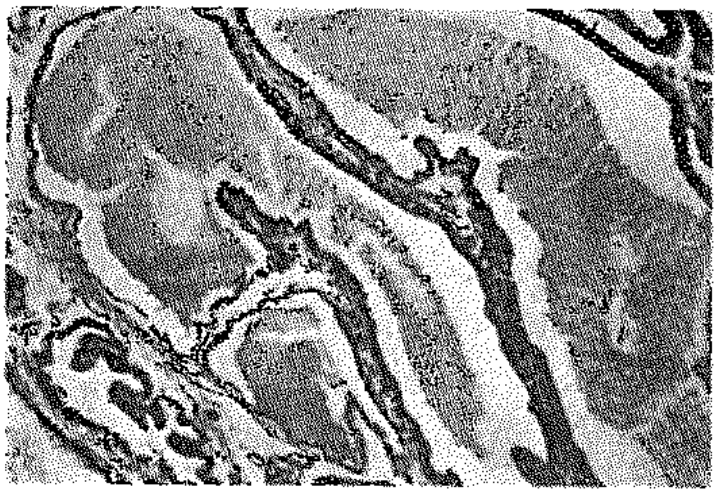

Photo 3. Prostate. Cernilton 12.6g/kg group, male (survival). Glandular ducts A were extensively degenerated and atrophied.

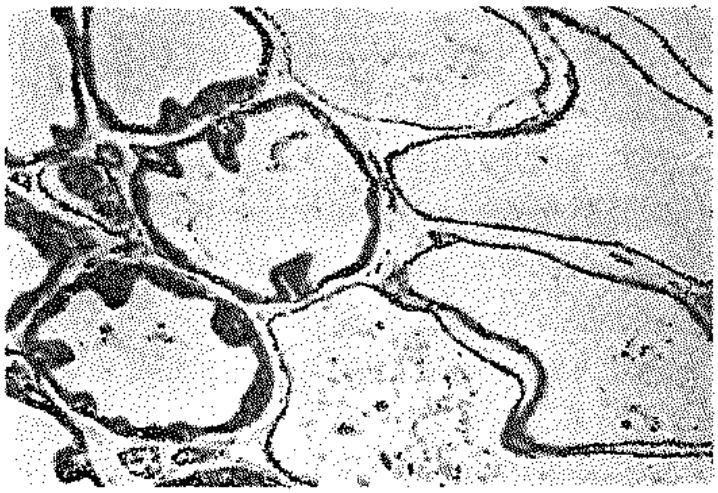

Photo 4. Prostate. Cernilton 12.6g/kg group, male (survival). Extensive vacuolation of the epithelium.

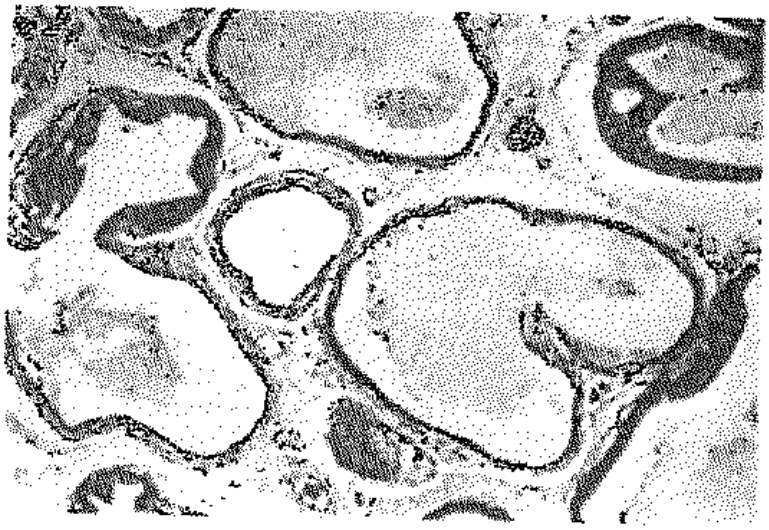

Photo 5. Prostate. Cernilton 12.6g/kg group, male (survival). Glandular ducts A were slightly dilated. Adjacent to them were dilated ducts that lost epithelium.

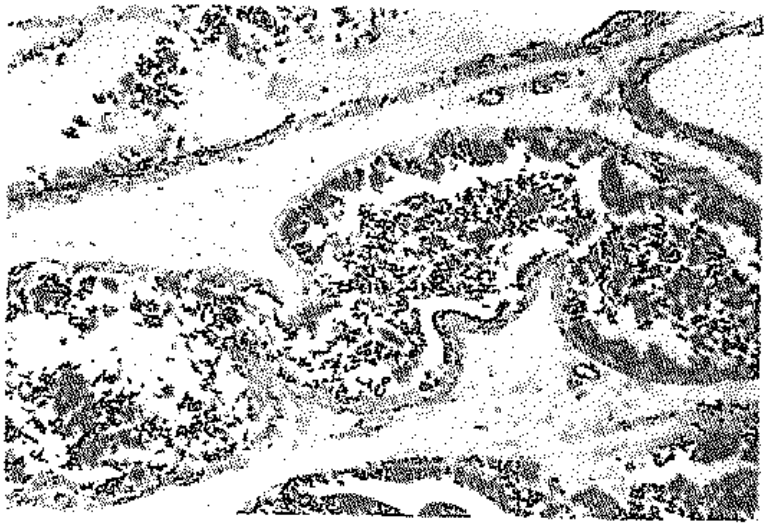

Photo 6. Prostate. Cernilton 6.3g/kg group, male (survival). Glandular ducts that lost most of the internal integument of the epithelium.

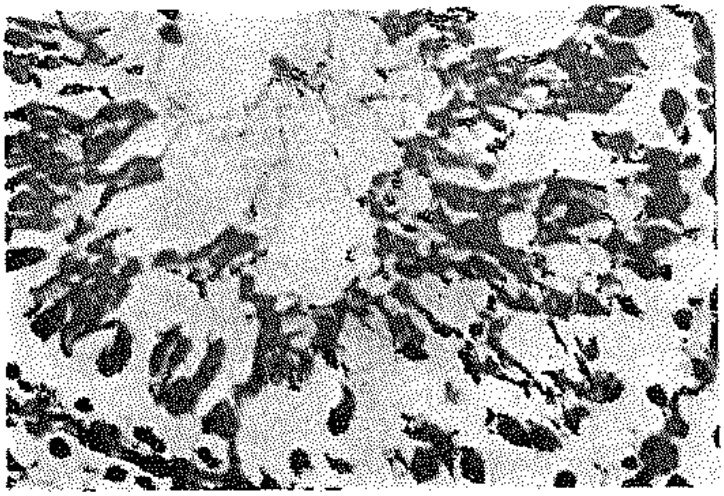

Photo 7. Testis. Cernilton 12.6g/kg group, male (survival). Spermatids showed hypoplasia and only Sertoli's cells were conspicuously seen. Suppression of naturation was of course noted.

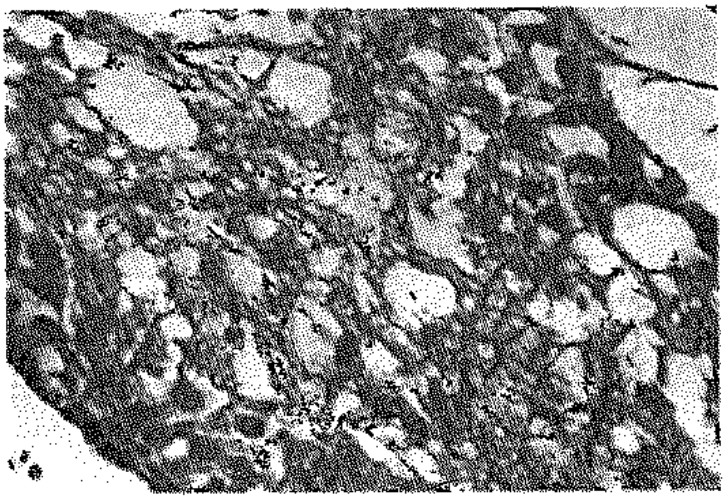

Photo 8. Testis. Cernilton 12.6g/kg group, male (survival). Maturation of spermatids was suppressed and the ducts underwent coagulative necrosis.