دراسة مقارنة عن تأثير الجرعة الواحدة والجرعات المتكررة من الثيامفينيكول والكلورامفينيكول فى الفيران

ع . شحاته ، روحيه غنيم ، ف . فركح

يعتبر الثيامفينيكول والكلورامفينيكول نوعان من العقاقير قريبة الشبه فى تأثيرها وان كان الاخير قد عرف عنه أنه أكثر سمية . وقد تم قياس تأثير الجرعة الواحدة والجرعات المتكررة فى الفيران على الكبد والطحال ونخاع العظم ، بالاضافة الى العضلات موضع الحقن .

ودراسة صورة الدم ومستوى الانزيمات فى الدم (الترانس أمينيز والفوسفاتيز القلوى) والفحص الهستوباثولوجى وجد أن الكبد هو أكثر الاعضاء تأثيرا بالجرعة الواحدة والجرعات المتكررة ايضا صحبها ارتفاع نسبة انزيمات الدم . وقد ثبت أن كلا العقارين لهما تأثير ضار على الكبد ونخاع العظم والطحال ، الا أن الفرق الوحيد كان فى درجة التأثير الناتجة عن حقنه واحدة أو جرعات متكررة من كلا من العقارين موضع البحث .

Pathology and Parasitology Department.
Faculty of Veterinary Medicine, University Cairo.
Head of Pathology Section, Prof. Dr. H.A. Shehata.

COMPARATIVE SYUDY ON THE EFFECT OF SINGLE AND REPEATED DOSES OF THIAMPHENICOL AND CHLORAMPHENICOL IN RATS

By
I.M. SHEHATA, R.E.M. DOGHAIM and F.I. FARKOUH
(Received at 24/3 /1977)

SUMMARY

Thiamphenicol and chloramphenicol are known as two related drugs. However, it was claimed that the latter is more toxic. Evaluation of the effect of single and repeated doses on liver. Spleen, bone marrow in addition to muscles at the site of injection was adopted. In addition, blood picture, serum glutamic oxal acetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were performed. Histopathological examination revealed that liver is the most affected organ with single and repeated doses of both drugs accompanied by increased level of SGOT and SGPT.Both drugs have the same drastic effect on liver and serum enzymes together with hemopoeitic organs. The difference lies only in the extent of change induced by single or, more particular, repeated doses of either drugs.

INTRODUCTION

Chloramphenicol, as a crystalline antibiotic, was first isolated BARTZ (1948) and known as chloromycitine. This was used in therapy of a variety of infectious diseases where sensetive bacteria are acted upon by its specific inhibition of protein synthesis, HAN, WISSMAN and HOPPS (1955). On the other hand, thiamphenicol which is also a broad spectrum antibiotic closely related to chloramphenicol was first syntesized by CHTLER, STEGNER and SUTER (1952).

The antibacterial effect of this relatively new drug is through interference with protein synthesis in bacterial cells. This is brought about by fixing the messinger R.N.A. on ribosomes, NISHIMURA, YAMAGUSHI and TANAKA, (1966). It was claimed that thiamphenicol is less toxic than chloramphenicol as both of them have similar effect. CONN, (1975) stated that chloramphenicol therapy have an errotic action.However, GOODMAN (1975) said that its sodium succinate must be intended for intravenous use. SMITH JOSLY, GRUHZIT,Mc LEAN,PENNER and EHRLICH (1948), recorded local ulceration at the site of repeated subcut aneous injection in mice and dogs. Due to, muscle degneration, trauma and necrosis transaminase enzymes are increased; DAYKIN (1960), WROBLEWSKI, (1959), ZIMMERMAN and WAST (1963) and CLERMONT and CHALMARS (1967). Thiamphenicol, on the other hand was said to have an exallent local tolerance and painless site of injection,QUINCY & PARTENSKY (1967), JAUNEAU, THABAT, PATIN and CANAYER (1967). It was mentioned LARGIER and BOISSER (1968), DULAC (1969) and Palmade, BEVEAR, ENJALBERT and COURTY (1970) that during treawith thiamphenicol injection in patients was well tolerated with no pain after its administration through intramuscular or intravenous routes. Regarding the effect of thiamphenicol upon liver and serum enzymes, LE LIVER DU THIOPHENICOL(1969) stated that no serious effects on liver tissue was reported.

Repeated doses of chloramphenicol was described by LICHMAN(1953) and REUTNER, MAXWELL, WESTON and WESTON(1955), to induce fatty changes in hepatic cells. Meanwhile KRUPP and CHATTON (1975) and GOTH (1968) added that liver diseases may be aggrevated by chloramphenicol. Both drugs was found to have toxic effect on bone marrow, KEISER and RACHEGGER (1973) and FIRKIN, SUMNER and BRADLEY (1974).

Drugs used for this study was:

1) Thismphenicol as " thiophenicol injectable, Clin-Midy, France ".
2) Chloramphenicol as " cidocitene injectable, Cid, Egypt ".

Experiment was performed using one hundred healthy adult male rats. Ten animals were left as control groups, 30 animals were divided into two groups that received single dose of each drug, then sacrificed after 1, 2 and 4 hours post injections. Single dose of either thiamphenicol or chloramphenicol was 1000 mg/kg body weight given intramuscularly in the gluteal muscle. Repeated dose adminstration was made by daily intramuscular injection with 250 mg/kg body weight of either thiamphenicol or chloramphenicol. Sixty rats used for repeated daily doses of thiamphenicol or chloramphenicol. Ten rats were killed by decapitation after 1, 2, and 3 weeks. Tissue samples were taken at once from liver spleens, bones, and muscles at the site of injection, fixation and further processing was done by paraffin embedding method. Bones were fixed in Spuler Maximow's fluid then decalcification was obtained by formic acid HCI solution, then processed as routine methods, CULLING, (1963). Blood was obtained from rats by decapitation for examination of blood picture. Meanwhile serum glutamic oxal acetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities were estimated according to the method of REITMAN & ERANKEL (1957). Histopathological examination of sections was performed.

RESULTS

Histopathological Results:

I- LIVER:

1) Single dose of thiamphenicol resulted in hyperemia with

cellular infiltration into the portal area. Early hepatic reaction was evident and consisted of dogenerative changes that proceeded to focal necrosis in animals left for 4 hours after injection, (Fig. 1).

2) Single dose of chloramphenicol resulted in marked hypremia of whole liver tissue with fatty change and cellular infiltration into the perivascular area of the congested protel vessels (Fig. 2) Hepatic dystrophy and coagulative necrosis was prominant accompanied by heavy cellular infiltration.

3) Repeated daily doses of thiampheicol for 1, 2 and 3 weeks: Gross examination have dark red colour and friable texture. Microscopic picture revealed congestion, compression and displacement of hepatic cells. Variable degrees of degenerations, necrosis and disintegration of affected cells were observed. Apperance of different necrobiotic changes in the nuclei were dominant finding in advancing cases (Fig. 3). Fibroblastic proliferation invading destroyed hepatic lobules was evident. Dense eosinophilic spherical bodies into some hepatic cells, as an indscation of regenerative activity was well seen (Fig. 4).

4) Repeated daily dose of chloramphenicol for 1, 2 and 3 weeks: Gross examination of livers revealed a dark colour with soft greesy texture. Minute greyish foci were noticed. These findings were found in a pronounced degree with advancing time and increasing number of daily doses. Microscopically, congestion and variable forms of degenerations were noticed (Fig. 5). These lesions were advanced in cases of animals that received 14 and 27 daily doses. Hepatic destruction was observed early, then followed by maked fibroblastic proliferation around the affected groups of cells (Fig. 6).

II- LOCAL REACTION AT THE SITE OF INJECTION:

Skeletal muscles at the site of daily injection with thiamphenicol did not show any abnormal finding. While, those animals received daily chloramphenicol injection, in the same manner, showed painful manifestation, inability to walk and draging their hind limbs as if paralized. This symptom was aggrevated during the second week, while gradually decreased in the third week.

Microscopically, acute suppurative myositis was evident in the first week, (Fig. 7) while during the second week this acute necrotizing reaction was much observed. Organisation and substitution of necrotic tissues in animals subjected to 21 daily dose of chloramphenicol were noticed in animals received 21 daily doses.

III- BONE MARROW:

Single dose of thimphenicol and chloramphenicol injection did not induce detectable lesion among groups of rats killed after 1, 2 & 4 hours post innoculation.

Repeated doses of thiamphenicol for 1, 2 and 3 weeks: Bone marrow of rats received daily doses showed and increased ratio of adipose tissue on the expense of the active hempoeitic tissue cells. Hemopoeitic foci were widely separated with irregularity in the cellular outline, especially of the erythrocytes. Megakaryocytes were decreased among the hypoplastic marrow tissue. Degenerative changes were also observed among the marrow cells, as shown by the faint stained cytoplasm and fragmented nuclei.

Repeated doses of chloramphenicol for 1, 2 and 3 weeks: Hemopoeitic foci were reduced in size and number, they were widely separated from each other. Few megakaryocytes were

observed among the hypoplastic marrow. Degeneration and disintigration of cells and nuclei were noticed in some foci. That change was observed in an increasing intensity among rats received 14 and 21 daily doses of chloramphenicol. Marked change in shape and size of erythrocytes was met with among the circulating blood inside the blood vessels,(Fig.8).

IV- SPLEEN:

Single dose of either thiamphenicol or chloramphenicol did not give a detectable lesion in the splenic parenchyma, examined after one, two and fours after the injection.

Repeated doses of thiamphenicol were characterized by increased activity of the white bulb of the spleen with simultaneous degeneration of their centers. Such changes was more pronounced in cases given 14 and 21 daily doses of the drug.

Repeated doses of chloramphenicol were found to induce hyperplasia of the white bulb in gradual increasing intensity accompanying the successive daily doses during the second and third week of administration. Degeneration of the central zone of the white bulb was observed especially at later stages of the experiment (Fig. 9).

V- SERUM ENZYMES AND BLOOD PICTURE:

Single and repeated doses of both thiamphenicol and chlor mphenicol resulted in an increased level of SGPT and SGOT in the serum. Marked rise of the level of both enzymes was noticed after the first and second week of chloramphenicol therapy, This rise of SGPT & SGOT was much more than that of thiamphenicol treated group. Slight insignificant decrease in erythrocytic, leucocytic count together with hemoglogbin contents were obtained after single dose of thiamphenicol treated group. This change was more pronounced in chloramphenicol treated group.

Repeated doses of thiamphenicol resulted in high significant decrease in R.B.Cs. and Hb. contents although in chloramphenicol treated animals marked drop in R.B.Cs. and Hb. contents. Leucocytic count did not show significant decrease in cases of repeated doses of either thiamphenicol or chloramphenicol treated animals.

DISCUSSION

Thiamphenicol was claimed to have similar antibiotic effect as chloramphenicol. However progressive increase in serum enzymes SGPT & SGOT was obtained between one and four hours after injection of either drugs. Repeated doses of thiamphenicol resulted in no signs of pain at the site of daily injection, while those of chloramphenicol suffered from severe errotic action at their gluteal muscles at the site of injection. These observations are in accordance with those of SMITH et al. (1948) , DAYIN, (1960) and CONN (1975). During this work it was noticed that suppurative myositis and even ulceration of the site of repeated injection, a finding which agreed well with that mentioned by these authors too. On the other hand, local tolerance of thiamphenicol injections was excellent among all animals used for this study, this observation coincides with those given by MACQUET & LAFFTTE (1967), QUINCY & PARTENSKY (1967), JANEAU et al. (1967) LARGIER & BOISSE, (1968), DULAC (1969), and PALMADE et al. (1970).

Single injection of thiamphenicol resulted in marked congestion with necrobiotic changes in examined liver after one, two and four hours post innoculation, these results are in contrast with that given by Book of Thiamphenicol (1960) which mentioned that thiamphenicol does not induce any interference with hepatic structure or function. Repeated doses

of either thiamphenicol or chloramphenicol resulted in severe and marked hepatic dystrophy which undergo further necrosis and even cellular substitution. These lesions were more pronounced in chloramphenicol treated group. Lesions induced from thiamphenicol are not in accordance with those mentioned by CLIN-COMAR (1969). Meanwhile, lesions produced from chloramphenicol in hepatic tissue are similar to those described by LICTMANN (1953), REUTNER et al. (1955), and KRUPP & CHATON, (1975).

Bone marrow and splenic lesions consisted of variable degrees of inhibition and hypoplasia of hempoeitic activity were obtained from cases treated with either thiamphenicol or chloramphenicol. These changes coincide with those given by RATZAN (1974) who mentioned that both drugs caused marked reduction in the activity of in vitro bone marrow colonies and suppression in their growth. However KEISER & BUCHEGAR, (1973) stated that thiamphenicol has direct effect of toxic nature on bone marrow and it is more toxic than chloramphenicol while this toxic effect is reversable after withdrowal of thiamphenicol. FIRKIN (1974) gave an identical results to that obtained during this study in that nucleated cells in bone marrow are markedly reduced. In addition, it was found that changes in the erythrocytic series are similar to the results mentioned by JANBON (1971). Marrow hypoplasia was a regular thiamphenicol and chloramphenicol, this result agreed well with that of KALTWASSEE (1974) who mentioned that significant decrease in packed cell volume and erythrocytopenia were noticed.

Serum enzymes transeaminases, SGPT & SGOT, were elevated in cases of thiamphenicol and chloramphenicol treated animals, especially those animals used for repeated daily doses of either drugs. This was a constant finding among

all cases examined in this investigation, this finding is in accordance with WROBLEWSKI (1963) and CLERMON & CHALMERS (1967). However, marked increase of enzyme level during repeated chloramphenicol injection could be claimed to be due to the combined effect of both hepatic dystrophy and necrosis of the skeletal muscles at the site of injection.

From this investigations it could be concluded that both thiamphenicol and chloramphenicol have the same drastic effect on hepatic structure and function. In addition, depressing effect on the hemopoeitic organs together with rise of serum enzymes SGPT & SGOT were a dominant findings in both drugs. The difference between these related drugs lies only in the extent of the change induced by single and more particular, repeated doses of either drugs. Whether the depressing effect of thiamphenicol on hemopoeitic organs is reversable or not needs a further investigations.

REFERENCES

Bartz, Q.R. (1948): Isolation and characterisation of chloromycitine J. Biol. Chem. 172, 445 - 450.

Clermont, R.J. and Chalmers, T.C. (1967): The transeaminase test in liver disease. Medicine Vol. 46, 179 - 207.

Conn, H.F. (1975): Latest approved methods of treatment for practicing physician. W.B.Saunders comp. Philadelphia; London & Toronto.

Culling, C.F.A. (1963): Handbook of Histopatholotical technique, 2nd Ed. London, Batterworth.

Cutler,R.A., Stegner, R.J. and Suter, C.M. (1952): New antibacterial agent. J. Am. Chem. Society, 73 , 5475.

Daykin, P.W.,(1960): Veterinary applied pharmacology and Therapeutics, 1st Ed. Bailliére, Tindall & Cox Ltd. London.

Dulac, J. (1969): Le thiamphenicol en partiaue Odontostomatologique L; Information Dentair, 4, 303 - 306.

Firkin, C.F., Sumner, A.M. and Bradley, R.T., (1974): The influence of chloramphenicol on the bone marrow hemopoeitic stem cells components Exp. Hemat. 2 - 5), 264 - 268.

Goodman, S.L. and Gilman, A., (1975): The pharmacological bases of therapeutics. 5th Ed. MacMillan Publishing Co. New York.

Goth, A. (1968): Medical Pharmacology 4th Ed. The C.V. Mosby Co. Saint Louis.

Hann, F.E., Wissman,C.L., Hopps, H.E., (1955): Mode of action of chloramphenicol. Action of Chloramphenicol on bacterial energy metabolism. J. Bact. 69, 215.

Jauneau, A., Thabat, A., Pattin, S. and Canayer, H. (1967): Intérét du thiamphenicol en partique Médicale courante Cohiers Médedicaux Lyonnais, T. 43 No. 27, 2515 - 2520.

Keiser, G., and Bachegger, U.,(1973): Hematological side effects of chloramphenicol and thiamphenicol,Helvetica Medica, Acta, 37, 265 - 279.

Krupp, M.A. and Chatton, M.J., (1975): Current Medical diagnosis and treatment. Lange Medical Publications Los Altos. California.

Largier, M.M., and Boissé, J.L. (1968): Essai dun nouvel antibiotique le glycinate de thiamphenicol en pneumophisiologie. Archives Mediterraneenes de Medecine, 6, 309 - 315.

Le Liver du Thiophenicol, (1969): a été édité a Paris parles Creation Elb pour les Laboratoires Clin-Comar.

Lichtman, S.S. (1953): Diseases of the liver, gall bladder and bile ducts, 3rd Ed. vol. I lea & Febiger U.S.A.

Nishimura, T., Yamaguchi, H. and Tanaka N.J., (1966): Biochemical studies on the activity of thiophenicol. Antibiotis S z 2 - 19, 166.

Palmade, M., Bevear,M., Enjalbert, M. and Courty. G., (1970): Le Thiophenicol en pathologie pulmonaire Bordeaux Medical No 7 - 8 P 1937 - 1944.

Quincy, R. and Partensky, (1967): Etude de laction due thiophenicol injectable en milieu obststrical. Cahier Medicaux Lyonnais T. 43 No 26 P 2375 - 2390.

Ratzan, J.R. Moore, S.A.M. and Yonis, A.A. (1974): Effect of Chloramphenicol and thiamphenicol on the in vitro colony forming cells " J. Haemat. 43? 3, 363 - 369.

Reitman, S. and Franel, S., (1957): Amer. J. Clin. Path. 28, 56.

Reutner, T.F., Maxwell, R.E., Weston, K.E. and Weston, J.K. (1955): Chloramphenicol toxicity studies in experimental animals Part 1, the effect of chloramphenicol and various other antibiotics on malnutrition in dogs with particular reference to hemopoietic system, Antibio. & Chemotherp. 5, 679.

Smith, R.M., Josly, D.A. Gruhzit, O.M., Mc Lean, I.W. , Penner, M.A. and Ehrlich, J., (1948): Chloromycitin Biological Studies, J. Bact. 55, 425.

Wroblewski, F., (1959): The clinical significance of transeaminase activities. Am. J. Med. 27, 911.

Zimmerman, H.J. and West, M. (1963): Serum enzyme levels in the diagnosis of hepatic diseases. Am.J. Gastroent. 40, 387.

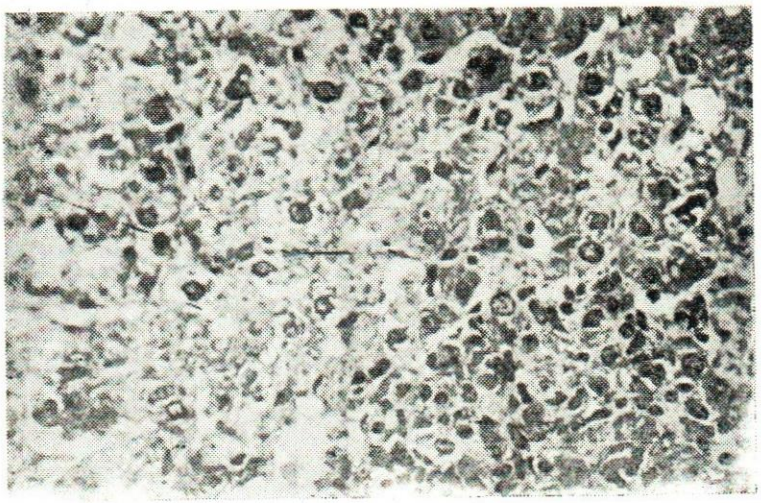

Fig. 1 : 4th hour thiamphenicol.
Focal necrosis among liver lobules
Degeneration of most of hepatic cells. [x. 100]
[Stain H & E]

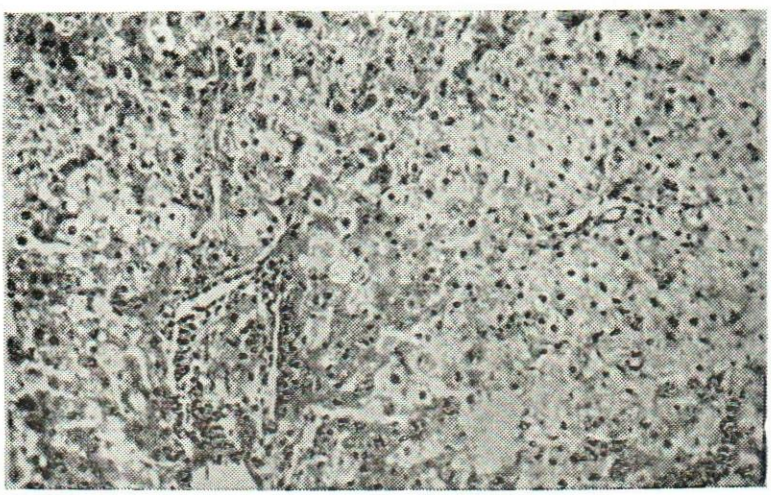

Fig. 2 : 4th hour chlorampnenicol
Degeneration and vacuolisation of hepatic cells and cellular infiltration into hepatic lobules. [x. 100] Stain H & E]

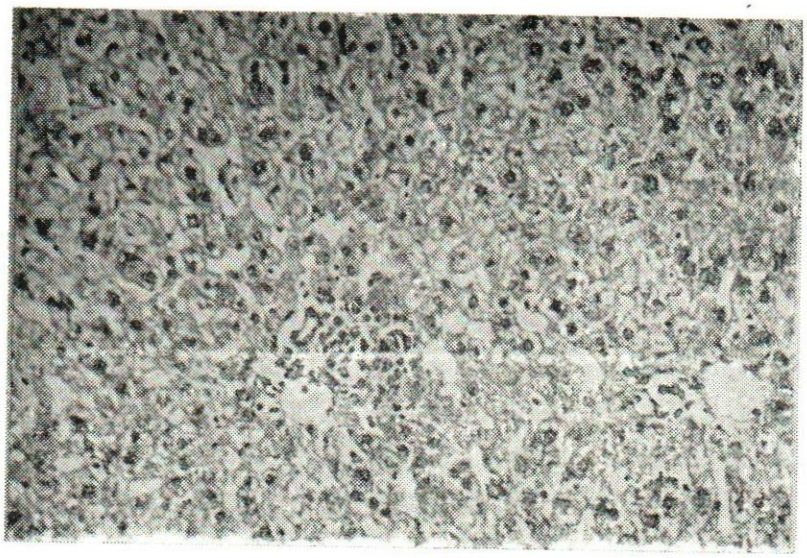

Fig. 3 : 3rd week thiamphenicol. Marked degeneration and disintegration of hepatic cells with cellular infiltration into some foci. [x. 200] [Stain H & E]

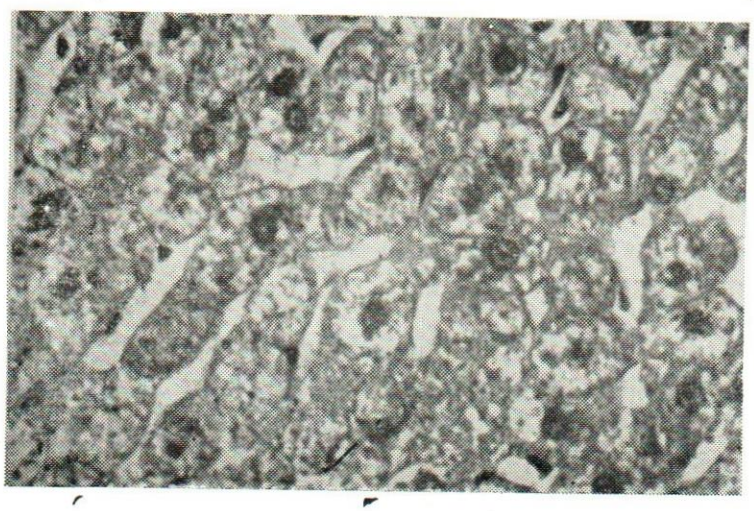

Fig. 4 : Necrotic liver cells showing granular cytoplasm with karyorrhexis and karyolysis of some nuclei. [x 600] [Stain H & E]

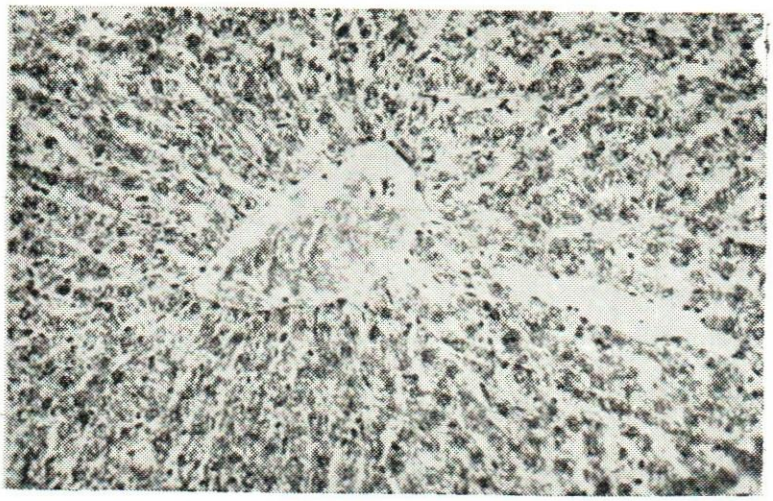

Fig. 5 : Ist week chloramphenicol. Congestion and compression of hepatic cells. [x. 200] [Stain H & E]

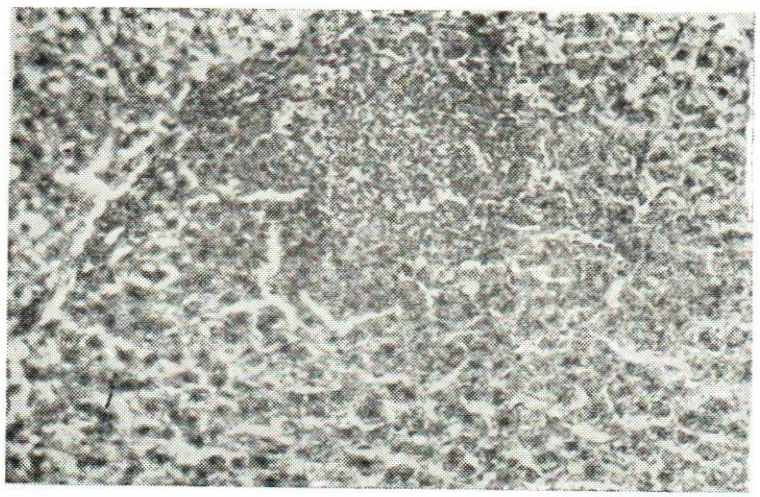

Fig. 6 : 3rd week chloramphenicol. Focal liver necrosis with accumulation of phagocytic cells together with fibroblastic activity into affected foci. [x. 200] Stain H & E]

Fig. 7 : Ist week chloramphenicol. Acute necrotizing suppurative myositis in skeletal muscles at the site of injection. [x. 100] [Stain H & E]

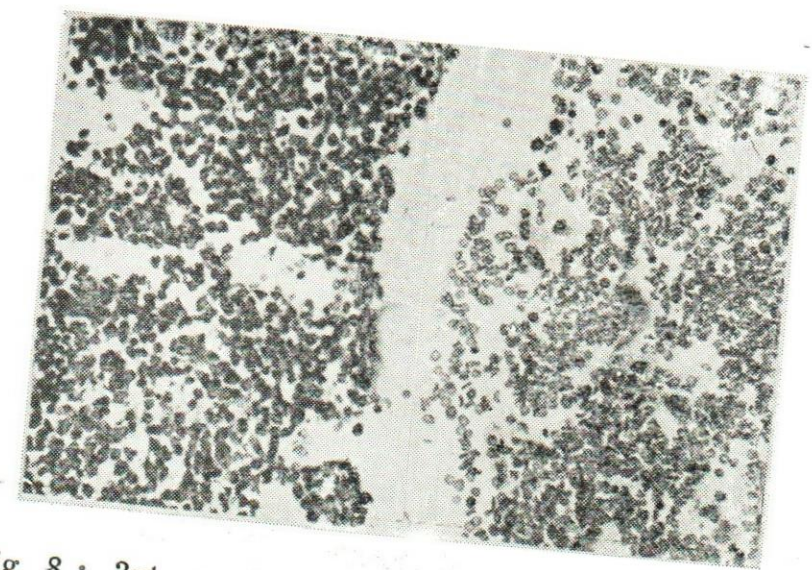

Fig. 8 : 3rd week chloramphenicol. Bone marrow showing deformed erythrocytes inside the blood spaees. [x 250] [Stain H & E]

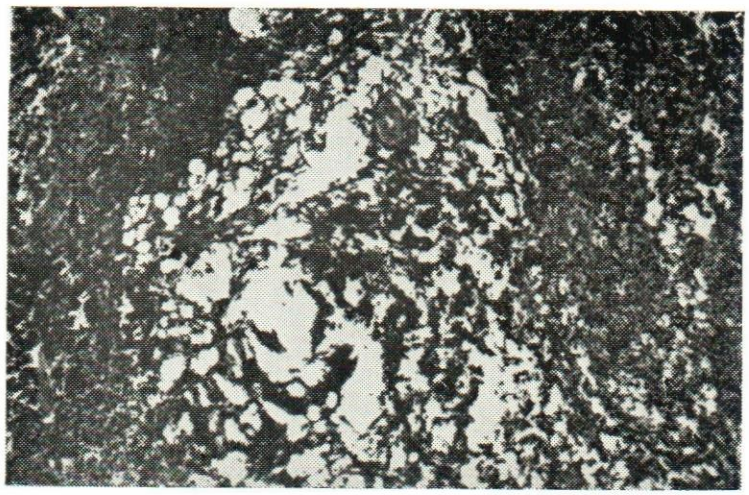

Fig. 9 : 3rd week chloramphenicol
Degeneration of the central zone of
malpigean corpuscles [x 100] [Stain H & E]