UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF LITHIUM CARBONATE ON CERTAIN HAEMATOLOGICAL AND URINE PARAMATERS IN MACACA FASCICULARIS

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ABSTRACT

A group of 6 monkeys (Macaca fascicularis) were used to determine the effect of lithium carbonate on certain haematological and urine parameters. Two monkeys were given the drug orally and one intraperitoneally. Three monkeys were not treated and served as controls. Blood and urine samples were collected. The blood urea nitrogen (BUN) concentrations, packed cell volume (PCV), total and differential white blood cell (WBC) counts, red blood cell (RBC) counts, urine protein and creatinine concentrations, and urine protein-to-creatinine ratios were determined on these samples.

Intraperitoneal lithium carbonate did not produce any specific pattern in urine protein-to-creatinine ratio, although there were marked elevations in BUN and urine creatinine concentrations. In contrast to the dogs, urine protein-to-creatinine ratio may not be useful parameter as an indicator of renal damage in monkeys. This experiment suggests that BUN concentration may still be a more accurate indicator of renal damage than the urine protein-to-creatinine ratio in monkeys.

It was found that oral lithium carbonate increased circulating white blood cell counts especially neutrophils. Other haematological

parameters did not show any observable change. It can be suggested that oral lithium carbonate may be used in alleviating neutropenic states in monkeys.

ASTRAK

Enam ekor kera *(Macaca fascicularis)* digunakan untuk menentukan kesan lithium karbonat ke atas parameter-parameter darah dan urin. Dua ekor kera diberi lithium karbonat melalui mulut manakala seekor lagi diberi secara intraperitonium. Tiga ekor kera lain tidak diberi lithium karbonat dan diguna sebagai kumpulan kontrol. Darah dan urin dikumpul. Konsentrasi urea nitrogen darah (BUN), isipadu sel padat (PCV), bilangan dan kiraan diferensial sel darah putih (WBC), bilangan sel darah merah (RBC), konsentrasi-konsentrasi protin dan kreatinin urin, dan nisbah protin-kreatininnya ditentukan.

Lithium karbonat yang diberi secara intraperitonium tidak menunjukkan apa-apa corak spesifik ke atas nisbah protin-kreatinin urin, walaupun konsentrasi BUN dan kreatinin urin menunjukkan peningkatan yang nyata. Berbeza dengan anjing, nisbah protin-kreatinin urin mungkin bukanlah satu parameter yang berguna sebagai penunjuk kerosakkan ginjal dalam kera. Percubaan ini mencadangkan bahawa bagi kera konsentrasi BUN adalah penunjuk yang lebih tepat daripada nisbah protin-kreatinin untuk kerosakkan ginjal.

Lithium karbonat diberi melalui mulut didapati meningkatkan bilangan sel darah putih, khususnya

nutrofil, dalam edaran darah. Parameter lain tidak menunjukkan perubahan yang dapat dikesan. Adalah dicadangkan lithium karbonat jika diberi melalui mulut bolih diguna untuk mengatasi keadaan nutropenia dalam kera.

INTRODUCTION

Determination of blood urea nitrogen (BUN) had long been used as an indicator of renal damage in human as well as in animals. There had been continuous studies carried out in laboratory animals to relate renal damage with certain urinary and haematological parameters.

It is a fact that lithium carbonate has a predilection towards bone marrow and kidney. In human, lithium carbonate stimulates pluripotential stem cell (CFU-s) proliferation which was reflected as neutrophilia in blood circulation. The drug can cause acute renal damage and this may be reflected by the changes in the urine and haematological parameters.

Urine protein-to-creatinine ratio of a single spontaneously micturited urine sample in the determination of true proteinuria were earlier suggested for dogs (White et al., 1984).This ratio was suggested since estimation of 24-hour urine concentration directly is time consuming and cumbersome. Thus in this experiment, the usefulness of using the same ratio in drug-induced proteinuria associated with renal damage in monkeys, was examined.

The effect of lithium carbonate on monkeys is relatively unknown. This experiment was designed to study the effect of lithium carbonate on certain haematological and urine parameters in Macaca fascicularis.

The objectives of this study were:

1. To gain experience in the performance of a controlled experiment involving monkeys.
2. To compare the changes in haematological and urinary parameters following lithium carbonate administration.
3. To compare the acute effect of a single dose of lithium carbonate administered orally and intraperitoneally on certain parameters.
4. To determine the possible use of urine protein-to-creatinine ratio in the assessment of renal damage.

This preliminary study in monkeys showed that the urine protein-to-creatinine ratio may not be useful as an indicator to renal disorders. Oral administration of lithium carbonate stimulated leucocytosis especially neutrophilia while intraperitoneal route did not show any characteristic pattern . The BUN concentrations were markedly elevated in monkeys given intraperitoneal lithium carbonate . In monkeys receiving the drug orally, the increase in BUN concentrations was only marginally higher than control animals . The results obtained in this study is consistent with those obtained in human.

LITERATURE REVIEW

Lithium carbonate, is a drug used extensively to treat manic psychosis in human (Venugopal and Luckey, 1978). It is also used in treatment of aggressive behavioural disorders in adolescents in chicken (Lazanoff et al., 1985). Lithium administration at therapeutic level (Ø.2 - 2.Ø meq/L) increased growth differentials between proximal and distal epiphysis (Lazanoff et al., 1985). The inclusion of lithium carbonate into complex therapy of ischemic heart disease decreased sympathetic manifestation and was not accompanied by any specific complications (Kurbanov et al., 1984). Lithium carbonate, when administered orally or intraperitoneally at doses higher than the therapeutic dose can cause acute renal toxicity (Kosuda et al., 1984). However, renal deterioration was not observed in human patients with chronic use of lithium carbonate therapy, although there was a tendency for older patients to have higher BUN and blood creatinine concentrations (Hwu et al., 1981).

Determination of BUN has long been used as an indicator of renal damage in human as well as in animals. The major disadvantage in the use of BUN is that, it is not a clear indicator of renal damage in cases of starvation, excessive protein intake, and excessive muscular damage (Duncan and Presse, 1977). In fact, urine creatinine in many respects may be a better parameter to use to assess renal damage. Recently, it was suggested that urine protein-to-creatinine ratio in a single specimen may be a better method for the detection of renal disorders. In dogs this ratio was found to be a sensitive, rapid and dependable diagnostic technique for the detection and quantitative estimation of proteinuria (White et al., 1984).However, there is no data to suggest that this ratio is as useful in the determination of renal disorders in non-human primates.

Lithium carbonate increases circulating polymorphonuclear leucocytes particularly the neutrophils (Greco and Brereton, 1977; Perez-munoz, et al., 1981). The effect of lithium on circulating white blood cells is a result of its effect on the bone marrow (Gomez et al., 1984). The influence of lithium carbonate on haematopoeitic stem cell proliferation in bone marrow occurs mainly at the colony forming unit (pluripotential stem cell or CFU-s) level (William and Dale, 1980; Gallicchio and Chen, 1981; Ninane et al., 1984).

Previous studies on the effects of lithium carbonate on haematological and urine parameters were performed mainly in dogs and humans. There were few studies on the effects of this drug on these parameters in monkeys. We undertook this study to determine the effects of lithium carbonate on certain haematological and urine parameters in monkeys. In this experiment six Macaca fascicularis were selected from a colony of 30 monkeys in primate unit, University Pertanian Malaysia.

MATERIALS AND METHODS

Animals

A total of six monkeys (long tailed macaque or Macaca fascicularis) which is commonly known as 'kera' were used in this study. They were females weighing between 1 to 2 kg and ranging between 2 to 3 1/2 years old.

Lithium Carbonate Adminstration

These monkeys were randomly selected from the primate unit, Universiti Pertanian Malaysia, Serdang, Selangor, and placed in individual metabolic cages equipped with urine collection funnels. These monkeys were on a normal diet of banana, papaya, bread, and monkey pellets. Two monkeys were given lithium carbonate suspension (0.5 gm in 5 ml distilled water/animal) orally with the aid of stomach tube (3 to 4 mm in diameter) and another intraperitoneally using a 21 G needle to induce renal damage. The remaining three monkeys did not receive any treatment and served as controls.

Blood Samples

The monkeys were restrained in a squeeze cage and anaesthetised with 10 mg ketamine (Ketelar, Parke-Davis) per kg body weight, intramuscularly. Two to three milliters of venous blood samples were collected daily in EDTA venoject tubes, for 5 days, alternately from right and left femoral vein. For the subsequent 6 days, blood was collected on alternate days. The blood samples were thoroughly mixed and the packed cell volume (PCV), plasma protein concentration (PP), complete blood count, and differential white blood cell counts were determined immediately. After these determinations the remaining blood sample was then spun at 1800 x g for 10 minutes, and plasma collected and frozen. Blood urea nitrogen concentration was determined spectrophotometrically using a standard diagnostic kit (Roche (M) Sdn. Bhd., 46870 Petaling Jaya, Selangor).

Urine Samples

Approximately 10 ml of spontaneous urine sample was also collected from each monkey in bottles. Urine samples were then spun at 1800 x g for 10 minutes and the supernatant collected and frozen. Protein and creatinine concentrations, and their ratios were determined on thawed urine samples using standard diagnostic kits (Roche (M) Sdn. Bhd., 46870 Petaling Jaya, Selangor).

Statistics

Statistical analysis was not performed on the data due to small sample size.

RESULTS AND DISCUSSION

Lithium Carbonate Administration

Intraperitoneal Route

In monkeys administered with intraperitoneal lithium carbonate, the blood urea nitrogen concentration increased rapidly to a maximum of 57 mg% at day 3 after administration of the drug, followed by a decrease to 13 mg% at day 8 (Figure 1). In the control monkeys the BUN concentrations

FIGURE 1. THE EFFECT OF INTRAPERITONEAL LITHIUM CARBONATE ON BLOOD UREA NITROGEN CONCENTRATION

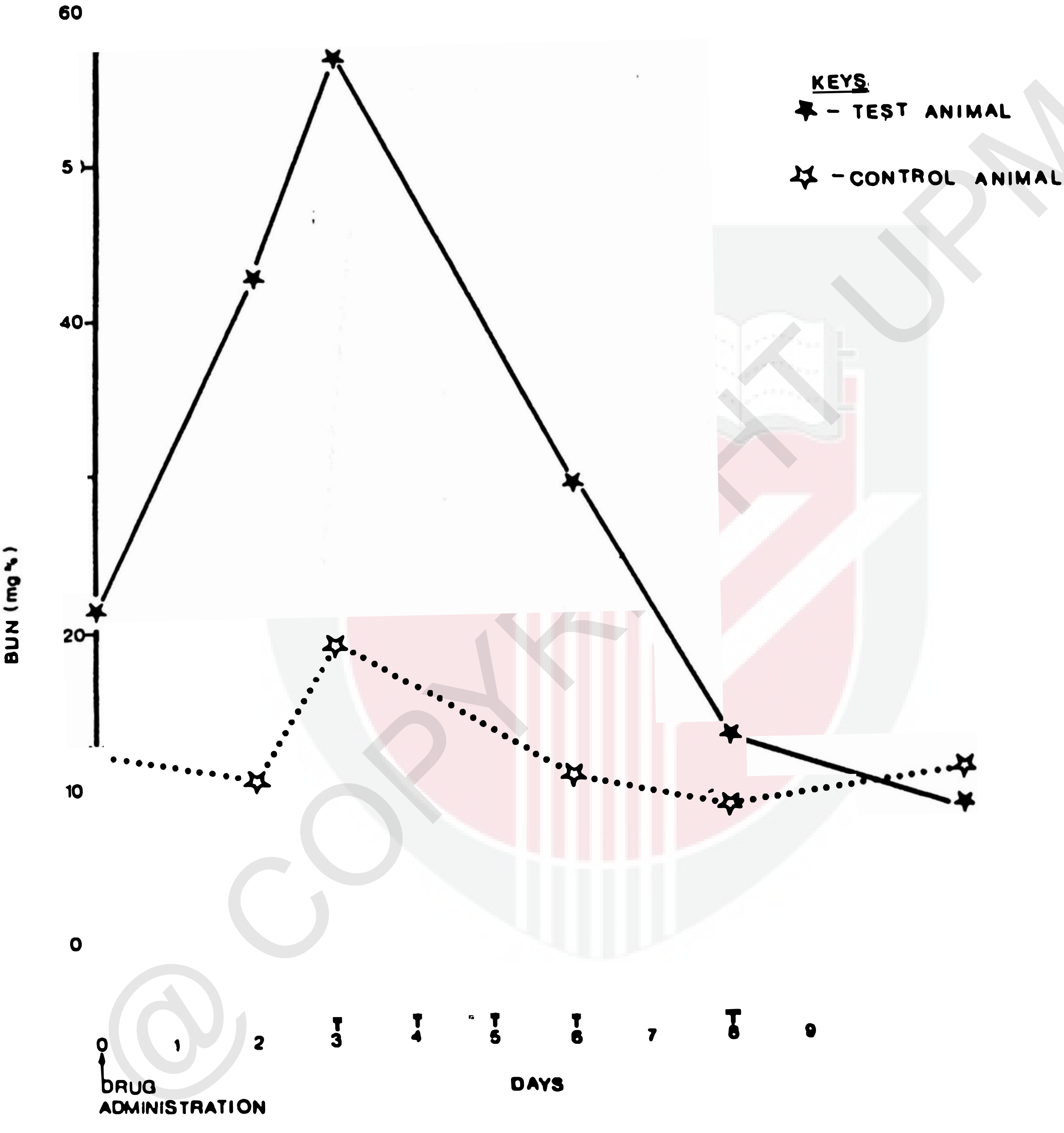


FIGURE 2. THE EFFECT ON INTRAPERITONEAL LITHIUM CRABONATE ON URINE CREATININE CONCENTRATION

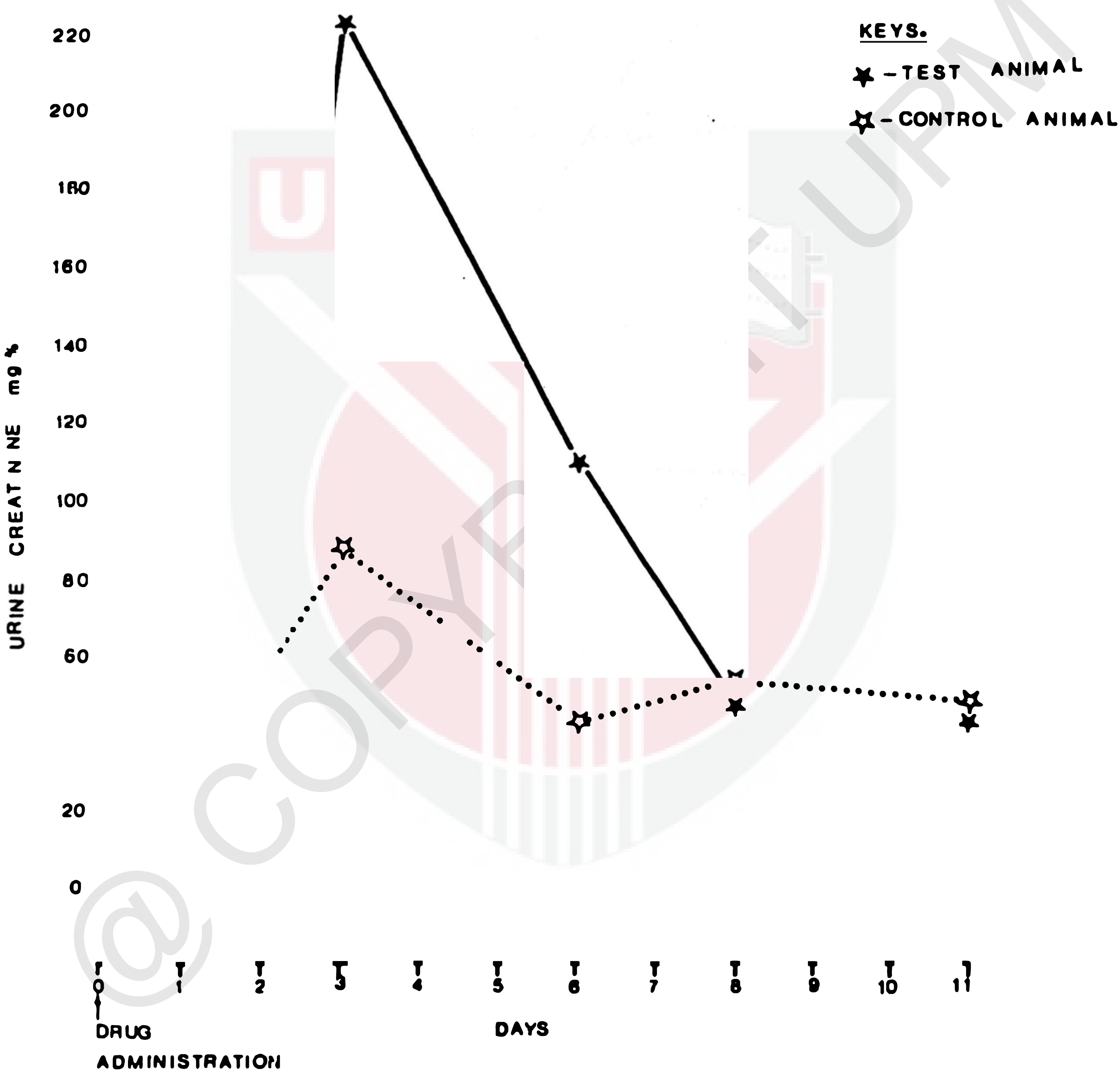


FIGURE 3. THE EFFECT OF INTRAPERITONEAL LITHIUM CARBONATE ON URINE PROTEIN-TO-CREATININE RATIO

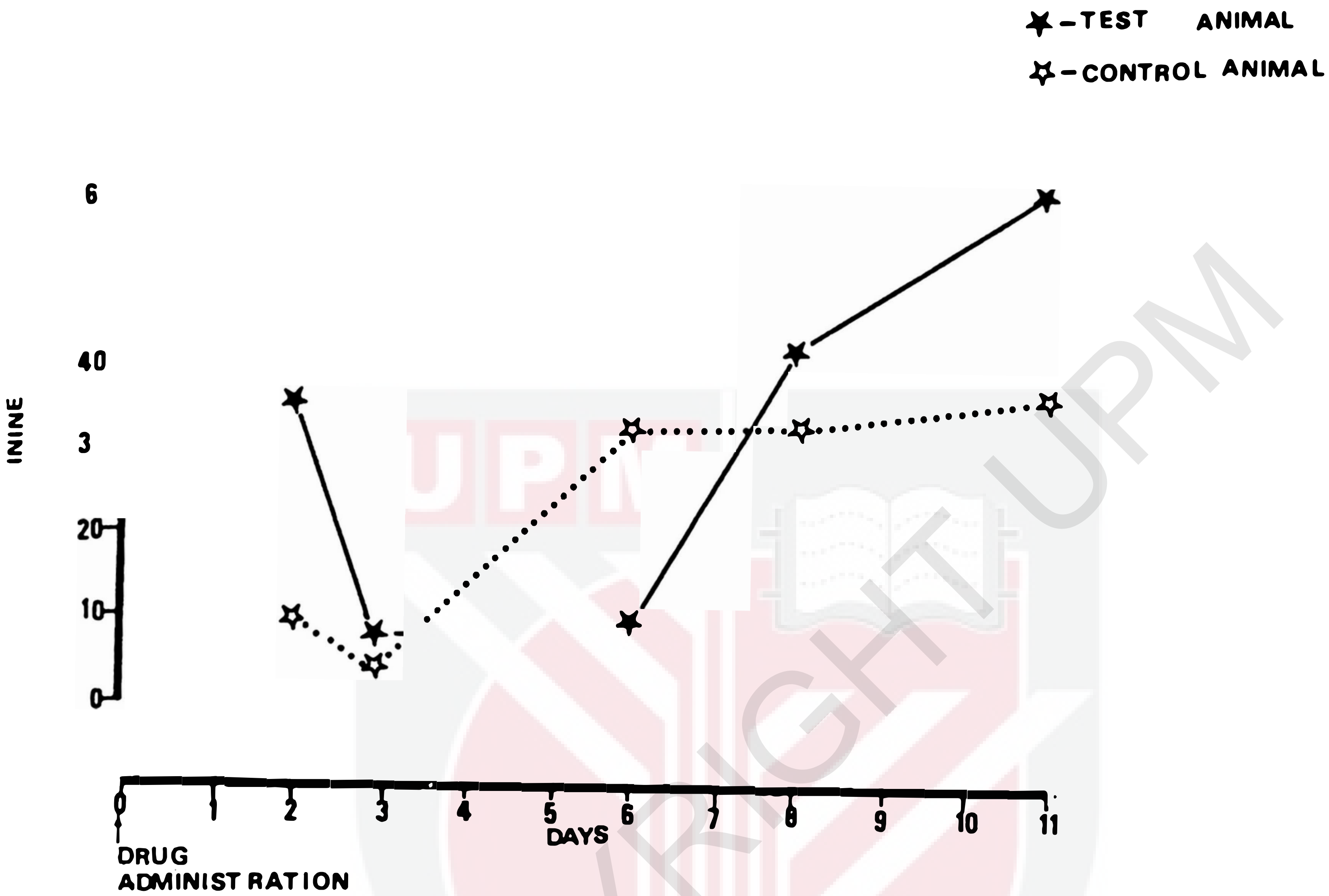


FIGURE 4. THE EFFECT OF INTRAPERITONEAL LITHIUM CARBONATE ON URINE PROTEIN CONCENTRATION

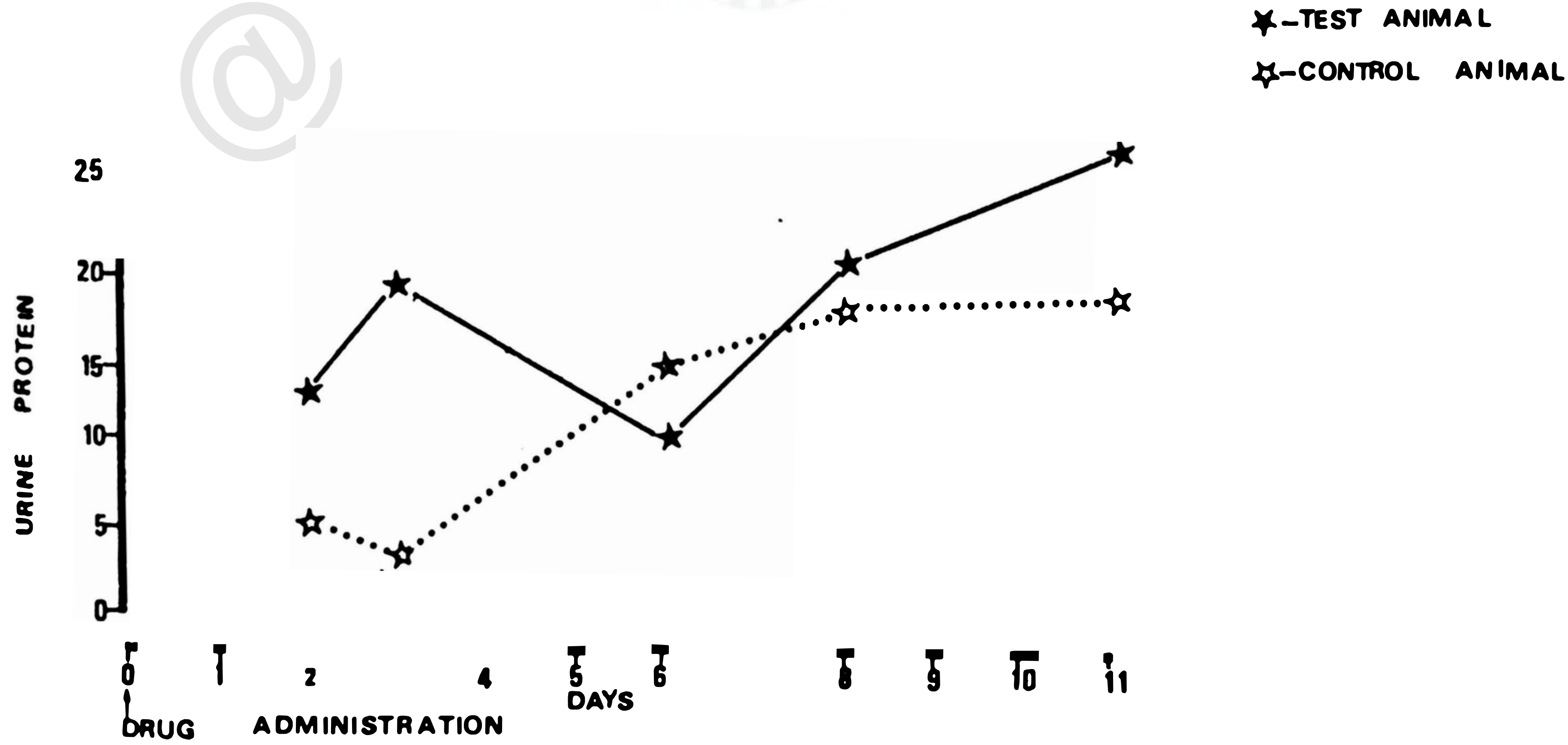


FIGURE 5. THE EFFECT OF INTRAPERITONEAL LITHIUM CARBONATE ON TOTAL WHITE BLOOD CELL NUMBER

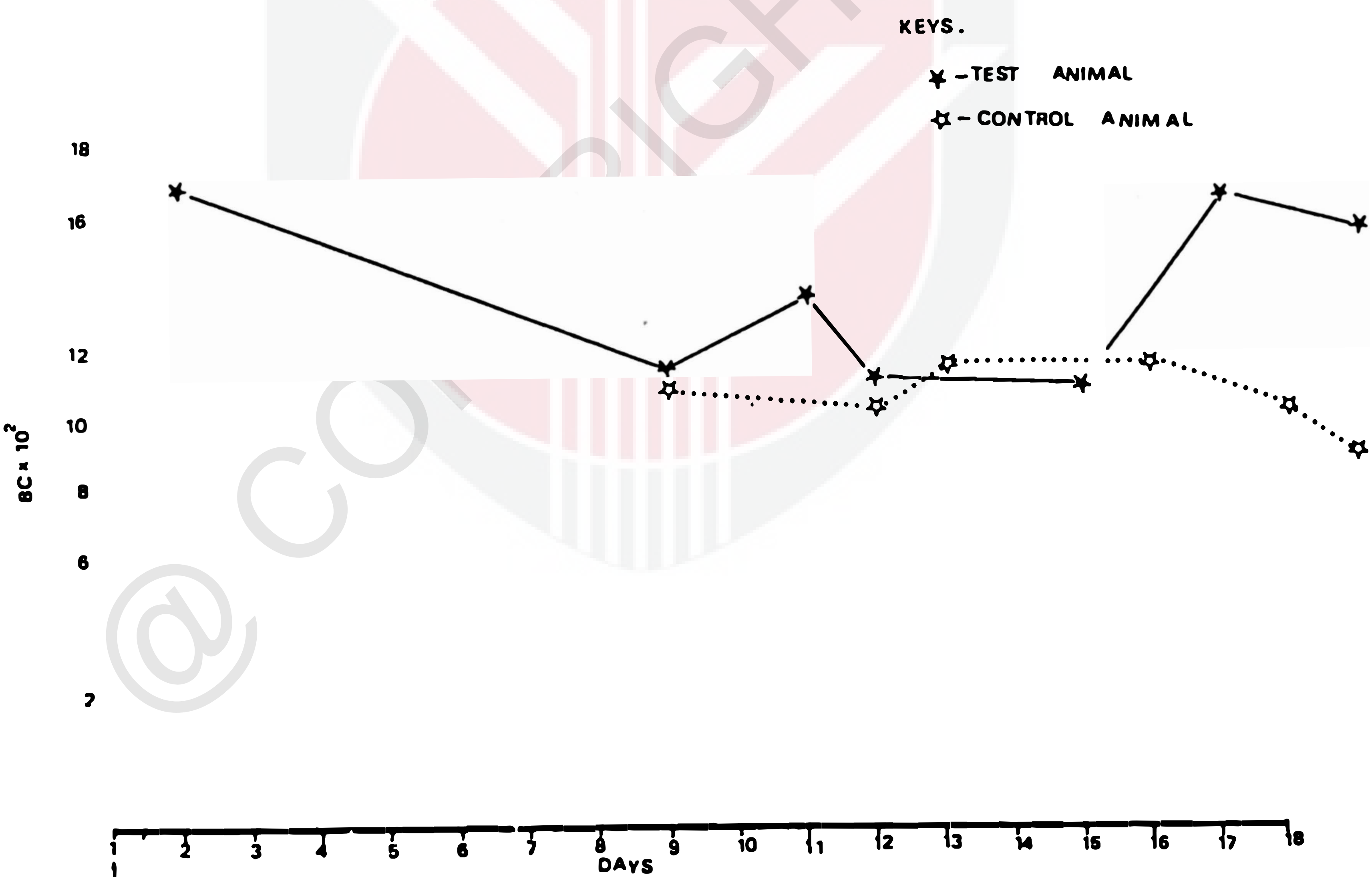


FIGURE 6. THE EFFECT OF INTRAPERITONEAL LITHIUM CARBONATE ON CIRCULATING NEUTROPHIL NUMBER

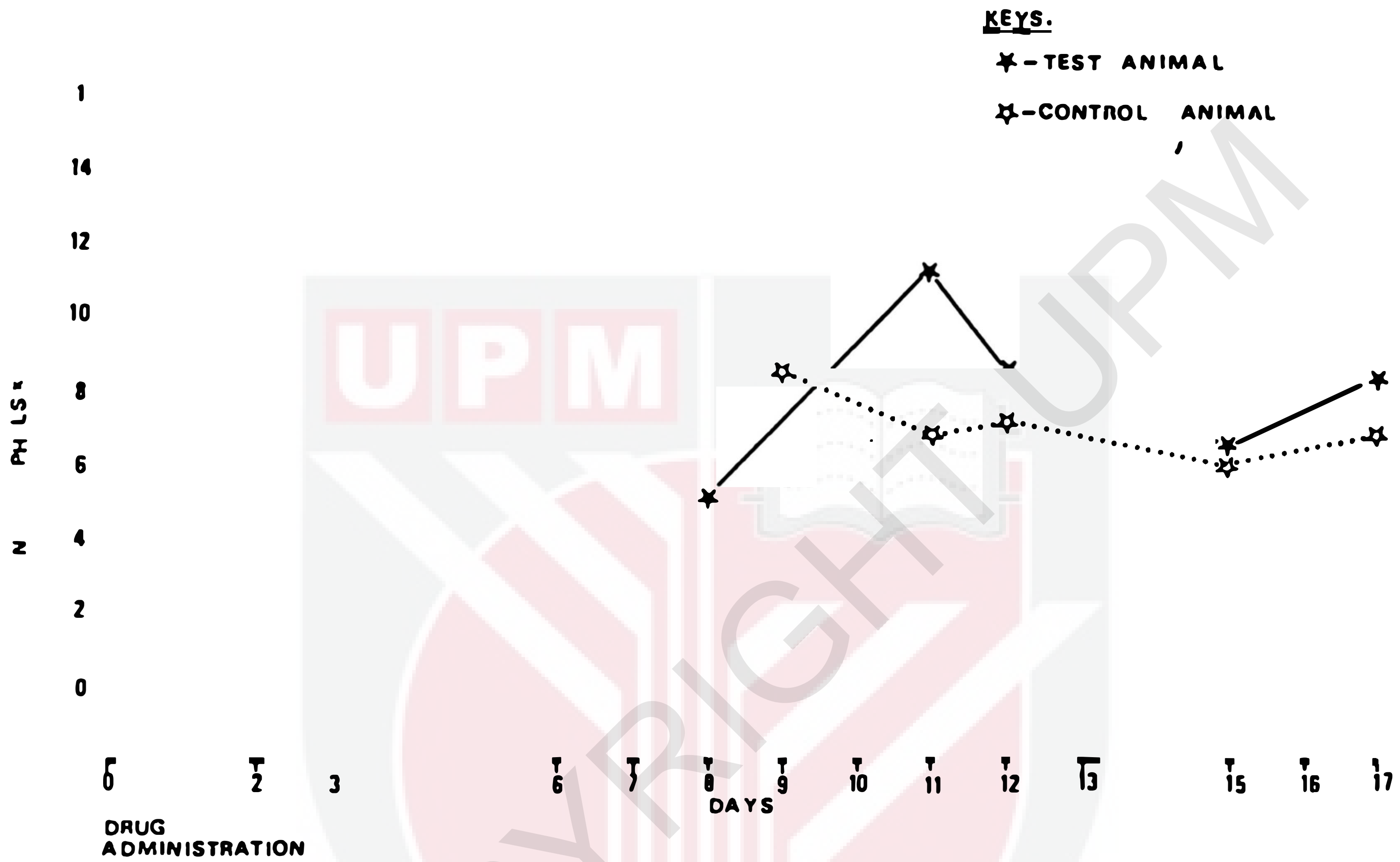


FIGURE 7. THE EFFECT OF INTRAPERITONEAL LITHIUM CARBONATE ON CIRCULATING LYMPHOCYTE NUMBER

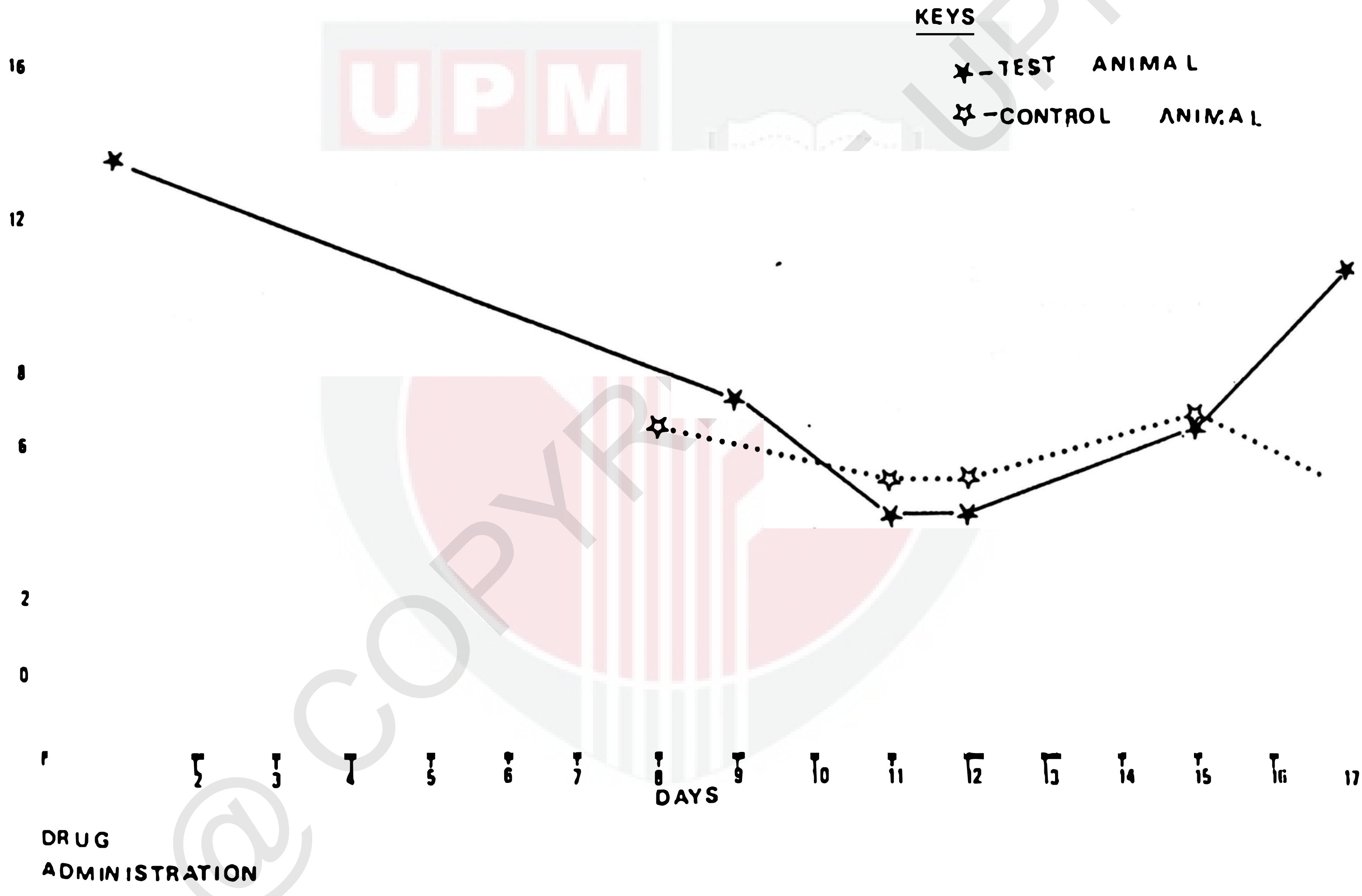


FIGURE 8. THE EFFECT OF ORAL LITHIUM CARBONATE ON BLOOD UREA NITROGEN CONCENTRATION

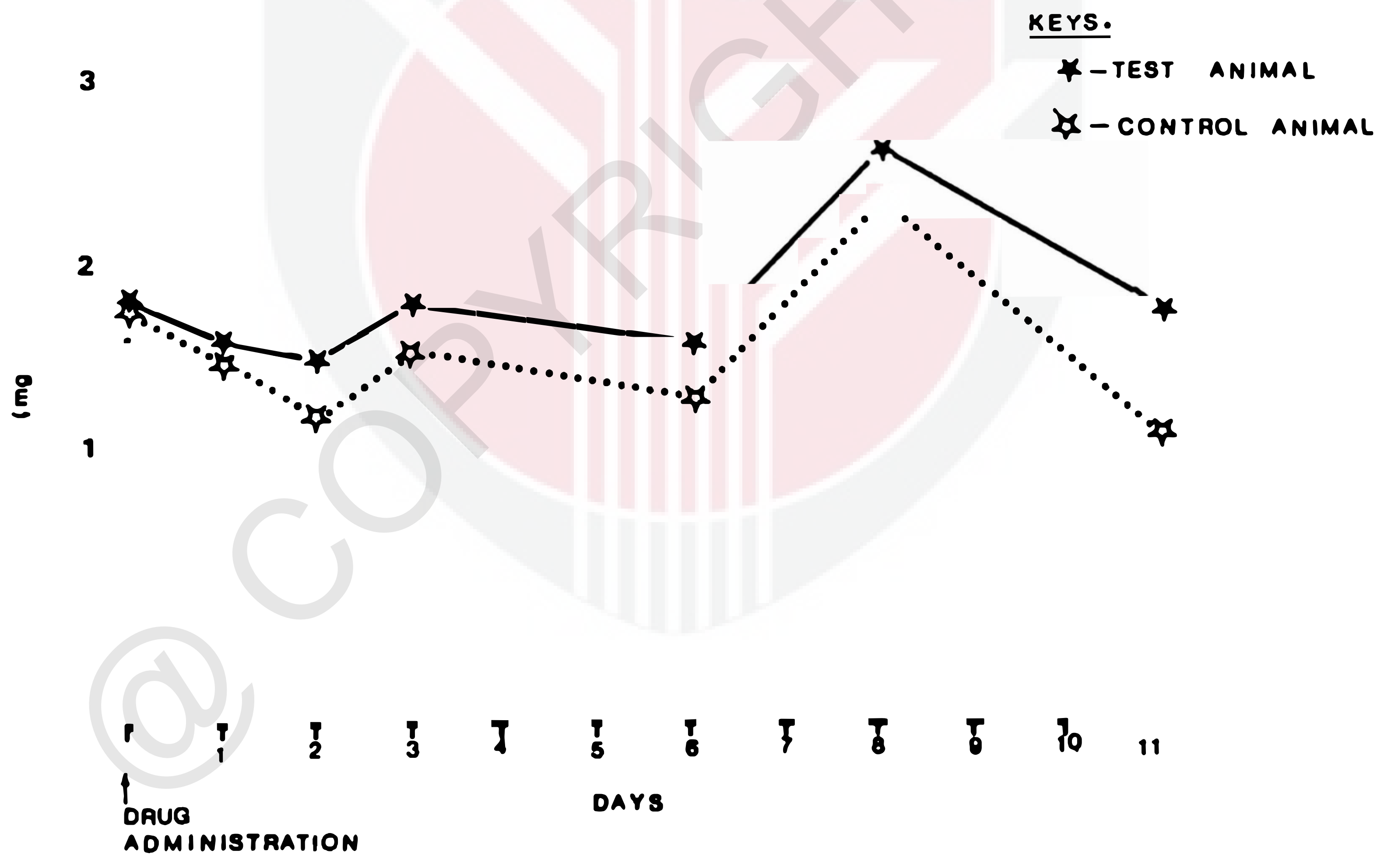


FIGURE 9. THE EFFECT OF ORAL LITHIUM CARBONATE ON TOTAL WHITE BLOOD CELL NUMBER

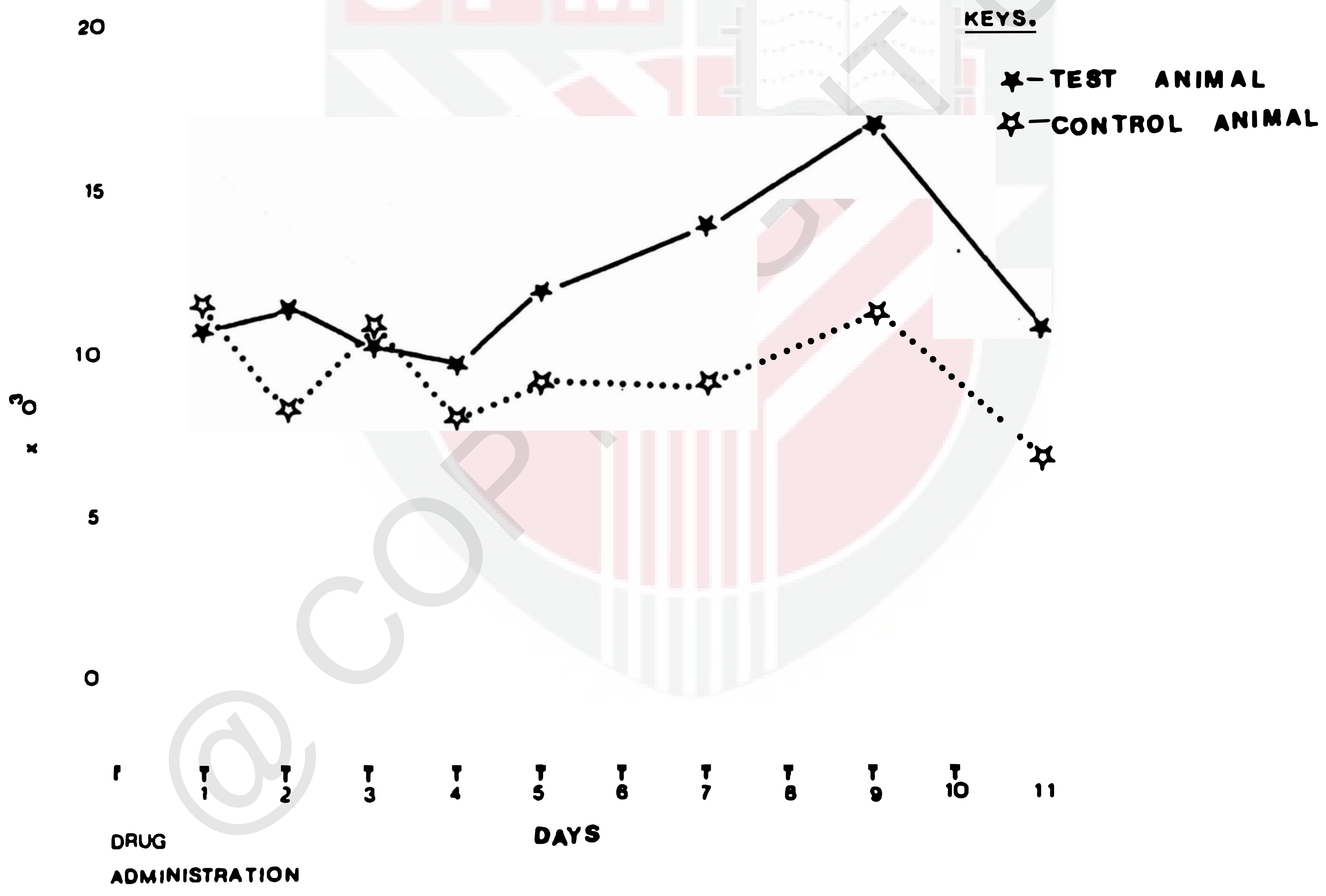


FIGURE 10. THE EFFECT OF ORAL LITHIUM CARBONATE ON CIRCULATING NEUTROPHIL NUMBER

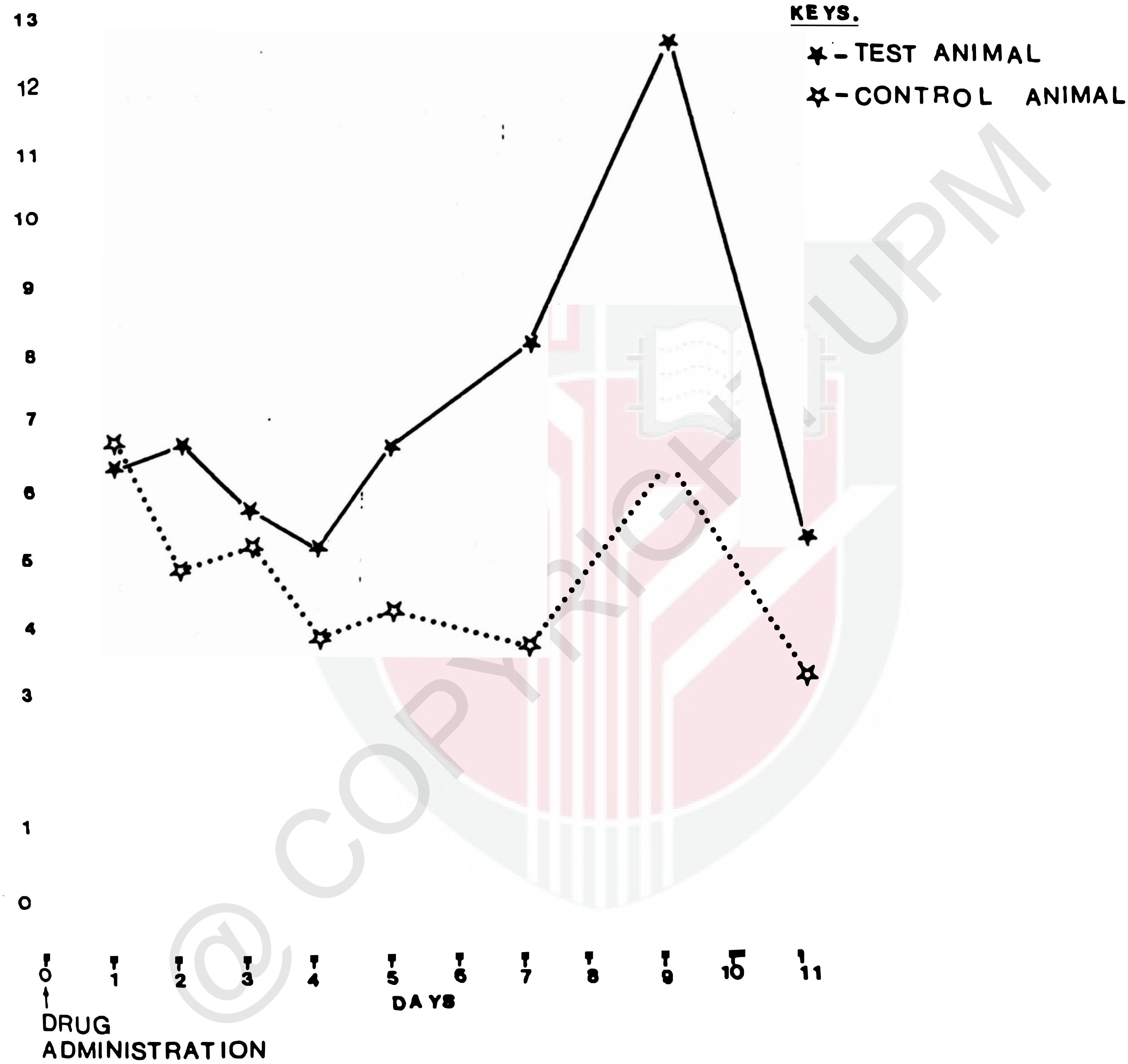


FIGURE 11. THE EFFECT OF ORAL LITHIUM CARBONATE ON CIRCULATING LYMPHOCYTE NUMBER

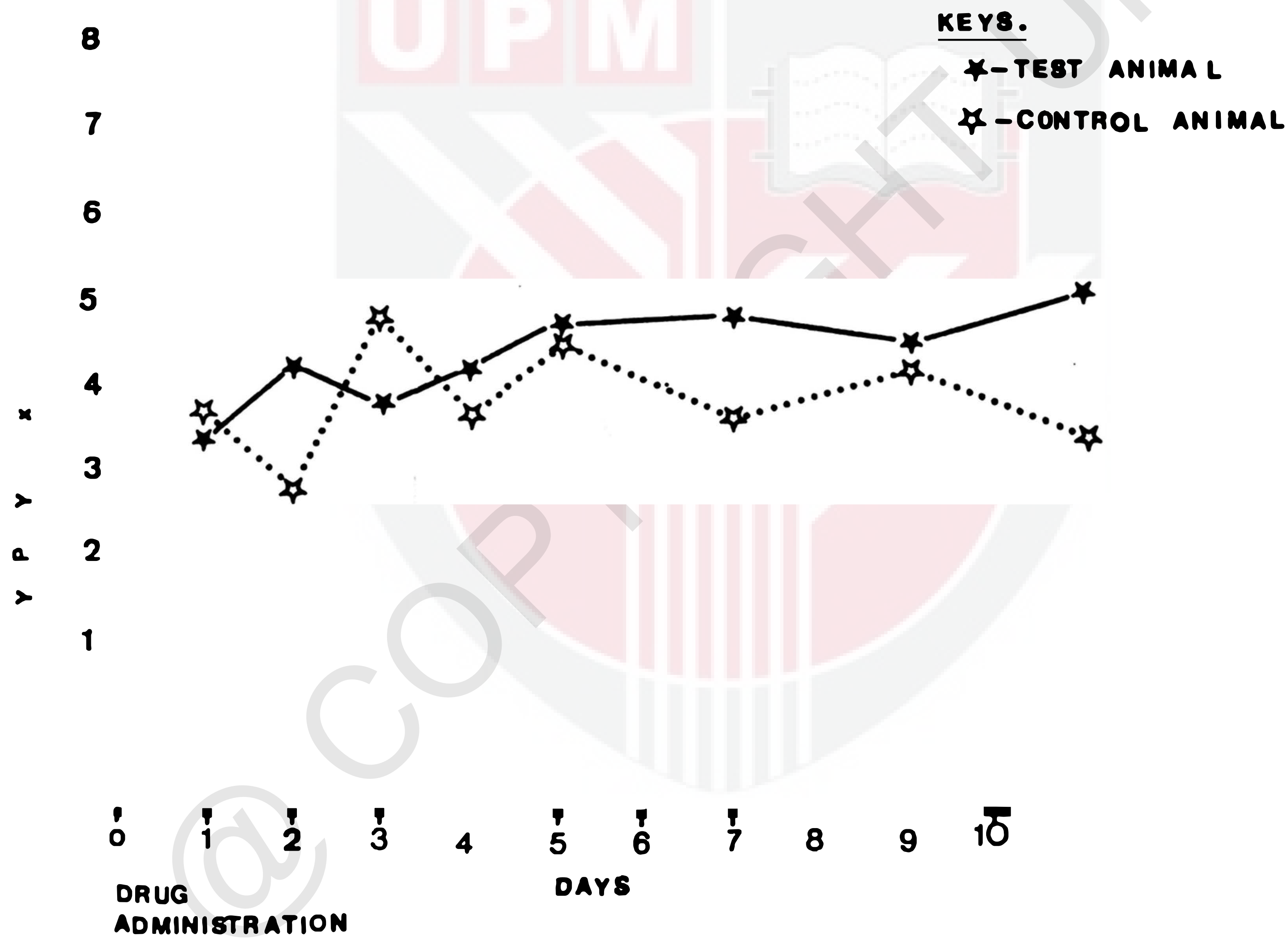


FIGURE 12. THE EFFECT OF ORAL LITHIUM CARBONATE ON CIRCULATING EOSINOPHIL NUMBER

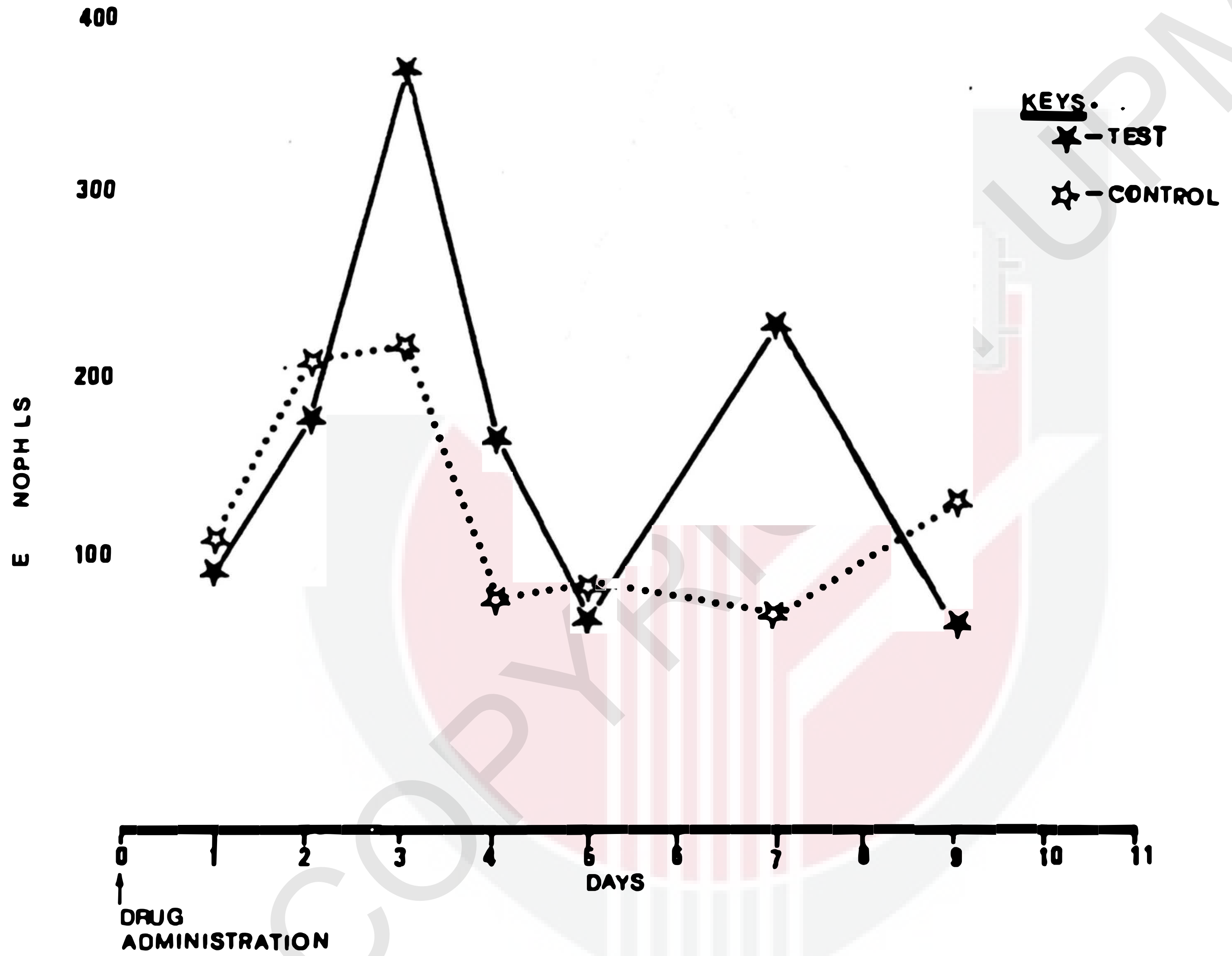
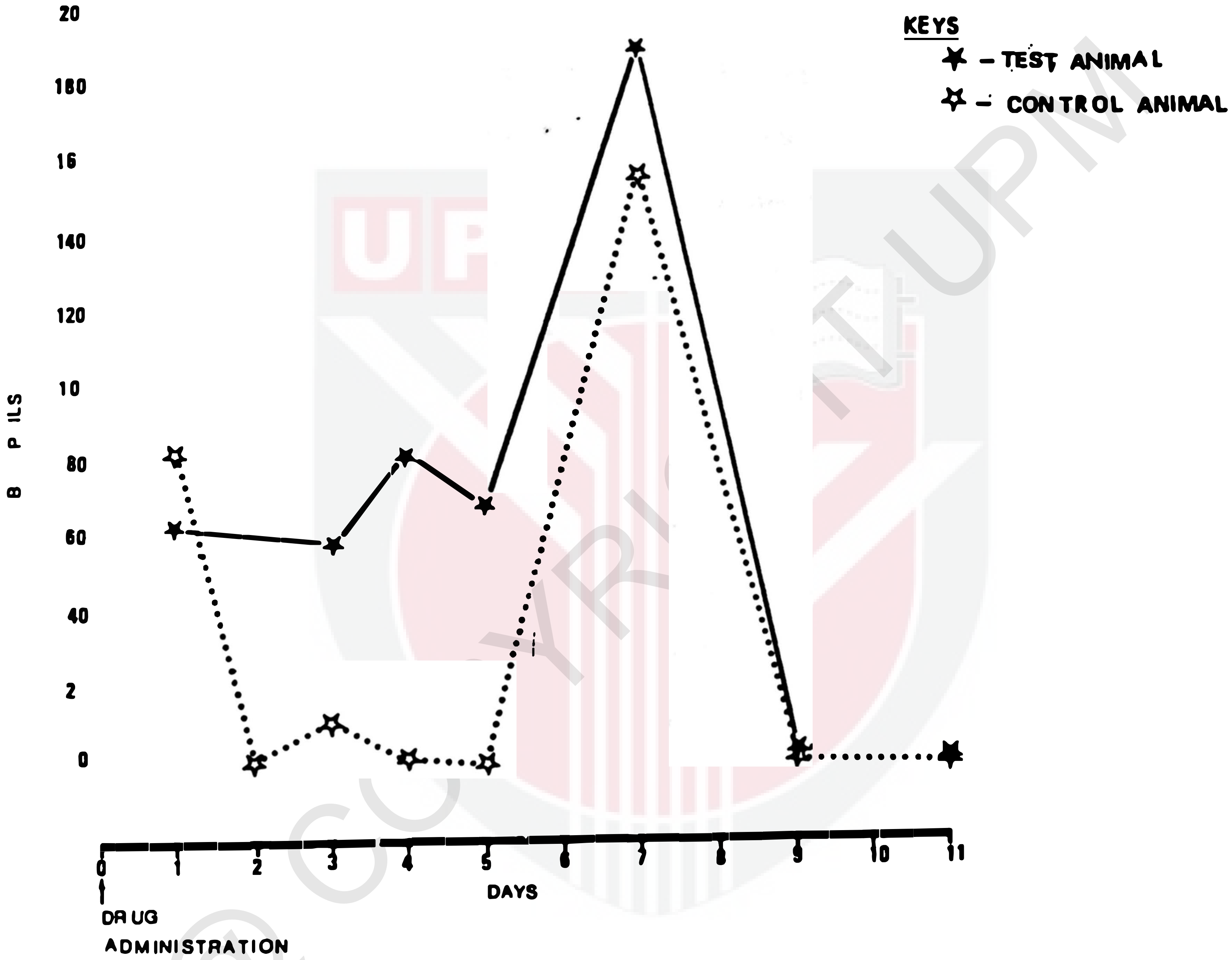


FIGURE 13. THE EFFECT OF ORAL LITHIUM CARBONATE ON CIRCULATING BASOPHIL NUMBER



remained normal with little daily fluctuations throughout the study. The urine creatinine excretion in these animals were markedly elevated (Figure 2). The urine creatinine concentrations were 34 mg% at day 2, rose quickly, reaching a maximum of 240 mg% at day 3. From thereon, the creatinine concentrations decreased rapidly to 40 mg% at day 8, and continued to decrease at a slower rate. The fact that the concentrations of BUN and urine creatinine peaked at the same time after intraperitoneal administration of lithium carbonate, indicated that both parameters were equally sensitive to the drug. Urine protein and urine protein-to-creatinine ratio in these animals, on the other hand, remained essentially unchanged. It should be noted that the most reliable parameter to assess proteinuria associated with renal damage is urine protein concentrations itself. However, estimation of protein in a single urine sample is still not a reliable parameter in the determination of renal damage in monkeys. The correct method to determine the extent of proteinuria is to analyse a 24-hour urine sample collected on ice. In most hospitals, this may not be a convenient method to perform. Thus, a group of workers suggested that in dogs, the use of the urine protein-to-creatinine ratio on a single urine sample as an accurate parameter of the assessment of proteinuria (White et al., 1984). In our study, although the elevated BUN concentration in monkeys receiving intraperitoneal lithium carbonate did suggest some measure of renal damage, the urine protein-to-creatinine ratio (Figure 3) and urine protein (Figure 4) did not change. The lack of proteinuria observed in a single urine sample may be due to diurnal fluctuations. This observation complicated our attempt to determine the usefulness of the urine protein-to-creatinine ratio in monkeys. However, if there is true proteinuria in these monkeys, subsequent to lithium carbonate administration, urine protein-to-creatinine ratio failed to record any change. It is still possible that this ratio may not be useful in the assessment of renal damage in monkeys.

Other blood parameters in monkeys receiving intraperitoneal lithium carbonate did not show any observable changes (Figures 5, 6, and 7).

Oral Route

The BUN concentrations were higher in monkeys receiving oral lithium carbonate than in the control animals. The BUN value in these test animals rose from day Ø reaching a maximum on day 8 post-lithium carbonate administration (Figure 8).

However this value is only marginally higher than the control values. These observations may still suggest that some renal abnormality had also occurred in these monkeys. Urine protein and creatinine concentrations and urine protein-to-creatinine ratio again failed to show observable difference between test and controls monkeys.

In oral administration of lithium carbonate, the total white blood cell counts appeared to be higher in test animals compared to the control animals (Figure 9). The increases in number of white blood cells were gradual, reaching a maximum on the ninth day after administration of lithium carbonate. This leucocytosis was clearly shown by neutrophilia (Figure 10). A very rapid increase in circulating neutrophils was seen from day 4 reaching a maximum on the ninth day, followed by a sharp drop to almost normal counts on the eleventh day. With the exception of lymphocytes which was slightly elevated (Figure 11), other leucocytes did not show any characteristic change (Figures 12 and 13). The changes in these white cell counts during the 2 weeks post-lithium carbonate administration may be explained by the fact that bone reflects lithium intake more than other tissues (Venugopal and Luckey,1978). In this study the effect of lithium carbonate on the bone marrow may be a selective stimulation of neutrophil and lymphocyte proliferation. In fact, it was shown by others that lithium carbonate increases circulating polymorphonuclear leucocytes and perhaps granulopoiesis in man (Greco and Brereton, 1977, Tisman et al.1973). This effect was suggested to be the influence of lithium on haematopoietic stem cell proliferation which occurs at the colony forming unit level (Ninane et al., 1984). The effect of lithium on the bone marrow would be manifested as leucocytosis especially neutrophilia by the sixth to the ninth day. In our experiment the circulating leucocytes particularly neutrophils increased gradually, reaching a maximum at day 9. From thereon, there was a sharp decrease in circulating leucocytes. This suggests that lithium carbonate may be used to alleviate the neutropenic state associated with bone marrow disorders.

Intraperitoneal versus Oral Route

It is obvious that there is a difference in the effect between lithium carbonate given intraperitoneally and orally.

Firstly, the BUN and creatinine concentrations in monkeys receiving intraperitoneal lithium carbonate were markedly elevated. Oral lithium carbonate did not produce the same effect. This phenomenon may be due to the rapid absorption of intraperitoneal lithium carbonate causing acute toxicity. Oral Lithium carbonate may not be absorbed that rapidly thus its accumulation and effect in kidney not as acute.

Secondly, there is a marked leucocytosis especially neutrophilia in monkeys receiving the drug orally, Whereas, those monkeys receiving the drug via intraperitoneal route did not show any observable change. This manifestation of the drug is enigmatic. The only possible explaination to this is, lithium carbonate when administered orally was degraded to its basic elements which may have better effects in the stimulation of bone marrow. Lithium carbonate absorbed from the intraperitoneal fluid was not subjected to the actions of gastric and intestinal juices.

CONCLUSION

Protein-to-creatinine ratio in urine specimens for quantitative estimation of renal damage may not be used in monkeys. In monkeys the use of BUN concentration as an indicator of kidney damage is still more reliable than other parameters. In these monkeys, the maximum effect of intraperitoneal lithium carbonate on urine creatinine and BUN concentrations were on day 3 post-lithium carbonate adminstration. By day 8, these parameters returned to normal. However, lithium carbonate when administered orally showed a similar effect on the above parameters only by day 8 post-lithium carbonate administration. It also took 8 days for oral lithium carbonate to fully exert its effect by increasing peripheral white blood cell counts.

Intraperitoneal route of lithium carbonate administration showed greater increases in blood urea nitrogen and urine protein or creatinine concentrations than the oral route of administration. However, monkeys given the drug orally showed greater increases in peripheral neutrophil and lymphocyte counts.

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