CHAPTER III
THE EFFECT OF CHLORAMPHENICOL
ON THE EMBRYONIC DEVELOPMENT OF THE ALBINO MOUSE

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REVIEW OF LITERATURE

Chloramphenicol was one of the earliest "broad-spectrum" antibiotics placed on the market. It was first prepared by Burkholder in 1947 by the fermentation process from Streptomyces venezuelae (Ehrlich, Bartz, Smith, Joslyn and Burkholder, 1947). In the following year it was synthesized, and subsequently the synthetic form and that obtained by fermentation were shown to have the same therapeutic action.

1. Chemical properties

According to Weinstein (1970), Chloramphenicol consists of yellowish-white crystals with an intensely bitter taste. It is highly soluble in alcohol and fairly soluble in ether. Its solubility in water is about 0.25 per cent at 25°C. Aqueous solutions have a pH of about 5.5 and are extremely stable. They can be kept indefinitely at ordinary room temperature if protected from light. Its structural formula is:
2. Pharmacological properties

Garrod and O'Grady (1968) stated that Chloramphenicol is completely absorbed by the oral administration. Its peak level is reached in two to four hours and it is about 8-12 µg per ml after a dose of 1 gm. Chloramphenicol succinate which is freely soluble, undergoes hydrolysis in the tissues with the liberation of chloramphenicol. Chloramphenicol succinate can be injected in a small volume either intramuscularly, intravenously, or subcutaneously.

About 60 per cent of chloramphenicol in the blood is bound to protein. Studies of organ distribution have shown a diminishing order of concentration in the kidney, liver, heart, spleen, muscle and brain. Free diffusion occurs into the serous effusions, and into the foetal circulation. Penetration into all parts of the eye has also been demonstrated. Perhaps more
important of all is the concentration attained in the cerebro-spinal fluid. It is higher than those attained in any other antibiotic and amount to 30-50 per cent of those of the blood even in the absence of meningitis. Glandular secretions also contain some of the antibiotic; its presence in the saliva occasions a bitter taste and accounts for changes in the oral flora.

In adults, Glazko, Dill and Rebstock (1950) had demonstrated that chloramphenicol is inactivated in the liver, mainly by glucuronide conjugation. Excretion is mainly renal; 90 per cent of the dose can be detected in the urine, but only 10 per cent of this amount is unaltered antibiotic. Chloramphenicol itself is excreted by the glomeruli and the inactive forms are excreted by the tubules. About 3 per cent of the administered dose is excreted in the bile but only 1 per cent appears in the faeces in the inactive forms.

Burns, Hodgman and Cass (1959) observed extremely high blood levels (170 μg per ml) in newborn infants receiving normal recommended doses. They ascribed this to poor tissue absorption, poor excretion or failure of
conjugation of the drug. Dorn and Smith (1959),
reported prolonged blood levels in the newborn and
the premature infants after a single dose of chlor-
amphenicol. Weiss, Glazko and Weston (1960) proposed
two mechanisms responsible for the high blood levels
in neonates; (1) failure of the drug to be conjugated
with glucuronic acid, due to the deficient glucuronic-
conjugating mechanisms in the liver characteristic of
the first weeks of life, and (2) inadequate renal ex-
cretion of unconjugated drug in the newborn. They also
stated that excessive plasma levels of the glucuronide
conjugate were also present, despite low rate of forma-
tion, because tubular secretion, the pathway of excre-
tion of this compound is also reduced in the neonate.

3. Mode of action

The mode of action of chloramphenicol had been
studied extensively particularly because of its selec-
tive ability to inhibit protein synthesis in bacteria.
Weisberger, Armentrout and Wolfe (1963 and 1964) had
demonstrated that chloramphenicol can inhibit protein
synthesis in mammalian cell-free systems as effectively
as it inhibits protein synthesis in analogous microbial
systems. Significant inhibition occurred only when
protein synthesis is stimulated by adding additional template RNA to ribosomes, there being comparatively little inhibition of protein synthesis in the absence of added stimulatory RNA. It was postulated from these studies that chloramphenicol may inhibit the function of messenger RNA by preventing its attachment to ribosomes.

Ambrose and Coons (1963) demonstrated that chloramphenicol also inhibits protein synthesis by intact mammalian cells in vitro. These authors demonstrated that chloramphenicol can inhibit antibody synthesis in cultures of lymph node fragments, and suggested that chloramphenicol might exert its inhibitory effect by blocking the function of messenger RNA formed in response to the antigenic stimulus.

Weisberger, Daniel and Hoffman (1964) studied the survival of homografts in rabbits administered chloramphenicol intramuscularly, to determine whether this drug inhibits de novo protein synthesis in vivo following antigenic stimulation. They concluded that chloramphenicol suppressed antibody synthesis by inhibiting de novo protein synthesis. Hypoplasia of antibody-
producing cells was excluded by histologic examination of spleen, lymph nodes, gastro-intestinal tract, pulmonary lymph follicles, and marrow of four animals receiving chloramphenicol in a dosage of 500 mg per kg. Optimum results were obtained when 500 mg per kg of chloramphenicol were employed. Dosages of chloramphenicol between 150 and 300 mg per kg were almost ineffective in suppressing antibody response.

Weisberger and Wolfe (1964), summarized some of the apparent differences in the effects of chloramphenicol on microbial and mammalian protein synthesis. They suggested that the inhibitory effect of chloramphenicol on protein synthesis in proliferating cells may be an important factor in the hematologic toxicity attributed to chloramphenicol.

4. Therapeutic uses

According to Weinstein (1970) chloramphenicol is currently the drug of choice in typhoid fever and other severe Salmonella infections. It is also useful in the treatment of Haemophilus influenzae meningitis, pneumonia, rickettsial disease, brucellosis and urinary tract infections.
The recommended doses, by the same author are; 1.5-3 gm daily in divided doses for adults, 25-50 mg per kg daily in divided doses for children, not more than 25 mg per kg daily for premature infants.

5. Toxicity

a) Marrow aplasia

Smadel (1949) stated that the presence of the nitrobenzene radical in the structure of chloramphenicol led to the suspicion that the drug might be toxic to the haematopoietic system.

Rich, Ritterhoff and Hoffmann (1950) reported the first case of aplastic anemia in association with chloramphenicol therapy, and the authors also claimed that the nitrobenzene radical is dangerous to the haematopoietic system.

Lewis, Putnam, Hendricks, Kerlan and Welch (1952) revealed that in 296 cases of aplastic anemia occurring between 1949 and 1952, 134 patients received chloramphenicol.

Hodgkinson (1954) described 26 cases of aplastic anemia and 3 cases of granulocytopenia associated with
the administration of chloramphenicol, in doses 4
times larger than those commonly used. Necropsy in
22 cases showed reduction of total blood cell-count.
The red blood cell and white blood cell precursors
were scanty. Megakaryocytes were reduced in number
or absent. A relative increase of lymphocytes was
found. Wide spread haemorrhages in the internal
organs was the cause of immediate death in many cases.
Bruising, petechiae of skin, and aplastic bone-marrow
were found. By postmortem examination in two cases,
damage to the liver, apart from fatty degeneration
was reported. In another case the surface of the liver
was found to be irregular owing to scarring. On sect-
ions, the scars were found to be recent, flabby and
produced by large areas of hepatic necrosis. In one
or two areas there were signs of multilobular-cirrhosis.

Krakoff, Karnofsky and Burchenal (1955) adminis-
tered large doses (6gms or more daily) of chloramph-
enicol to four patients with carcinoma. The drug caused
reticulocytopenia and anemia. In one, in whom leuko-
penia and thrombocytopenia also developed, the bone-
marrow revealed vacuolization apparently involved only
the granulocytes.
Rigdon, Martin and Crass (1955) administered chloramphenicol to white Pekin ducks. The drug caused anemia and the percentage of erythroid cells in their bone marrows showed degenerative changes.

Rubin, Weisberger, Botti and Storeasli (1958) using radio-iron techniques, found evidence for red blood cell suppression in 5 of 15 patients treated with chloramphenicol. They observed delayed incorporation of Fe$^{59}$ in the circulating red cells and an elevated plasma iron.

Rosenbach, Caviles and Mitus (1960) found that during the administration of chloramphenicol, when reticulocytopenia and anemia developed, the bone marrow showed maturation arrest of the erythroid elements. The authors claimed that these effects probably represent toxic effects on the haematopoietic system, which at this stage is still reversible with the discontinuation of the drug.

McCurdy (1961) presented 15 patients with bone marrow depression associated with administration of 44 - 105 mg per kg chloramphenicol. Erythropoiesis was most often affected, followed by suppression of thrombo-
poiesis and leukopoiesis.

Saidi, Wallerstein and Aggelor (1961) described the presence of vacuoles in the primitive erythroblasts of 12 of 22 patients receiving 40-85 mg per kg of chloramphenicol per day, whereas 12 subjects who received 11-45 mg per kg per day had no such changes. They suggested that occurrence of toxicity is partially dose dependent.

Gussoff, Lee and Lichtman (1962) described four cases of anemia crisis occurring during the course of chloramphenicol therapy. The outstanding feature was vacuole formation chiefly in the cytoplasm and also in the nucleus in early erythroid cells.

McCurdy (1963) found correlation between bone marrow depression and elevated levels of free chloramphenicol serum. The author presented evidence that toxicity is more common in patients receiving high doses or prolonged treatments of chloramphenicol.

Suhrland and Weisberger (1963) investigated chloramphenicol toxicity in liver and renal disease. In 16 patients with liver disease treated with the drug,
8 developed erythropoietic depression. In 19 patients with moderately severe renal disease, six showed signs of toxic effects. A group of 16 patients without renal or liver disease showed no evidence of bone marrow toxic effects. An elevated level of serum free chloramphenicol was found in all instances of erythropoietic depression. There was no correlation between the metabolic products of chloramphenicol and toxicity. Drug hypersensitivity did not appear to be a factor in the development of erythropoietic depression. The authors suggested that those who develop toxic effects were either unable to conjugate at a normal rate or unable to excrete the free form of the drug.

Colombo and Micciarelli (1964) demonstrated the effects of chloramphenicol on disembyronated chick blastoderm. A clear inhibition of differentiation and development of blood islands was observed. This inhibition was more evident in the younger treated blastoderms, and proportional to the concentration of the drug and the length of treatment. The histological analysis of chloramphenicol-treated blastoderms showed the inhibition of blood island growth and haemoglobin synthesis due to the degeneration of angioblas-
tic cells in the earlier stages and of blood cells in later stages. The cells did not divide and degenerated.

Rosenthal and Blackman (1965) observed hypoplasia of the marrow with pancytopenia following the use of chloramphenicol eye drops in a patient with a family history of a niece who succumbed to aplastic anemia following ingestion of chloramphenicol. They suggested that inherited hypersensitivity or enzyme abnormality disposed the patient to the unusual occurrence of marrow toxicity following topical medication.

b) The "Grey Syndrome" in infants

Chloramphenicol had in the past been considered to be free from serious side effects, other than those associated with haematopoietic system and hypersensitivity. The drug was recommended for use in the treatment and prophylaxis of infections in newborn infants. The doses given were from 100 to 150 mg per kg daily by intramuscular injection. In 1959, Sutherland described three newborn infants who died of cardiovascular collapse after receiving large amounts of chloramphenicol (about 200 mg per kg daily). Then several workers (Kretzner, 1959; Van Gelder, 1959; Kent, 1959;
Sutherland et al., 1959; and Lambin et al., 1960) observed gastro-intestinal symptoms followed by acute cardio-vascular collapse and increased death rates among newborn infants administered this drug. They presented evidence which suggests that administration of chloramphenicol in previously recommended doses (100-160 mg per kg) was responsible for the so-called Grey Syndrome.

Burna, Hodgman and Cass (1959) treated groups of 50-53 premature infants within 12 hours of birth with (1) no antibiotics, (2) penicillin and streptomycin, (3) chloramphenicol 100 to 160 mg per kg daily by intramuscular injection, (4) all of these antibiotics in the same doses. The mortality in these groups were 19, 18, 60 and 68 per cent respectively. The illness usually began 2 to 9 days (average 4 days) after treatment was started. The manifestations in the first 24 hours were vomiting, refusal to suck, irregular and rapid respiration, abdominal distension, attacks of cyanosis and passage of loose green stools. All the children were severely ill by the end of the first day and, in the next 24 hours, developed flaccidity, an ashen-grey colour, and a decrease in temperature.
Death occurred in about 40 per cent of the patients, most frequently on the fifth day of life. Those who recovered exhibited no sequelae. Blood chemical studies were done in some infants. All the infants studied had an elevated non-protein nitrogen. As their condition deteriorated, the serum carbon dioxide fell, and the serum potassium rose. Spinal fluid chemical findings in two of the infants were normal. Chloramphenicol blood levels were very high in two cases tested. One of them showed about 8 fold higher level than normal. Autopsies were done on 34 infants, or 68 per cent of the deaths. Gross findings were not remarkable in 25 infants, the cause of death was obscure. Of the remainders, two had bronchopneumonia, five had cerebral haemorrhage, and two died after surgical correction of a tracheoesophageal fistula and a duodenal stenosis. The authors attributed the Grey Syndrome to the poor liver function especially in the glucuronide conjugation system, as well as decreased kidney function, allowing normally safe doses to accumulate to toxic levels. This toxicity manifested itself by a characteristic picture of circulatory collapse.
Weiss, Glazko and Weston (1960) instituted chloramphenicol to newborn infants within the first 48 hours of life. The symptoms first appeared after 3 to 4 days of continued treatment with 100 mg per kg of body weight daily or more. Progression of symptoms from onset to death was accelerated with higher doses. They found no pathological changes attributable to the use of chloramphenicol in any of the organs or systems, including the haematopoietic system. The authors emphasized the variations in metabolic disposition of chloramphenicol in the light of current knowledge concerning the development of renal and hepatic functions in the newborn infants.

Lischner, Seligmen, Krammer and Parmelee (1961) reported a death rate of 67 per cent for 12 term infants who received over 110 mg per kg chloramphenicol daily and a cumulative dose over several days of 400 mg per kg or more. Death occurred at 2 to 5 days of age, after the sudden appearance of vascular collapse.

Iossifides, Smith, Inez and Keital (1963) stated that toxicity may develop in neonates especially premature babies, when they are exposed to excessive doses of the drug. Chloramphenicol tends to accumulate in the
blood of such children and reaches high levels at about the fourth day of treatment.

a) Optic neuritis

Among the rare toxic effects produced by chloramphenicol are the optic neuritis and blurring of vision. Dietman, Di Sant'Agnese and Wong (1964) emphasized the need to pay attention to the visual acuity during the chloramphenicol therapy.

Cocke, Brown and Geppert (1966) described optic neuritis in children receiving prolonged chloramphenicol treatment for pulmonary infection. Stopping the drug and administration of large doses of vitamin B-complex resulted in partial restoration of sight.

Later Cocke, in 1967, described a case of optic neuritis of 12-years-old girl with cystic fibrosis. She experienced two consecutive episodes of chloramphenicol optic neuritis following total doses of 135 and 47 gm of the drug respectively. The patient has been spared a third occurrence of 200 gm chloramphenicol, by simultaneous administration of large doses of vitamins B₆ and B₁₂. The author suggested that large doses of group B vitamins given concomitantly with large
doses of chloramphenicol may serve as a preventive treatment against chloramphenicol optic neuritis.

In spite of the above severe damages exerted by chloramphenicol, the drug is still widely used in general practice. However, the literature is entirely lacking its effects on the foetus when it is maternally administered. Thus the present study was proposed to investigate the effect of chloramphenicol on the foetus of an inbred strain of the albino mouse, when it is maternally administered, during the different periods of embryonic development.
RESULTS OF MATERNAL ADMINISTRATION
OF CHLORAMPHENICOL

A- GROSS EXAMINATION

In this investigation gross examination included; the number of females that continued pregnancy, the total number of foetuses obtained from the females that continued pregnancy, and the number of living and dead foetuses either normal or abnormal.

Vaginal bleeding followed by reduction in abdominal swelling was diagnostic of abortion.

The number of living foetuses means the number of foetuses surviving for a few minutes before they were fixed in a suitable fixative for further detailed studies.

By the term abnormal is meant only morphological deviation from normality in development.

The controls were subjected to the same treatment, except the drug, from the 1st to the 16th day of gestation. No abortions were observed and the average number of foetuses per litter was 8.4.
1. MATERNAL ADMINISTRATION OF 1/10 LD\(_{50}\)

The results of maternal administration of 1/10 LD\(_{50}\) of chloramphenicol during the different periods of gestation by three different routes of administration, are summarised in table 1.

a) Animals subjected to treatment from the 1st to the 6th day of gestation (group 1).

Orally treated ten females continued pregnancy except one. The nine females that continued pregnancy gave 63 living and morphologically normal foetuses.

Intramuscularly treated ten females continued pregnancy except two. The eight females that continued pregnancy gave 67 living and morphologically normal foetuses.

Intraperitoneally treated ten females continued pregnancy. They gave 88 living and morphologically normal foetuses.

b) Animals subjected to treatment from the 7th to the 12th day of gestation (group 2).

Orally treated ten females continued pregnancy. They gave 65 living normal foetuses.
Intramuscularly treated ten females continued pregnancy except one. They gave 60 living normal foetuses.

Intraperitoneally treated ten females continued pregnancy and gave 61 living normal foetuses.

a) Animals subjected to treatment from the 13th to the 18th day of gestation (group 3).

Orally treated ten females continued pregnancy. They gave 87 living normal foetuses.

Intramuscularly treated ten females continued pregnancy. They gave 80 living normal foetuses, and 4 dead foetuses.

Intraperitoneally treated ten females continued pregnancy. They gave 84 living normal foetuses, and 2 dead foetuses.
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**Table 1**

Results of Gross Examination after Treatment with 1/10 TDP50
2. MATERNAL ADMINISTRATION OF 1/3 LD₅₀

The results of maternal administration of 1/3 LD₅₀ of chloramphenicol during the different periods of gestation by three different routes of administration, are summarised in table 2.

a) Animals subjected to treatment from the 1st to the 6th day of gestation (group 1).

Orally treated ten females continued pregnancy except one. They gave 61 living normal foetuses.

Intramuscularly treated ten females continued pregnancy except two. They gave 59 living normal foetuses.

Intraperitoneally treated ten females continued pregnancy except two. They gave 64 living normal foetuses.

b) Animals subjected to treatment from the 7th to the 12th day of gestation (group 2).

Orally treated ten females continued pregnancy. They gave 54 living normal foetuses, 3 living abnormal and 2 dead. The abnormal foetuses were smaller than the controls (figs. 2-4). The reduction in size was
marked in the body while the head appeared normal.

The abnormal foetus (fig. 2) showed shortness of the medial and terminal parts of the fore-limbs and hind limbs (brachymely). The digits of both limbs were short (brachydactyly), specially the fore-limbs. The fore-limbs were flexed posteriorly at the wrist joints. The hind limbs were flexed anteriorly in the place of the ankle joint.

The abnormal foetus (fig. 3) had the left hind limb atrophied and flexed posteriorly. The fore and hind limbs showed brachydactyly.

The abnormal foetus (fig. 4) had the fore-limbs flexed posteriorly at the wrist joint. They showed brachymely and brachydactyly. One hind limb was flexed posteriorly at the knee joint and showed brachydactyly. The other hind limb was normal.

Intramuscularly treated ten females continued pregnancy. They gave 61 living normal foetuses.

Intraperitoneally treated ten females continued pregnancy except one. They gave 55 living normal foetuses.
c) Animals subjected to treatment from the 13th to the 18th day of gestation (group 3).

Orally treated ten females continued pregnancy. They gave 81 living foetuses and 4 dead foetuses. Two of the dead foetuses were morphologically abnormal. They were smaller in size than the controls.

One of the abnormal foetuses (fig. 5) had the fore-limbs flexed posteriorly at the wrist joints. One hind limb was markedly flexed posteriorly and upwards. The fore- and hind limbs showed brachydactyly.

The abnormal foetus (fig. 6) showed atrophy of the medial and distal parts of the fore-limbs (brachymely). The digits of the fore-limbs showed brachydactyly. The hind limbs were normal.

Intramuscularly treated ten females continued pregnancy. They gave 80 living normal foetuses and one dead foetus.

Intraperitoneally treated ten females continued pregnancy. They gave 84 living normal foetuses and 4 dead foetuses.
<table>
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Results of Gross Examination after Treatment with T/3 1% Cholorphenicol
B-MACROSCOPIC EXAMINATION OF FOETAL SKELETON

Examination of the foetal skeleton was possible by preparing the specimens according to Dawson's method. The soft tissues were transparent while the skeleton, even very small parts, were stained pink.

Preparations from the control foetuses were first studied (fig. 7). It was possible to recognize the following bones:
Bones of the pectoral girdle scapula, clavicle, and sternum which appeared as dotted line (6 sternebrae).
Bones of the fore-limbs; humerus, radius and ulna, four bones of metacarpus, and phalanges.
Bones of the pelvic girdle; ilia, ischia and pubis.
Bones of the hind limbs; femur, tibio-fibula as two bones which united at the distal extremity, two bones in the tarsus (astragalus and calcaneous), five bones in the metatarsus, and phalanges.
The vertebral column; 7 cervical segments, 13 thoracic, 6 lumbar, 4 sacral, and 20-25 caudal segments. The ribs were 13 pairs.
The skull with the upper and lower jaws were also examined.
B-MACROSCOPIC EXAMINATION OF FOETAL SKELETON

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Bones of the pectoral girdle: scapula, clavicle, and sternum which appeared as dotted line (6 sternabrae).
Bones of the fore-limbs: humerus, radius and ulna, four bones of metacarpus, and phalanges.
Bones of the pelvic girdle: ilia, ischia and pubis.
Bones of the hind limbs: femur, tibio-fibula as two bones which united at the distal extremity, two bones in the tarsus (astragalus and calcaneous), five bones in the metatarsus, and phalanges.
The vertebral column; 7 cervical segments, 13 thoracic, 6 lumbar, 4 sacral, and 20-25 caudal segments. The ribs were 13 pairs.
The skull with the upper and lower jaws were also examined.
Preparations of the treated foetuses were similarly examined in order to study these skeletal structures. No major skeletal defects were observed in any foetus among those prepared for this study. However, some foetuses which were externally normal, showed absence of some of the small bones. The caudal vertebrae were in most cases reduced, minimum to two vertebrae. The two tarsus bones (astragalus and calcaneous) were mostly missing, and in some cases the astragalus was absent while the calcaneous was present (fig. 8).

The malformed foetus, fig. 2, had the long bones in the fore-limbs and hind limbs extremely shorter than normal. The two tarsus bones were missing. The caudal vertebrae were reduced to 4, (fig. 9).

The flexed hind limb of the malformed foetus, fig. 3, had all the long bones present although relatively smaller than normal. The two tarsus bones were missing. The caudal vertebrae were reduced to 6.

The malformed foetus, fig. 4, had the bones of the flexed fore-limbs complete but shorter than normal. The long bones of the flexed hind limb were all present but markedly shorter than normal. However, the two
tarsus bones, astragalus and calcaneous, were missing from both limbs. The caudal vertebrae were reduced to two segments (fig. 10).

The malformed foetus, fig. 6, had the bones of the short fore-limbs present but shorter than normal. The bones of the sternum were reduced to 5 instead of 6 in the normal foetuses. The caudal vertebrae were reduced to 6 vertebrae.

It should be stated here that some bones are missing in the photographs, because they are out of focus.
G - MICROSCOPIC EXAMINATION

Microscopic examination of sections, prepared from foetuses of mothers subjected to either chloramphenicol or phenylbutazone during different periods of embryonic development, showed that the two drugs exerted histopathological effects on some internal organs. These effects were marked in the foetal liver, kidney and spinal cord.

The general results of the pathological changes observed in the foetal liver and kidney due to maternal administration of either chloramphenicol or phenylbutazone can be summarised as follows:

Liver lesions

The foetal liver cells showed different phases of degeneration in the form of cloudy swelling, hydropic degeneration, fatty degeneration, focal necrosis and complete necrosis. These pathologic changes appeared either singly or in combination.

Cloudy swelling:

The liver cells appeared swollen and their cytoplasm contained many pink-stained granules in the eosin and haematoxylin stained sections. These were coarse
irregular granules scattered throughout the entire cytoplasm. The nuclei were swollen, but remained central in position (fig. 11).

**Hydropic degeneration:**

The liver cells appeared swollen and the cytoplasm became foamy in appearance, while the nuclei were still central in position (fig. 12).

**Fatty degeneration:**

This lesion appeared in the paraffin sections stained with eosin and haematoxylin as vacuoles or empty spaces, due to dissolution of the fat in the reagents used, as xylol and alcohol. The nuclei of the cells were flattened and pushed against the cell wall by the accumulated fat (fig. 13), so the cells acquired a signet ring appearance (fig. 14). Frozen sections stained with Sudan black demonstrated the fats as variable sized black globules. The fat globules were irregularly distributed in the liver cells (fig. 15).

**Necrosis:**

The most striking change was the disappearance of many nuclei, while the remainder showed one or
other of the postnecrotic changes; pyknosis, karyolysis or karyorrhexis. (fig. 17).

In pyknosis, the nucleus through shrinkage and condensation was converted into a single rounded mass of uniformly intense basophilic quality.

Karyolysis appeared as solution of the chromatin and thereafter diffusion of the solute through the nuclear membrane. Thus the nucleus appeared as empty vesicle, or disappeared completely if the solution of the nuclear membrane took place.

Karyorrhexis entailed disintegration of the chromatin network into several deeply stained fragments. These were arranged under the nuclear membrane and on disappearance of the latter, they were left irregularly distributed in the cytoplasm.

Complete necrosis:

Areas of complete necrosis were seen mostly, in the periphery of the liver lobules (fig. 18) and rarely in the centrilobular parts (fig. 19). They appeared as collapsed reticular framework entangling many cellular and nuclear debris. Some foci with
complete necrosis showed haemorrhages demonstrated as many intact and haemolysed red cells dispersed in the liver (fig. 20).

Many of the surviving liver cells were abnormally large with abundant cytoplasm and large hyperchromatic dividing nuclei denoting regenerative activity (fig. 25).

**Bile pigment retention:**

Sometimes the cytoplasm of the degenerated and necrotic liver cells showed fine yellowish green to dark brown retained bile pigments (figs. 21 and 22). The Kupffer cells in the walls of the sinusoids were swollen and distended by these bile pigments (fig. 11).

**Lymphocytic infiltration:**

In most cases of necrosis lymphocytes and few polymorphonuclear leucocytes appeared in the sinusoids and portal tracts (figs. 23 and 24).

**Congestion:**

Independent of the above mentioned degenerative effects, congestion sometimes appeared in the central veins and sinusoids (figs. 26 and 27). This appeared as dilatation and engorgement of these vessels, which
were packed by a large number of intact red cells, haemolysed cells and haemosiderin pigment (fig. 28). The Kupffer cells were swollen and distended with haemosiderin (fig. 11).

Kidney lesions

Microscopic examination of the foetal kidneys showed two degenerative effects; cloudy swelling and necrosis. These degenerative changes mainly appeared in the convoluted tubules and the ascending limbs of Henle's loops. Necrosis was a further step after cloudy swelling and thus the two lesions mostly appeared combined.

Cloudy swelling:

In this case the cells lining the affected tubules were swollen and became conical in shape (fig. 29). In some cases they were seen separated from each other and sometimes from the basement membrane. The cells also projected inwards and consequently the lumina of the convoluted tubules became small and irregular. The cytoplasm of the affected cells appeared granular and more acidophilic. The top portions of the
cells in some of the tubules were seen detached (fig. 30). The nuclei of the affected cells were slightly swollen when compared with the nuclei of the cells lining the collecting tubules which showed no pathological effects.

**Necrosis:**

The lining cells of the convoluted tubules and the ascending limbs of Henl's loops were swollen, with ill-distinct cell borders and the neighbouring cells were fused together in a homogeneous pink mass. Some cells were detached and appeared in the lumina of the tubules as granular and epithelial casts (fig. 31).
Results of maternal administration of chloramphenicol

1. Foetal liver

The results of microscopic examination of serial transverse sections of foetuses from mothers received chloramphenicol by different routes; oral, intramuscular, and intraperitoneal, during early, mid, or late gestation with 1/10 LD<sub>50</sub> and 1/3 LD<sub>50</sub> are represented in tables (2) and (4) respectively.

All the foetuses examined were morphologically normal and surviving, before they were prepared for examination.

The following pathological lesions were recognized in the liver of almost all foetuses examined; cloudy swelling, hydropic degeneration, fatty degeneration, and necrosis. These lesions mostly appeared in combination. In some cases areas of complete necrosis were seen in the peripheries of the liver lobules and rarely in the centriloculobular parts. The necrotic cells in few cases contained excessive amounts of granular bile pigment. Congestion of the central veins and the sinusoids commonly accompanied severe fatty degeneration
and necrosis. In some cases of necrosis the portal tracts and sinusoids showed lymphocytic infiltration. The surviving liver cells showed different phases of division to form regenerating liver cells.

The incidence of these lesions in foetuses examined from each identically treated group is recorded in tables 3 and 4. The code number of each examined foetus is recorded under every lesion observed in it.

The total results obtained are again summarized in table 5, independent on the route of administration. The number and percentage of foetuses affected were recorded under every lesion observed in each group.
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I.1</td>
<td>I.2</td>
<td>I.3</td>
<td>I.4</td>
<td>I.5</td>
<td>I.6</td>
<td>I.7</td>
<td>I.8</td>
</tr>
</tbody>
</table>

**Legend:**
- T.P. = Transpontine route
- T.I. = Transiron route
- D.I. = Direct route
- T.H. = Transhum route
- H.Z. = H. Z. route
- Z.Z. = Z. Z. route
- D.D. = Direct route
- D.Z. = D. Z. route

---

**Results of the clinical examination at 1/10 T/250 of the liver:**

<table>
<thead>
<tr>
<th>Veins</th>
<th>Lymphatics</th>
<th>Portal areas</th>
<th>Splenic areas</th>
<th>Mesenteric areas</th>
<th>Focals</th>
<th>Fatty degeneration</th>
<th>Hydropic degeneration</th>
<th>Swelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen.</td>
<td>Gen.</td>
<td>General</td>
<td>General</td>
<td>General</td>
<td>Focal</td>
<td>Fatty</td>
<td>Hydropic</td>
<td>Swelling</td>
</tr>
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</table>

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**Table:**

- Complete
- Complete
- Complete
- Complete

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**Conclusion:**

The examination revealed no significant abnormalities. Results of the clinical examination were within normal limits.
<table>
<thead>
<tr>
<th>Route</th>
<th>0 = Oral route</th>
<th>i.m. = Intramuscular route</th>
<th>i.p. = Intraperitoneal route</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Results of treatment administered on 1/31/59 of Case 4.</th>
<th>Lymphocytic Infiltration</th>
<th>Central Sinus</th>
<th>Central Nephritis</th>
<th>Central Peri-renal Nephritis</th>
<th>Central Nephrocalcinosis</th>
<th>Central Necrosis</th>
<th>Hydropic Swelling</th>
<th>Cloudy Examinations</th>
<th>Foetuses</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
<td>% No.</td>
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<td>(-)</td>
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<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table:**

| Time of treatment | Dose (mg) | No. of foetuses examined | Incidence of the histopathological lesions in the foetal liver
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td>****</td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td></td>
<td>40</td>
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<td>TP</td>
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<td></td>
<td>80</td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

**Note:**
- Different periods of gestation.
- After maternal administration of chlorpromazine.
2-Foetal kidney

Results of microscopic examination of serial sections of foetuses from mothers treated with chloramphenicol during the different periods of pregnancy; early from the 1st to the 6th day, mid from the 7th to the 12th day, or late from the 13th to the 18th day of gestation, by the different routes of administration; oral, intramuscular and intraperitoneal, using 1/10 and 1/3 LD$_{50}$ are demonstrated in tables (6) and (7) respectively.

The basic lesions met with were; cloudy swelling and necrosis of the convoluted tubules and the ascending limbs of Henl's loops (Figs. 29-31). However, the glomeruli, collecting tubules and the descending limbs of Henl's loops showed normal histological appearance.

The total results obtained were again summarized in table (8), independent on the route of administration. The number and percentage of foetuses affected was recorded under every lesion observed in each group.
Table 6

Results of maternal administration of 1/10 LD$_{50}$ of chloramphenicol on the foetal kidney

<table>
<thead>
<tr>
<th>Group</th>
<th>Route</th>
<th>Foetuses examined</th>
<th>Cloudy swelling</th>
<th>necrosis</th>
<th>Congestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.</td>
<td></td>
<td>$A_1$ &amp; $A_2$</td>
<td>$A_1$ &amp; $A_2$</td>
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</tr>
<tr>
<td>I</td>
<td>i.m.</td>
<td>$B_1$ &amp; $B_2$</td>
<td>$B_1$ &amp; $B_2$</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>$C_1$ &amp; $C_2$</td>
<td>$C_1$ &amp; $C_2$</td>
<td>$C_1$</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>i.m.</td>
<td>$D_1$ &amp; $D_2$</td>
<td>$D_1$ &amp; $D_2$</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>$E_1$ &amp; $E_2$</td>
<td>$E_2$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
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<td>$F_1$ &amp; $F_2$</td>
<td>$F_1$ &amp; $F_2$</td>
<td>$F_1$</td>
<td>$F_2$</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>$G_1$ &amp; $G_2$</td>
<td>$G_1$ &amp; $G_2$</td>
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<td>-</td>
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<tr>
<td></td>
<td>i.m.</td>
<td>$H_1$ &amp; $H_2$</td>
<td>$H_1$ &amp; $H_2$</td>
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<tr>
<td></td>
<td>i.p.</td>
<td>$I_1$ &amp; $I_2$</td>
<td>- $I_2$</td>
<td>$I_1$</td>
<td>-</td>
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</tbody>
</table>


Table 7.

Results of maternal administration of $1/3\text{ LD}_{50}$
of Chloramphenicol on the foetal kidney.

<table>
<thead>
<tr>
<th>Group</th>
<th>Route</th>
<th>foetuses examined</th>
<th>Cloudy swelling</th>
<th>Necrosis</th>
<th>Congestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>-</td>
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<tr>
<td>I</td>
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<tr>
<td></td>
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<td>$L_1 &amp; L_2$</td>
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<td>$M_2$</td>
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<td>-</td>
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<tr>
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<td>$O_1 &amp; O_2$</td>
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<td>$O_2$</td>
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<td>$P_2$</td>
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<td>III</td>
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Table 8.

Incidence of the histopathologic lesions in the foetal kidney after maternal administration of chloramphenicol during the different periods of gestation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of Treatment</th>
<th>Dose</th>
<th>Number of foetuses examined</th>
<th>Cloudy swelling</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number affected</td>
<td>% Number affected</td>
</tr>
<tr>
<td>I</td>
<td>1-6</td>
<td>1/10</td>
<td>6</td>
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<td>100</td>
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<tr>
<td></td>
<td></td>
<td>1/3</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>II</td>
<td>7-12</td>
<td>1/10</td>
<td>6</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
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<td></td>
<td>1/3</td>
<td>6</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>III</td>
<td>13-18</td>
<td>1/10</td>
<td>6</td>
<td>5</td>
<td>83</td>
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<tr>
<td></td>
<td></td>
<td>1/3</td>
<td>6</td>
<td>4</td>
<td>67</td>
</tr>
</tbody>
</table>
3- Foetal Spinal Cord

The external configuration of the foetal spinal cord was first studied in serial transverse sections of the control groups throughout the cervical, thoracic and lumbar regions (figs. 32, 33 and 34).

Treatment from the 1st to the 6th day of gestation:

Foetuses maternally treated from the 1st to the 6th day of gestation, with 1/10 LD50 of the drug by the oral, intramuscular and intraperitoneal routes showed, malformations in the spinal cord in the cervical region. The cord showed asymmetry of its halves in the affected regions. (figs. 35, 36 and 37).
However, the thoracic and lumbar spinal cord showed normal external configurations. The vertebral arch was internally destructed throughout the different regions. Also the vertebral canal showed deficiency in the regions of the asymmetrical cord (figs. 36 and 37).

Foetuses of mothers treated from the 1st to the 6th day of gestation with 1/3 LD50 of the drug by the different routes, showed asymmetry of the spinal cord. This effect on the cord was marked in the cervical and thoracic regions, while the lumbar cord showed
normal external configurations. Dorsal herniation of one half was the cause of the asymmetry of the cervical and thoracic spinal cord. One of the dorsal gray horns, white matter and the meninges were included in this herniation (figs. 38, 39 and 40). The vertebral canal showed marked deficiency in the asymmetrical region of the cord (figs. 39 and 40). The vertebral arch was internally destroyed throughout the different regions. The ossifying centres were markedly lacking specially ventral to the cord. (fig. 40).

Treatment from the 7th to the 12th day of gestation:

Fœtuses maternally treated from the 7th to the 12th day of gestation with 1/10 LD$_{50}$ of the drug by the different routes showed shrinkage of the spinal cord inside the dura mater. This effect was marked in the cervical and thoracic regions while it was slightly detected in the lumbar region. The outer contours of the affected regions of the cord were crenated. The spinal cord was shifted dorsally leaving a large subdural space ventrally (figs. 41, 42 and 43). In one foetus, the cervical spinal cord showed rupture of the wall of the central canal and the tissues of
the dorsal commissure. Thus the spinal cord was longitudinally splitted from the dorsal median level till the position of the canal (fig. 43). The vertebral arch was intact throughout the different regions. No haemorrhages were detected in any of the structures related to the cord.

Fetuses treated by \( \frac{1}{3} \text{LD}_{50} \) of chloramphenicol showed marked shrinkage of the spinal cord inside the dura mater throughout the cervical and thoracic regions (figs. 44, 45, 46 and 47). The outer contours of the spinal cord in the affected regions showed marked crenation of the ventral and lateral sides. In some cases the central canal was disrupted in the cervical and thoracic regions (figs. 44 and 45). However, these defects were not detected in the lumbar region. The vertebral arch was intact throughout the different regions. No haemorrhages were observed in any of the structures related to the spinal cord.

**Treatment from the 13th to the 18th day of gestation:**

Fetuses maternally treated from the 13th to the 18th day of gestation with \( \frac{1}{10} \text{LD}_{50} \) of chloramphenicol by the different routes, resulted in the formation of
focal subdural invaginations in some areas along the cervical and the thoracic regions of the spinal cord. (figs. 48, 49, 50, 51 and 52). Red blood corpuscles indicating haemorrhages, were sometimes detected inside these invaginations. The dorsal and ventral spinal arteries were found to be the cause of these haemorrhages (figs. 49, 50 and 53).

The spinal cord showed also moderate shrinkage in the different regions inside the duramater (figs. 48 and 53). However, the lumbar spinal cord showed normal external configurations in all the affected foetuses. The vertebral arch was intact around the cord throughout the different regions.

Foetuses maternally treated with 1/3 LD₅₀ by the oral route showed also subdural invaginations throughout the cervical and thoracic regions. Red blood corpuscles indicating haemorrhages were sometimes observed inside these invaginations. The dorsal spinal artery was seen to be the origin of these haemorrhages (figs. 54, 55 and 56).

In another foetus from mother treated by the intramuscular route, the cervical spinal cord showed
dorsal destruction in the white matter and red blood corpuscles were detected mixed with the destructed tissue (figs. 57 and 58). The dorsal spinal artery and the fine blood vessels of the spinal cord were seen to be the origin of these haemorrhages. The thoracic and lumbar spinal cord showed normal external configurations. The vertebral arch was destructed in different regions around the spinal cord (fig. 57).

In a foetus maternally treated intraperitoneally the spinal cord was severely affected throughout the cervical and thoracic regions. In the cervical region only remnants of the cordal tissue were detected ventrally. The dorsal portion of the cord was replaced by a horny-like tissue (fig. 59). In the thoracic region the cord was represented by dorsal and ventral ill-defined cordal tissue. At the site of the central canal and the surrounding area, there present a necrosed tissue mixed with red blood corpuscles (fig. 60). However, the lumbar spinal cord of this foetus showed normal external configurations. The vertebral canal showed great deficiency in different regions. The centra of the thoracic vertebrae appeared
huge with two remnants of notochordal tissue (fig. 60).

The vertebral arch surrounding the spinal cord again was the site of haemorrhages, among this group of foetuses maternally treated at the later period of pregnancy. Subperiosteal haemorrhages were detected in many of the specimens. The discontinuity of the periosteum of the vertebral canal permitted the extravasated blood to leak into the cavity of the vertebral space around the spinal cord (figs. 61 and 62).
DISCUSSION

Wilson (1959 and 1961) has put forward some generalizations applicable to experimental teratology. The effect of a drug on the embryonic development may depend upon the developmental stage at which it is applied to the embryo. Each organ may be thought of as having a susceptible period occurring early in the formation of its perimordium. However, it must be taken into consideration that these periods vary with the drug used, as it will determine which facet of the developmental process is disturbed. Moreover, during the late development susceptibility to teratogens is reduced, nevertheless development is not complete and changes continue to take place until after birth. Another aspect which must be taken into account, in mammals, is the relation of the time of treatment to the state of development of the materno-foetal exchange mechanisms.

Taking into consideration the above-mentioned factors, it was thought that the study may be of more practical value if it covers the whole period of preg-
nancy. Continuous administration of the drugs tested for long periods is, however, far from clinical use. Consequently, the test animals were divided into three groups and each group was subjected to daily treatment during one trimester.

The majority of investigations in experimental teratology have been performed in rodents. Tuchmann-Duplessis (1965) stated that the mouse is usually more susceptible to teratogens than the rat. He added, that the spontaneous malformations in the mouse has to be taken into account. However, the incidence of spontaneous malformations was not observed during the breeding of the mice used in the present experiments in order to get a large number of inbred strain of animals. As a control experiment 21 females were treated by the drug solvent. No deviation from normal was observed among 176 foetuses extracted.

Choice of the drug:-

Alamanni (1957) stated that chloramphenicol, among other antibiotics, is able to pass easily through the placental barrier, and appear in elevated levels in the foetal blood and amniotic fluid. Several
investigators established that some antibiotics disturb the development of the embryo in mammals. Their teratogenic effect may be either due to, their antibiotic properties, allergo-toxic manifestations, or interference with the metabolic activity of the different elements of the vitamin B complex.

Since Rich, Ritterhoff and Hoffmann (1950) reported the first case of aplastic anemia in association with chloramphenicol therapy, several investigators reported irreversible toxic effects on the haematopoietic system as well as cases of hypersensitivity. In 1959 about five papers were published that reported gastro-vascular collapse and increased death rates among premature and newborn infants received chloramphenicol in doses previously recommended for children on body weight bases. Weiss, Glazko and Weston (1960) proposed that the premature infant is unable to metabolize chloramphenicol because its liver cannot form the less toxic glucuronide. This results in a high concentration of the drug in the blood.
Colombo and Micciarelli (1964) by histological analysis of chloramphenicol-treated blastoderms of chick embryo observed inhibition of blood island growth and haemoglobin synthesis. They referred this to the degeneration of angioblastic cells in the earlier stages and of blood cells in the later stages.

Weisberger, Armentrout and Wolfe (1963 and 1964) had demonstrated that chloramphenicol can inhibit protein synthesis in mammalian cell-free systems as effectively as it inhibits protein synthesis in analogous microbial systems. Ambrose and Coons (1963) demonstrated that chloramphenicol also inhibits protein synthesis by intact mammalian cells in vitro. Weisberger and Wolfe (1964) suggested that the inhibiting effect of chloramphenicol on protein synthesis in proliferating cells may be an important factor in the haematologic toxicity attributed to chloramphenicol.

Although the pertinent literature suspects chloramphenicol to possess some teratogenic effects in mammals, no direct evidence for such effect has so far been reported.
The General Effects of Maternal Administration of Chloramphenicol

Treatment during early gestation.

Females treated from the 1st to the 6th day of gestation were the most susceptible for abortion; 10 per cent were aborted when administered \(\frac{1}{10} \text{LD}_{50}\) of chloramphenicol and this incidence increased to 16.7 per cent when the dose was increased to \(\frac{1}{3} \text{LD}_{50}\). The average number of foetuses per litter only slightly decreased from 8.4 in the controls to 8.1 in the group subjected to \(\frac{1}{10} \text{LD}_{50}\), but this effect became more pronounced in the group subjected to the higher dose (\(\frac{1}{3} \text{LD}_{50}\)) as this value decreased to 7.4. No malformed foetuses were obtained in this group of animals. All the foetuses extracted were living and survived for few minutes after extraction before they were fixed for further detailed studies.

According to Snell (1956); the implantation of the ova in the mouse uterine mucosa occurs after about 5 days. Up to the time of implantation there has been no growth in size of the egg. Cleavage has resulted in numerous smaller cells, but little if any new
protoplasm has been formed in this process. After implantation rapid growth commences. One day after implantation there is an appreciable swelling in the uterus at the implantation site. Meanwhile the zona pellucida has been lost from around the egg. Tuchmann-Duplessis (1965) stated that during the short pre-implantation period, exogenous agents do not in general produce malformations. The dividing ovum may be killed by physical or chemical injury, but when this is less severe the damaged cells can be repaired or replaced so that development is resumed without impairment of organogenesis.

The results obtained in this study are concordent with what was mentioned by the above authors as well as the generalization made by Beck and Lloyd (1965) that before implantation, the ovum enclosed in the zona pellucida, is relatively impermeable to substances in its immediate environment and, for this reason, most teratogenic agents except physical agents are incapable of attacking it.
Treatment during mid-gestation:

The females treated from the 7th to the 12th day of gestation showed marked decrease in the number of foetuses per litter; from 8.4 in the controls to 6.4 and 5.0 corresponding to 1/10 and 1/3 LD_{50} respectively. One case of abortion occurred among the females treated by each dose, representing about 3.3 per cent of the cases. Two dead and three malformed foetuses were obtained in the group subjected to the higher dose (1/3 LD_{50}).

Tuchmann-Duplessis and Mercier-Parot (1959 and 1960), showed that actinomycin was teratogenic in rat and the period in which the teratogenic activity became most evident was between the 7th and the 9th day of gestation. Also Erikson, Strandvik and Gyllensten (1963) administered streptomycin to mice from the 9th until the 13th day of gestation. They were able to observe, in 9 out of a total of 52 embryos, cerebral haemorrhage of slight extent, ependymal polypi, and further ocular anomalies. Filippi (1967) investigated the effect of penicillin and streptomycin combinations on the embryonic development in rats. The
malformations were observed in 35–45 per cent of the young when the drug was administered from the 5th to the 9th day of gestation.

The early embryology of the mouse was described in detail by Snell (1956), and is summarized in table (9). This table shows that the foetuses in the present study (subjected to treatment from the 7th to the 12th day of gestation) received the drug after implantation. Beck and Lloyd (1965) stated that once implantation of the ovum has ensured that the nutritional requirements for rapid growth and differentiation can be satisfied, gastrulation begins to take place. At this stage the embryo is highly susceptible to teratogenic agents. Later on Tuchmann-Duplessis (1965) stated that during the formation of the main layers, the appearance of the "anlage" and the differentiation into organs, the embryo is most susceptible to the effects of exogenous agents. However, in the present investigation the anomalies obtained among animals subjected to the drug during early gestation are not related to impairment of organogenesis.
Table 9

Summary of the early embryonic development of the mouse

<table>
<thead>
<tr>
<th>Event</th>
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<th>Hours</th>
</tr>
</thead>
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</tr>
<tr>
<td>Morula stage</td>
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<td>Primitive streak</td>
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<td>Head process</td>
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<td>Fore-gut</td>
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<tr>
<td>Neural groove</td>
<td>7 1/4</td>
<td>24</td>
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<td>7 1/4</td>
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<td>Allantois</td>
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<td>Head fold</td>
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<td>Heart</td>
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* After Snell (1956). The females were hybrids between two strains and the males were from a third strain. The stages as described here are usually earlier, often by as much as a day or more, than comparable stages described by other authors.
Treatment during late gestation:

The group of females administered chloramphenicol from the 13th to the 18th day of gestation gave nearly the same number of foetuses per litter as the controls. Also, like the controls, no abortions were observed. However, the striking feature in this group was a relatively high mortality rate among foetuses. The percentage of dead foetuses in the females treated with $1/10 \text{LD}_{50}$ and $1/3 \text{LD}_{50}$ was 2.4 and 3.7 per cent respectively. The absence of abortions and the normal size of litter obtained in this group are apparently due to the fact that treatment started after implantation by an enough long period. However, the drug affected the late development of the limbs in two foetuses of the same litter.

The present study demonstrated that chloramphenicol can affect the embryonic development of the mouse when it is administered during the second or the third trimesters. The anomalies observed are characterised by shortening of the distal segments (radius and ulna, tibia and fibula) and digits. Genetic factors were excluded as the mouse strain used in this study never presented these anomalies spontaneously.
It is noteworthy that the malformations of the limbs observed in this study are similar to those obtained experimentally with vitamin \( B_2 \) deficiency in the rat by Giroud and Boisselot (1948), Warkany and Schraffenberger (1943) and by Bologna and Piccioni (1950). Filippi and Mela (1958) administered to the pregnant rats tetracycllin in doses calculated to be equivalent to those used therapeutically in man. They observed in the embryos; cleft palate, hypotrophy of the mandible, syndactyly and abnormal shortness of the limbs. They referred these anomalies to deficiency of vitamin-B complex. Later Filippi (1967) repeated the same study and obtained similar results. In his study, the animals administered vitamin-B complex with the antibiotic restored normal development. Carter and Wilson (1962) published a report that a child having congenital malformation of the hand was born to a mother who had been treated with tetracyclline during pregnancy.

Since chloramphenicol, like tetracycllin, inhibits vitamin syntheses by affecting the normal bacterial flora of the large intestine, vitamin deficiency may be considered responsible for the anomalies observed.
in the present study. Maldevelopment of the limbs observed in this work is a common feature among anomalies reported in the literature after administration of antibiotics. It is noteworthy that the malformed foetuses obtained in the present investigation were from females administered the drug by the oral route. This offers a strong evidence that vitamin deficiency is responsible for the anomalies observed since the concentration of the drug in the intestine should be maximum by the oral route relative to the other two routes. No malformations were observed in the offspring of the females administered the drug either intramuscularly or intraperitoneally.

Examination of the foetal skeleton showed that the long bones were all present although shorter in the affected limbs. Among the small bones some caudal vertebrae, the astragalus and the calcaneous were frequently missing.

The results obtained, in this study, show that chloramphenicol has no major effects on the embryonic development of the albino mouse, although the current knowledge suspect this drug to be teratogenic.
The anomalies obtained were not due to impairment of organogenesis but may be related to later interference with the development of the limbs.

The effect of chloramphenicol on the foetal liver

Hodgkinson (1954) described cases of fatty degeneration and large areas of hepatic necrosis after post-mortem examination in two cases associated with administration of chloramphenicol in doses four times larger than the therapeutic dose.

In the present study it was found that the drug had affected the liver cells of the foetuses with varying severity, since they showed the different phases of cell degeneration. The pathological changes observed ranged from cloudy swelling to complete necrosis of the cells.

Treating the mother during the early days of gestation with $1/10 \text{ LD}_{50}$ resulted in cloudy swelling in 50 per cent of cases, hydropic degeneration in 33 per cent, fatty degeneration in 67 per cent, and necrosis in 83 per cent.
Treating the pregnant females with $1/10 \text{LD}_{50}$ of chloramphenicol during mid-gestation resulted in a slight increase in the more severe types of degeneration and necrosis. So fatty degeneration appeared in 83 per cent of cases as compared to 67 per cent in foetuses delivered from mothers treated during early gestation with the same dose. The incidence of liver cell necrosis was equal in both cases. The higher dose in this period caused fatty degeneration in 100 per cent of the cases and necrosis in 83 per cent.

Treatment during late gestation with either $1/10$ or $1/3 \text{LD}_{50}$ resulted in more marked degenerative changes mainly fatty degeneration and necrosis which were demonstrated in 100 per cent of the cases examined.

It could be concluded that chloramphenicol when administered during early days of embryonic development while the foetal liver had not yet been completely formed, the effect of the drug on the liver cells was a combination of mild degeneration and necrosis. Probably these effects are secondary to the effect of the drug on the mother. The effect of the drug
during the last days of embryonic development, after the liver has been completely formed, was more severe accounting to necrosis in all cases. No appreciable difference was observed between the effects of the two doses of the drug used which denotes that the lower dose as well as the higher one were able to produce the degenerative and necrotic changes mentioned.

No changes in foetal liver were observed related to maldevelopment after the administration of chloramphenicol during the different periods of embryonic development.

The effect of chloramphenicol on the foetal kidney

The pathological lesions that occurred in the foetal kidney after treatment of the pregnant females by 1/10 and 1/3 LD$_{50}$ of chloramphenicol in the different periods of embryonic development were cloudy swelling and necrosis of the lining cells of the convoluted tubules and the ascending limbs of Henl's loops (figs. 29, 30 and 31).
Using 1/10 LD$_{50}$ of chloramphenicol, the incidence of cloudy swelling was decreased from 100 per cent in foetuses of the first group to 83 per cent in those of the second and third groups respectively. At the same time the incidence of necrosis was 17 per cent in foetuses of any of the three groups.

Using the high dose of chloramphenicol (1/3 LD$_{50}$) resulted in marked decrease in the incidence of cloudy swelling from 100 per cent in foetuses of the first group to 67 per cent in those of the second and the third groups respectively. Since most of the affected tubules showed more degenerative effects such as necrosis. The incidence of necrosis was increased from 17 per cent in foetuses of the first group to 33 per cent in the second, and lastly to 50 per cent in the third group respectively.

Treatment of the mothers by the low dose of chloramphenicol (1/10 LD$_{50}$) was enough to cause the pathological changes of the foetal kidney at any period of embryonic development. None of the maternally treated foetuses showed normal kidney structures. However, treatment of the mothers with the drug caused no con-
gestion or haemorrhages in the foetal kidney.

The effect of chloramphenicol on the foetal spinal cord

Treatment during early gestation

Maternal administration of the lower dose of chloramphenicol (1/10 LD<sub>50</sub>) was capable to cause asymmetry of the two halves of the foetal spinal cord (figs. 35, 36 and 37). This effect was independent on the route administration. The higher dose of chloramphenicol (1/3 LD<sub>50</sub>) induced the same effect and asymmetry in this condition resulted from marked herniation in one half of the spinal cord (figs. 38, 39 and 40). Herniation comprised the covering meninges, the grey matter and the white matter.

The vertebral arch was also affected in foetuses subjected to the lower dose as well as the higher dose. It was internally destructed and showed deficiency in the region of the malformed cord (figs. 38, 39 and 40). The vertebral arch showed lacking of ossification specially in the ventral side since these regions re-
mained mesenchymatous (fig. 40). The vertebral arch has either the same asymmetrical configuration of the spinal cord (fig. 37) or even destructed in the region opposite to the protruding part of the cord (fig. 39). Consequently the malformation in the spinal cord is not a secondary feature following anomalies in the vertebral arch. These results are in accordance with those of some workers who studied the relation between the spinal cord and the vertebral column. Watterson and Spiroff (1949) stated that in chick embryo, the shape of the vertebral canal was determined, to considerable extent, by the shape of the spinal cord. They came to this conclusion after observing that modifications of the shape of the spinal cord reflected corresponding modifications in the shape of the vertebral canal.

Fowler and Watterson (1953) extirpated segments of the neural tube from two days chick embryo and noticed complete absence of the vertebral elements in the operated region.

Grobstein and Parker (1954) implanted somite mesoderm combined with pieces of spinal cord in the
anterior chamber of the eye in the mouse. They found that the mesoderm differentiated cartilage module in culture and produced bone including haemopoietic tissue. Somite mesoderm without pieces of spinal cord failed to develop cartilage or bone.

Therefore an explanation of the deformity of the vertebral canal in the present study might be that the distortion in the shape of the spinal cord have been reflected on the integrity of the vertebral canal.

Treatment during mid-gestation

Both doses of chloramphenicol administered by the different routes caused marked shrinkage of the spinal cord inside the duramater (figs. 41 - 45). This effect was more pronounced in the cervical and thoracic regions, while in the lumbar region shrinkage was slightly detected. The central canal of the cervical and the thoracic spinal cord was frequently ruptured (figs. 43, 44 and 45). In some cases the spinal cord lost its smooth surface and showed irregularity in its outlines (figs. 42, 44, 45 and 47). In spite of these changes the two halves of the spinal cord remained symmetrical. The vertebral canal was
intact around the spinal cord and no features of haemorrhages were detected.

Snell (1956) stated that in the mouse, closure of neural tube occurred between the 8th and 10th day of gestation. Thus the results obtained in the spinal cord of one foetus (fig. 43) in which the cord was longitudinally splitted may be explained as failure of the neural tube to close during treatment of the drug at this period.

On the ground of the straight line relation between the size of the spinal cord and the vertebral canal, demonstrated by Watterson and Spiroff (1949), Fowler and Watterson (1953) and Grobstein and Parker (1954), the normal area of the vertebral canal and the large subdural space observed in the present study indicated that the spinal cord succeeded to develop till it occupied a normal area then it shranked later. Shrinkage of the spinal cord is indicated by crenations in its outlines. Shrinkage of the spinal cord and rupture of the central canal may be due to degenerative effect of the drug following normal development.
If maldevelopment was the cause of the reduction in the diameter of the spinal cord observed, then the diameter of the vertebral arch might correspondingly become reduced. Holtzer (1952) reduced the diameter of the spinal cord in the urodele embryos in the brachial, trunk and tail regions. The operations were performed well in advance of the first appearance of the precartilage cells. In all cases the transverse diameter of a given vertebral arch was proportionally reduced to the size of its spinal cord. Also Wattersson, Fowler and Fowler (1954) reduced the diameter of the spinal cord of chick embryos, and observed the same effect in the vertebral canal.

Treatment during late gestation:

Maternal administration of chloramphenicol during late gestation caused deformations in the foetal spinal cord. Deformations were restricted to the cervical and the thoracic regions, while the lumbar spinal cord appeared normal. Large subdural invaginations were seen in one or more sides of the spinal cord. In most cases a slight amount of red blood corpuscles was detected inside these invaginations indicating haemorrhages. The dorsal and ventral spinal arteries were seen to be the origin of these
haemorrhages (figs. 54, 55, 56, 58 and 61).

In addition to these effects, the high dose of chloramphenicol (1/3 LD$_{50}$) caused destruction of the white matter and gray matter in the central and peripheral areas of the foetal spinal cord. (figs. 57, 58 and 60). A few number of red blood corpuscles were seen entangled with the destructed tissues.

The vertebral canal surrounding the spinal cord again showed haemorrhages since subperiosteal haemorrhages were noticed in many of the specimens. In such cases the discontinuity of the vertebral canal permitted the extravasated blood to leak into the vertebral space around the spinal cord (figs. 61 and 62).

Congestion of the blood vessels and haemorrhages were common features in the liver and spinal cord of foetuses maternally treated by the drug during this later period of pregnancy.

Haemorrhages from the dorsal and ventral spinal arteries caused cystic subdural haemorrhages that pressed upon the surface of the spinal cord in some
areas and caused the focal invagination.

Haemorrhages of the fine blood vessels inside the grey matter and the white matter caused the destruction of the tissues of these areas.

Burns, Hodgman and Cass (1959) studied the role of antibiotics on premature infants. They treated groups of 30-33 premature infants within 12 hours of birth with (1) no antibiotics, (2) penicillin and streptomycin, (3) chloramphenicol 100 to 160 mg per kg daily by intramuscular injection and (4) all of these antibiotics. The mortality in these groups were 19, 18, 60 and 68 per cent respectively. Autopsy was done in 34 infants. Gross findings were not remarkable except cerebral haemorrhages in 5 infants.
ATLAS WITH EXPLANATION
Fig. 1: Photograph of a side view of a foetus from the control group subjected to the same treatment except the drug (chloramphenicol), from the 1st to the 18th day of gestation.

Fig. 2: Photograph of a side view of a malformed foetus, after maternal administration of 880 mg/kg (1/3 LD₅₀) chloramphenicol by the oral route from the 7th to the 12th day of gestation. The foetus has reduced size and abnormal short limbs.

Fig. 3: Photograph of a side view of a malformed foetus, after maternal administration of 880 mg/kg (1/3 LD₅₀) chloramphenicol by the oral route from the 7th to the 12th day of gestation. The foetus has reduced size and one abnormal hind limb.
Fig. 4: Photograph of a side view of an abnormal foetus, after maternal administration of 880 mg per kg (1/3 LD₅₀) chloramphenicol by the oral route from the 7th to the 12th day of gestation. The fore-limbs and one hind limb are malformed.

X 3

Fig. 5: Photograph of a side view of an abnormal foetus, after oral maternal administration of 880 mg per kg (1/3 LD₅₀) chloramphenicol from the 13th to the 18th day of gestation. The foetus has reduced body size. The fore-limbs and one hind limb are abnormally flexed posteriorly.

X 3

Fig. 6: Photograph of a side view of an abnormal foetus, after oral maternal administration of 880 mg per kg (1/3 LD₅₀) chloramphenicol from the 13th to the 18th day of gestation. The distal segments of the fore-limbs are markedly short.

X 3

Th. 4155
Fig. 7: Photograph of a side view of an alizarin red skeletal preparation of a normal foetus from the control group. It is possible to recognise all parts of the foetal skeleton.

X 3

Fig. 8: Photograph of a side view of an alizarin red skeletal preparation of a morphologically normal foetus, after intraperitoneal maternal administration of 880 mg per kg, chloramphenicol, from the 7th to the 12th day of gestation. The astragalus bone is absent from the hind limbs. The caudal vertebrae are reduced to 5 segments.

X 3

The arrow is denoting to the calcaneous bone.
Fig. 9: Photograph of a side view of an alizarin red skeletal preparation of the abnormal foetus (fig. 2), after oral maternal administration of 880 mg per kg (1/3 LD₅₀) chloramphenicol, from the 7th to the 12th day of gestation. The bones of the forelimbs are shorter than normal. The two tarsus bones are absent and the caudal vertebrae are reduced to 4 segments.

Fig. 10: Photograph of a side view of an alizarin red skeletal preparation of the abnormal foetus (fig. 4), after maternal administration of 880 mg per kg, (1/3 LD₅₀) chloramphenicol orally, from the 7th to the 12th day of gestation. The bones of the hind-limbs are short and the tarsus bones are absent. The caudal vertebrae are reduced to 2 segments.

The arrow is denoting to the place of the tarsus bones in the two figures.
Fig. 11: A photomicrograph of a section of liver of a foetus maternally treated by 100 mg/kg phenylbutazone, showing cloudy swelling. The liver cells are swollen and the cytoplasm appears granular. The Kupffer cells are swollen and distended with bile pigments and haemosiderin.

Rosin and haematoxylin X 675
- The arrows are denoting to the swollen Kupffer cells.

Fig. 12: A photomicrograph of a section of liver of a foetus maternally treated by 200 mg/kg phenylbutazone showing hydropic degeneration. The liver cells are swollen and the cytoplasm appears foamy. Some of the liver cells are showing necrosis.

Rosin and haematoxylin X 1350
- The arrows are denoting to the necrotic liver cells.
Fig. 13: A photomicrograph of a paraffin section of liver of a foetus maternally treated by 100 mg per kg phenylbutazone, showing fatty degeneration of the liver cells. The fat appeared as empty vacuoles due to its dissolution in the reagents used. The nuclei are pushed against the cell walls.

Gomori stain X 1100

1: Fat vacuole.

2: Nucleus pushed against the cell wall.

Fig. 14: A photomicrograph of a paraffin section of liver of a foetus maternally treated by 1/10 LD_{50} chloramphenicol, showing fatty degeneration as described above. The fat vacuoles acquired a signet-ring appearance.

Haematoxylin and Eosin X 540.

The arrows are denoting to the fat vacuoles.
Fig. 15: A photomicrograph of a frozen section of liver of a foetus maternally treated by 1/10 LD$_{50}$ chloramphenicol showing fats as variable sized black globules which are irregularly distributed in the liver cells. Sudan black stain X 405

Fig. 16: A photomicrograph of a frozen section of liver of a foetus from the control group. The fat globules appeared less than those of the above treated specimen. Sudan black stain X 405
Fig. 17: A photomicrograph of a section of liver of a foetus maternally treated by 1/3 LD<sub>50</sub> chloramphenicol, showing necrosis of the liver cells. Many nuclei had disappeared while the remainder showed one or other of the postnecrotic changes; pyknosis, karyolysis or karyorrhexis.

Haematoxylin and eosin X 675
1: Pyknosis (nucleus as dark dense mass).
2: Karyolysis (chromatin of the nucleus on the nuclear membrane).
3: Karyorrhexis (dissolution of the nuclear membrane).

Fig. 18: A photomicrograph of a section of liver of a foetus maternally treated by 100 mg/kg phenylbutazone, showing area of complete necrosis in the periphery of the liver lobule. This area comprised collapsed reticular framework entangling many cellular and nuclear debris with haemorrhages in some regions.

Haematoxylin and eosin X 540
1: Peripheral area of complete necrosis
2: Intact red blood cells
3: Haemolysed blood
Fig. 19: A photomicrograph of a section of liver of a foetus maternally treated by $1/3 \text{LD}_{50}$ chloramphenicol showing area of complete necrosis in the centrilocular region with increase in the number of lymphocytes.

Gomori stain \hspace{1cm} X 405
1: Centrilobular area of complete necrosis
2: Lymphocytes
3: Haemolysed blood in a central vein

Fig. 20: A photomicrograph of a section of liver of a foetus maternally treated by 200 mg/kg phenylbutazone showing areas of complete necrosis with haemorrhages demonstrated as many intact or haemolysed red cells.

Haematoxylin and eosin \hspace{1cm} X 163
1: Areas of necrotic tissue overshadowed with haemorrhages
2: Congested central vein
Fig. 21: A photomicrograph of a section of liver of a foetus maternally treated by 100 mg/kg phenylbutazone, showing retention of bile pigments in the cytoplasm of some of the degenerated and necrotic liver cells.

Haematoxylin and eosin  X 675

- The arrows are denoting to bile pigment retention.

Fig. 22: A photomicrograph of a section of liver of a foetus maternally treated by 1/10 LD$_{50}$ chloramphenicol, showing retention of bile pigment as fine granules in the cytoplasm of some of the degenerated and necrotic liver cells.

Haematoxylin and eosin  X 945

- The arrows are denoting to bile pigment retention.
Fig. 23: A photomicrograph of a section of liver of a foetus maternally treated by $1/3 \text{LD}_{50}$ chloramphenicol showing diffuse necrosis with lymphocytes and polymorphonuclear leucocytes dispersed between the liver cells in the sinusoids.

Haematoxylin and eosin  X 405

- The arrows are denoting to lymphocytes.

Fig. 24: A photomicrograph of a section of liver of a foetus maternally treated by $1/10 \text{LD}_{50}$ chloramphenicol, showing plenty of white blood cells in the portal tract and sinusoids.

Gomori stain  X 675

- The arrows are denoting to white blood cells.
Fig. 25: A photomicrograph of a section of liver of a foetus maternally treated by 200 mg/kg phenylbutazone showing necrosis of many liver cells while the surviving cells were abnormally large with abundant cytoplasm and large hyperchromatic dividing nuclei denoting regenerative activity.

Gomori stain X 1350

1: Large dividing liver cell
2: Necrotic liver cell
3: White blood cell

Fig. 26: A photomicrograph of a section of liver of a foetus maternally treated by 100 mg/kg phenylbutazone showing congestion of the central vein. The liver cells are showing diffuse necrosis with lymphocytic infiltration in the sinusoids.

Haematoxylin and eosin X 270

1: Congested central vein
2: Lymphocytes
Fig. 27: A photomicrograph of a section of liver of a foetus maternally treated by 200 mg/kg phenylbutazone, showing congestion of the sinusoids.

Gomori stain X 405

- The arrows are denoting to the congested sinusoids

Fig. 28: A photomicrograph of a section of liver of a foetus maternally treated by 200 mg/kg phenylbutazone showing congested central vein with intact red cells, haemolysed cells and haemosiderin pigments.

Haematoxylin and eosin X 540

- The arrows are denoting to the haemosiderin inside the central vein
Fig. 29: A photomicrograph of a transverse section of kidney of a foetus maternally treated by 1/10 LD₅₀ of chloramphenicol showing cloudy swelling of the convoluted tubules and the ascending limbs of Henle's loops. The cells lining these tubules are swollen and appear conical in shape. Thus the lumina of these tubules became narrow and irregular.

Haematoxylin and eosin  X 540  
- The arrows are denoting to the swollen cells with granular cytoplasm.

Fig. 30: A photomicrograph of a transverse section of kidney of a foetus maternally treated by 1/3 LD₅₀ of chloramphenicol. The convoluted tubules and the ascending limbs of Henle's loops show cloudy swelling. The lining cells have the top portions detached.

Haematoxylin and eosin  X 405  
- The arrows are denoting to the detached borders of the lining cells of the affected tubules.
Fig. 31: A photomicrograph of a transverse section of kidney of a foetus maternally treated by \( \frac{1}{3} \text{LD}_{50} \) chloramphenicol showing necrosis of the lining cells of the convoluted tubules and the ascending limbs of Henl's loops. The cells have ill-distinct cell borders and are fused together in homogeneous pink masses which appear in the lumina of the tubules.

Haematoxylin and eosin X 270

- The arrows are denoting to the ill-defined homogeneous masses of the affected tubules.
Fig. 32: A photomicrograph of a transverse section through the cervical spinal cord of a normal foetus from the control group, showing its normal external configuration.

Haematoxylin and eosin  X 50

Fig. 33: A photomicrograph of a transverse section through the thoracic spinal cord of a normal foetus from the control group, showing its normal configuration.

Haematoxylin and eosin  X 40
Fig. 34: A photomicrograph of a transverse section through the lumbar spinal cord of a normal foetus from the control group, showing the normal configuration.

Haematoxylin and eosin  X 100

Fig. 35: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated orally by 1/10 LD₅₀ of chloramphenicol during early gestation showing asymmetry of the two halves.

Haematoxylin and eosin  X 30
Fig. 36: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated intramuscularly by 1/10 LD₅₀ of chloramphenicol during early gestation, showing asymmetry of the two halves. The vertebral arch is destructed ventrally.

Haematoxylin and eosin  X 65
- The arrows are denoting to the destructed part of the vertebral arch.

Fig. 37: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated intraperitoneally by 1/10 LD₅₀ of chloramphenicol during early gestation, showing asymmetry of the two halves. The vertebral arch shows deficiency in the region of the herniated cord.

Haematoxylin and eosin  X 50
- The arrows are denoting to the deficient part of the vertebral arch.
Fig. 38: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated orally by $1/3 \text{LD}_{50}$ of chloramphenicol during early gestation. It shows dorsal herniation in one of its halves. The vertebral arch is internally destructed and shows deficiency of ossification.

Haematoxylin and eosin X 55
- The arrows are denoting to the herniated part of the spinal cord.

Fig. 39: A photomicrograph of a transverse section through the thoracic spinal cord of a foetus maternally treated intramuscularly by $1/3 \text{LD}_{50}$ of chloramphenicol during early gestation. It shows dorsal herniation in one half which resulted in its asymmetry. The vertebral arch is internally destructed and shows deficiency in the same region of the affected cord.

Haematoxylin and eosin X 90
- The arrows are denoting to the deficient part of the vertebral arch.
Fig. 40: A photomicrograph of a transverse section through the thoracic spinal cord of a foetus maternally treated intraperitoneally by $1/3 \text{LD}_{50}$ chloramphenicol during early gestation. It shows dorsal herniation of one half which resulted in its asymmetry. The vertebral arch is internally destructed and shows deficiency of ossification. Haemorrhages of blood are present in the vertebral canal which extravasated through the destructed arch.

Haematoxylin and eosin  X 75
1 : Herniated region of the spinal cord
2 : Destructed vertebral arch
3 : Extravasated blood in the vertebral canal

Fig. 41: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated orally by $1/10 \text{LD}_{50}$ of chloramphenicol during mid-gestation.

It shows shrinkage of the cord which results in large ventral subdural space and the outer surface of the cord shows ctenation.

Haematoxylin and eosin  X 65
- The arrows are denoting to a ventral subdural space
Fig. 42: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated intramuscularly by 1/10 LD$_{50}$ of chloramphenicol during mid-gestation. It shows shrinkage of the cord which resulted in a large ventral subdural space. The outer surface of the cord shows crepation at the ventral and lateral sides.

Haematoxylin and eosin  X 50

- The arrows are denoting to a large subdural space.

Fig. 43: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated orally by 1/10 LD$_{50}$ of chloramphenicol during mid-gestation. It shows the cord longitudinally splitted from the dorsal surface till the position of the central canal. Large subdural space is shown in the ventral and lateral sides.

Haematoxylin and eosin  X 50

- The arrows are denoting to a large subdural space.
Fig. 44: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated intraperitoneally by 1/3 LD$_{50}$ of chloramphenicol during mid-gestation. It shows the shrinkage of the cord with crenation in its outer surface and large subdural space around it. The central canal shows disruption of its wall.

Haematoxylin and eosin  X 75
1: Disrupted walls of the central canal
2: Crenated surface of the spinal cord
3: Large subdural space

Fig. 45: A photomicrograph of a transverse section through the thoracic spinal cord of a foetus maternally treated intraperitoneally by 1/3 LD$_{50}$ of chloramphenicol during mid-gestation. It shows shrinkage of the cord with crenation in its outer surface and a large subdural space around it. The central canal shows disruption of its wall.

Haematoxylin and eosin  X 75
1: The disrupted walls of the central canal
2: The crenated surface of the cord
3: The large subdural space
Fig. 46: A photomicrograph of a transverse section cervical through the spinal cord of a foetus maternally treated intramuscularly by 1/3 LD₅₀ of chloramphenicol during mid-gestation. It shows shrinkage of the cord inside the dura mater and crenation in its outer surface.

Haematoxylin and eosin X 75

Fig. 47: A photomicrograph of transverse section through the thoracic spinal cord of a foetus maternally treated intraperitoneally by 1/3 LD₅₀ of chloramphenicol during mid-gestation. It shows shrinkage of the cord inside the dura mater and the outer surface of the cord is highly crenated.

Haematoxylin and eosin X 75

- The arrows are denoting to the crenated surface of the spinal cord.
Fig. 43: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated orally by 1/10 LD₅₀ of chloramphenicol during late gestation. It shows subdural invagination in one side and a large subdural space from the ventral side.

Haematoxylin and eosin  X 70
- The arrows are denoting to the subdural invagination

Fig. 41: A photomicrograph of a transverse section through the thoracic spinal cord of a foetus maternally treated intramuscularly by 1/10 LD₅₀ of chloramphenicol during late gestation. It shows subdural haemorrhages and a small invagination in one side of the cord. The vertebral arch shows deficiency of ossification specially ventral to the cord.

Gomori stain  X 90
1: Subdural haemorrhage
2: Subdural invagination
3: Region of the vertebral arch showing deficiency of ossification.
Fig. 50: A photomicrograph of a transverse section through the thoracic spinal cord of a foetus maternally treated intraperitoneally by 1/10 LD$_{50}$ of chloramphenicol during late gestation. It shows lateral subdural invagination and subdural haemorrhages from the dorsal and ventral spinal arteries.

Gomori stain X 55
1: Subdural haemorrhages
2: Subdural invagination

Fig. 51: A photomicrograph of a transverse section through the thoracic spinal cord of a foetus maternally treated intraperitoneally by 1/10 LD$_{50}$ of chloramphenicol during late gestation. It shows a large subdural invagination in one half. The vertebral arch is internally destructed and shows deficiency of ossification.

Haematoxylin and eosin X 55
- The arrows are denoting to the subdural invagination.
Fig. 52: A photomicrograph of a transverse section through the thoracic spinal cord of a foetus maternally treated intramuscularly by 1/10 LD$_{50}$ of chloramphenicol during late gestation, showing one lateral subdural invagination. Subdural haemorrhage from the dorsal spinal artery is also present.

Haematoxylin and eosin  X 55
- The arrows are denoting to the subdural invagination.

Fig. 53: A photomicrograph of a transverse section through the lumbar spinal cord, of a foetus maternally treated intramuscularly by 1/10 LD$_{50}$ of chloramphenicol during late gestation. It shows subdural haemorrhages and the vertebral arch is internally destructed and shows lack of ossification.

Gomori stain  X 95
- The arrows are denoting to the subdural haemorrhages.
Fig. 54: A photomicrograph of a transverse section through the cervical spinal cord of a foetus maternally treated orally by 1/3 LD₅₀ of chloramphenicol during late gestation. It shows subdural haemorrhage from the dorsal spinal artery. Subdural invaginations are also present. The vertebral arch is destructed and red blood cells are seen inside the vertebral canal.

Haematoxylin and eosin  X 75
1: Subdural haemorrhages
2: Subdural invaginations
3: Haemolysed blood inside the vertebral canal

Fig. 55: A photomicrograph of T.S. through the cervical spinal cord of a foetus maternally treated orally by 1/3 LD₅₀ of chloramphenicol during late gestation. It shows large subdural invagination and subdural haemorrhages from the dorsal spinal artery. The vertebral arch is destructed and red blood cells are seen inside the vertebral canal.

Gomori stain  X 75
1: Subdural haemorrhages
2: Subdural invaginations
Fig. 56: A photomicrograph of T.S. through the lumbar spinal cord/maternally treated orally by 1/3 LD$_{50}$ of chloramphenicol during late gestation. It shows subdural haemorrhages from the dorsal spinal artery. The vertebral arch is internally destructed and shows deficiency of ossification specially in its ventral side.

Haematoxylin and eosin 95

1: Subdural haemorrhages
2: Parts of vertebral arch showing deficiency of ossification

Fig. 57: A photomicrograph of T.S. through the cervical spinal cord of a foetus maternally treated intramuscularly by 1/3 LD$_{50}$ of chloramphenicol during late gestation. It shows destruction of the white and gray matter from the dorsal side. Subdural invagination is shown at one side of the cord. The vertebral arch is seen destructed specially at the regions of the affected cord.

Gomori stain  X 65

1: Destructed region of the spinal cord
2: Subdural invagination
3: Destructed vertebral arch
Fig. 58: A photomicrograph of T.S. through the cervical spinal cord of a foetus maternally treated intra-muscularly by 1/3 LD\textsubscript{50} of chloramphenicol during late gestation. It shows destruction in the dorsal white matter and the dorsal spinal artery is destroyed and haemolysed blood is seen entangling with the destructed tissue of the cord. It shows also an invagination in one lateral side of the cord.

Haematoxylin and eosin  X 75

- The arrows are denoting the destructed tissue of the spinal cord entangling with the haemolysed blood.

Fig. 59: A photomicrograph of T.S. through the cervical spinal cord of a foetus maternally treated intraperitoneally by 1/3 LD\textsubscript{50} of chloramphenicol during late gestation. The cord consists of a horny-like tissue dorsally and ill-defined cordal tissue ventrally. The vertebral arch is highly destructed in this region and haemorrhages are seen in the vertebral canal.

Haematoxylin and eosin  X 80

1: Ill-defined cordal tissue
2: Horny-like tissue
3: Destructed regions of the vertebral arch
Fig. 60: A photomicrograph of T.S. through the thoracic spinal cord of a foetus maternally treated intraperitoneally by 1/3 LD$_{50}$ of chloramphenicol. It shows highly destructed cord. The cordal tissue is ill-defined and is entangled with haemolysed blood. The vertebral arch is highly destructed. The centrum is seen huge than normal and there are two remnants of notochord.

Haematoxylin and eosin  80

1: Ill-defined cordal tissue
2: Destructed cordal tissue with haemolysed blood.
3: Huge centrum
4: Two remnants of notochord

Fig. 61: A photomicrograph of T.S. through the lumbar spinal cord of a foetus maternally treated orally by 1/3 LD$_{50}$ of chloramphenicol during late gestation. It shows haemorrhages from the dorsal spinal artery of the cord. The vertebral arch shows subperiosteal haemorrhage.

Gomori stain  X 95

1: Subdural haemorrhages from the dorsal spinal artery
2: Subperiosteal haemorrhages
Fig. 62: A photomicrograph of a T/S. through the thoracic spinal cord of a foetus maternally treated orally by 1/3 LD$_{50}$ of chloramphenicol during late gestation. It shows destruction of the vertebral arch and haemorrhages are seen inside the vertebral canal.

Haematoxylin and eosin  X 55

1: Haemolyzed blood inside the vertebral space
2: Regions of the destructed vertebral arch