CHAPTER IV

THE EFFECT OF PHENYL BUTAZONE
ON THE EMBRYOIC DEVELOPMENT OF THE ALBINO MOUSE

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REVIEW OF LITERATURE ON PHENYL BUTAZONE

Phenylbutazone is one of the pyrazolone derivatives, it is chemically related to antipyrine and aminopyrine. The drug was first introduced by Belart in 1949 for the treatment of rheumatoid arthritis and allied disorders.

1- Chemical properties

Woodbury (1970) stated that phenylbutazone is a white or very light yellow powder with a slight bitter taste. Its solubility in water is about 0.7 mg per ml at 22.5°C. Chemically phenylbutazone is: 3,5-dioxo-1,2-diphenyl-4-n-butylpyrazolidine. The structural formula of this drug is as follows:

\[
\begin{align*}
\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2
\end{align*}
\]

Any slight change in the chemical structure of the drug affects its pharmacological properties. Modifications that increase the acidity of the molecule enhance the uricosuric activity, whereas changes that decrease the acidity cause a loss of this activity.
The more acidic compounds are excreted more rapidly in the urine and the less acidic derivatives are very slowly eliminated.

2- Pharmacological properties

Welhelmi (1949) studied the general pharmacological actions of phenylbutazone in animals. The author found that the drug was an effective antipyretic, reduced inflammatory responses to ultraviolet irradiation by causing constriction of the dilated capillaries.

Domenjoz (1960) found that phenylbutazone inhibits extravasation of various dyes into an area of experimentally induced inflammation and oedema. The author postulated that it may act by decreasing capillary permeability.

Woodbury (1970) stated that the drug is not generally employed as analgesic, but only for pain associated with inflammatory diseases in which the basis for the relief is mainly the anti-inflammatory activity of the drug. In man, the anti-inflammatory activity of phenylbutazone are demonstrable in rheumatoid arthritis and related disorders. The author
claimed that the drug produces a minor effect on the adrenohypophyseal-adrenocortical axis, and is also an effective anti-inflammatory agent in adrenalectomized animals.

According to Woodbury (1970), phenylbutazone has a mild uricosuric effect in experimental animals and man. The same author attributed the uricosuric effect to diminished reabsorption of uric acid by the proximal renal tubules, probably as a result of the ionic form of the drug competing with uric acid for reabsorption. The drug also competitively inhibits the active renal tubular transport of para-aminohippurate, para-aminosalicylate, and phenolsulfonphthalein in the proximal renal tubules.

The same author recorded that administration of phenylbutazone caused a significant retention of sodium and chloride, accompanied by reduction in urine volume, oedema may result in some cases.

Phenylbutazone reduces the uptake of iodine by the thyroid gland; in most cases, this does not appear to be associated with clinical signs of hypothyroidism but goiter and myxedema occasionally result.
Phenylbutazone also influences certain aspects of intermediary metabolism. It inhibits the oxidative decarboxylation of pyruvate and α-ketoglutarate in relatively low concentrations and depresses succinoxidase in even lower concentrations, inhibition of these enzymes leads to diminished energy synthesis of many cellular substances and might explain the multiplicity effects of the drug. Phenylbutazone also uncouples oxidative phosphorylation and inhibits the ATP dependent biosynthesis of mucopolysaccharides sulphates in cartilage, effects probably related to its anti-inflammatory activity.

3- Absorption, Fate and Excretion

Phenylbutazone is rapidly and completely absorbed from the gastro-intestinal tract but it is slowly absorbed from intramuscular depots. After therapeutic doses, the drug is found in the plasma bound to protein. It is almost completely metabolised in the liver microsome system, and only a trace of unchanged drug is excreted in the urine.
4- Therapeutic Uses

Woodbury (1970) stated that phenylbutazone often relieves pains associated with rheumatoid arthritis and allied disorders. It is also used for the therapy of acute gout, and this effect is attributed to a combination of its anti-inflammatory, analgesic, and uricosuric properties. Phenylbutazone is useful in the management of certain musculo-skeletal disorders; it reduces joint tenderness, may increase the range of movement, reduces swelling, and causes drop of temperature. Phenylbutazone has also been used in rheumatic fever, ankylosing spondylitis, osteoarthritis and some of the less well-defined rheumatic conditions. The author stated also that there was evidence that phenylbutazone acts via the anterior pituitary or suprarenal cortex. He also claimed that in man, doses of 400 to 600 mg per day for one week provides maximum therapeutic effects; higher doses for more longer periods do not increase effectiveness but only increase toxicity.
5- Toxicity

a- Hematological toxicity

Brown and Currie (1952) mentioned that in over 280 cases treated by phenylbutazone, they were impressed by the lack of toxic side-effects. They used small maintenance doses instead of continued full dosage.

Hart and Johnson (1952 a) although they had noticed no complications apart from reaction to injection, they offered early warning that phenylbutazone is not so free from serious toxic effects. The same authors later (1952 b) reported three cases of melena and hematemesis, 2 cases of gastritis and 6 cases of skin rashes among 60 patients treated with about 400-800 mg phenylbutazone daily.

Nassim (1953) described 46 cases suffering from toxic reactions, among 109 patients given phenylbutazone for various rheumatic conditions. The drug was given by mouth, average dose 0.6 gm daily, and little were receiving the drug intramuscularly of one gm on four alternate days, followed by weekly injections. The author described toxic findings; anorexia, upper
abdominal pain, looseness of the bowels, dryness of the mouth and throat, actual ulceration, punctate erythematous rashes, enlargement of the glands in the posterior triangles of the neck, severe vertigo and granulopenia.

Leonard (1953) reviewed the published reports. Among 1326 patients treated with phenylbutazone, 356 patients suffered toxic effects. The side-effects reported included oedema, nausea, vomiting, diarrhoea, hematemesis, activation of peptic ulcer, jaundice, skin rashes, anemia, agranulocytosis purpura and thrombocytopenia. A fatal case of aplastic anemia was reported. The author concluded that the incidence of toxic effects did not seem to be related to the dosage of phenylbutazone, as reactions commonly developed in patients with small doses.

Mauer (1955) reviewed the literature, including the reports of individual cases and of various series of patients who had been treated with phenylbutazone. Among 3954 patients at risk in these series, he found 6 cases of agranulocytosis (0.15%), 32 of leukopenia (0.8%), and 32 of thrombocytopenia (0.8%). None of
these hematological disturbances were fatal, although three deaths from other causes were reported. In the individual case reports he found 19 fatalities attributable to phenylbutazone toxicity and added two cases from his own experience. Ten of these fatalities were due to agranulocytosis, one to aplastic anemia and one to thrombocytopenia.

Bean (1960) reported six cases of leukemia developed in patients who had received phenylbutazone of maximum dose of 600 mg per day. Postmortem examination of five of these patients revealed, pale fatty bone marrow, with little or no erythropoietic tissue.

McCarthy and Chalmers (1964) described aplastic anemia and agranulocytosis in two patients, given 300 mg of phenylbutazone daily for 11 weeks, and 100–200 mg daily for 2 months respectively. They reviewed the English literature on the hematological toxic effects of phenylbutazone. They examined reports of individual cases of toxicity following the use of the drug as well as recorded observations on toxic effects occurring in series of patients under treatment of phenylbutazone. Series of reports in 1955–
1964 added 1763 patients at risk, and among these 16 instances of leukopenia were recorded. Three cases of thrombocytopenia were reported, one in association with a fall in haemoglobin and leukocyte count. Agranulocytosis was not encountered. Individual cases reported in the same period added the records of six patients with aplastic anemia, nine with leukemia, four with agranulocytosis, and isolated cases of leukopenia, megaloblastic anemia, leukemoid reaction and depression of the erythroid elements of the bone marrow.

B- Gastro-intestinal toxicity

Hart and Johnson (1952b) described a case of duodenal ulceration of a man, age 34 years, with ankylosing spondylitis, administered phenylbutazone, 400-800 mg per day by mouth during two months of uncontinued treatment.

Jarvis (1952) described a case of stomatitis and pharyngitis of a woman, aged 59, with rheumatoid arthritis after administration of phenylbutazone, 1200 mg daily by mouth for a period of one month.
Loxton, Le Vay and Wilson (1952) observed that about 20 per cent of 50 patients given phenylbutazone, in a dosage about 400 mg per day, suffered side-effects including gastritis, melaena and hematemesis.

Benstead (1953) stated that agranulocytosis and haemorrhages, usually from the gastro-intestinal tract were the principal recognized toxic reactions caused by phenylbutazone. Further more, the author reported fatal peritonitis after perforation of duodenal peptic ulcerations in two patients receiving phenylbutazone.

Bhatia, Zaidi and Sigh (1965) stated that species variation appeared to be an important factor for the ulcerogenic effect of phenylbutazone. In their work no ulcer could be produced in mice, rats, and monkeys after 30 days of administration of 100 mg per kg of body weight, but it was produced in dogs even after 18 days of administration of the drug. They concluded that individual susceptibility and species variation seemed to be important factors that influenced the ulcerogenic effect of phenylbutazone.

Bucciarelli, Biliotti, Andreoli and Petruzzi (1966) injected rats with phenylbutazone, subcutan-
eously with 150 mg per kg body weight for 10 days. They found that both the hydrochloric acid and pepsin were significantly decreased in the gastric juice of the treated animals as compared with controls. The hyposecretion observed in 100 per cent of the animals treated for a long period with phenylbutazone was due to the numerous and large ulcers on the gastric mucosa.

Larsen and Bredahl (1966) treated female rabbits with 60 mg per kg phenylbutazone for 20 days by subcutaneous injection in the back. Histological examination of 5 animals showed that 2 of them had chronic ulcers.

Max and Menguy (1969) found that large amounts of DNA appeared in washings of Heidenhain pouches of dogs given aspirin or phenylbutazone. They postulated that these drugs induce gastric mucosal damage and/or bleeding may represent in part a failure of the gastric mucosa to replace the surface cells which are being lost at a more rapid rate.
c- Liver and kidney toxicity

Bean (1960) reported some cases of liver and kidney enlargements among patients administered 60 mg per day of phenylbutazone. Microscopic examination revealed widespread infiltration of the marrow, kidneys, liver and spleen with lymphocytes.

Jørgen (1965) described a case of acute poisoning with phenylbutazone in a 15-month-old child. The acute phase was dominated by hyperpnea, oliguria and serious disturbances of the acid-base equilibrium. Later the author found a passing effect on the liver with jaundice.

d- Embryotoxic effects

Triebold, Stamm, Küng and Müller (1957), had found no reduction in fertility in experiments with female rats which were given 33.3 mg per kg phenylbutazone subcutaneously within the first 18 days of pregnancy.

Larsen and Bredahl (1966) studied the embryotoxic effect of phenylbutazone (30-60 mg per kg), monophenylbutazone (60-150 mg per kg), and thalidomide (60 mg per kg) in rabbits. Each drug was administered daily from the first to the 20th day after mating by
subcutaneous injection in the back. Only thalidomide definitely reduced fertility and the average weight of the young, beside it increased foetal mortality. With regard to anomalies they were much more frequent in the thalidomide group than in any other groups. Both the phenylbutazone and monophenylbutazone caused anomalies in the skeletal system, whereas anomalies in the soft tissues were only definitely seen in the phenylbutazone groups. Among 35 foetuses of the group receiving the higher dose of phenylbutazone 3 foetuses showed anomalies. One had an umbilical hernia with intestinal loops and a lobe of the liver in it, the second had no tail and abnormal sternebrae; only 3 sacral and no caudal segments, the third had an abnormally short tail and only 3 caudal segments.

Even though phenylbutazone has been used clinically since 1949, only a few publications seem to deal with the embryotoxic effect of this drug. The aim of the present work is to investigate the effect of phenylbutazone on the foetus of an inbred strain of the albino mouse, when it is maternally administered.
RESULS OF MATERNAL ADMINISTRATION
OF PHENYL BUTAZONE

A-GROSS EXAMINATION

Gross examination in this investigation includes; the number of females that continued pregnancy, the number of females that aborted, the total number of foetuses obtained from the females that continued pregnancy and the number of living and dead foetuses either normal or abnormal.

The results of oral maternal administration of either 100 or 200 mg per kg phenylbutazone during the different periods of gestation are summarised in table (10). Foetuses that survived for few minutes, before they were fixed in a suitable fixative for further detailed studies, were recorded living. Morphologically normal foetuses were recorded normal. Vaginal bleeding followed by reduction in abdominal swelling was diagnostic of abortion.
The controls were subjected to the same treatment except the drug, from the 1st to the 18th day of gestation. No abortions were observed and the average number of foetuses per litter was 8.2.
a- Animals subjected to treatment from the 1st to the 5th day of gestation:

of the ten females, treated with 100 mg per kg daily, one aborted and nine continued pregnancy. They gave 67 living normal foetuses and 2 dead foetuses that were also morphologically normal.

Of the ten females, treated with 200 mg per kg daily, only seven continued pregnancy. They gave 37 normal living foetuses, 2 dead and 3 abnormal. The 3 abnormal foetuses were of one litter and one of them was dead. They had their fore-limbs shorter than normal, attached to their abdomen till nearly the elbow joint and flexed posteriorly at the wrist joint. (fig. 64).

b- Animals subjected to treatment from the 7th to the 12th day of gestation.

All the females, treated with 100 mg per kg daily, continued pregnancy and gave 61 normal living foetuses and one dead morphologically normal foetus.

Among the ten females, treated with 200 mg per kg daily, eight continued pregnancy. They gave
46 normal living foetuses and five dead foetuses, three of them were morphologically abnormal (figs. 65 to 67). The size of these abnormal foetuses was markedly small, one of them had about half the size of the controls.

The first abnormal foetus (fig. 65) had atrophied fore-limbs while the hind limbs appeared normal. The fore-limbs had well pronounced shortening of the medial and terminal segments (brachymely), with short digits (brachydactyly).

The second abnormal foetus (fig. 66) had ventrally flexed body with reduction in its posterior half. The fore-limbs had brachydactyly. The hind limbs appeared normal.

The third abnormal foetus (fig. 67) had its fore-limbs attached to the body till the elbow joint. The fore-limbs had brachydactyly. One of its hind limbs was flexed at the knee joint while the other appeared normal.

- Animals subjected to treatment from the 13th to the 18th day of gestation:

The ten females that were administered 100 mg per kg daily, continued pregnancy and gave 78 normal
living foetuses.

The ten females that were administered 200 mg per kg daily, continued pregnancy and gave 83 morphologically normal living foetuses.
<table>
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<th>7 - 12</th>
<th>13-18</th>
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<td>I</td>
<td>100</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>II</td>
<td>6.2</td>
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<td>III</td>
<td>8</td>
<td>7.6</td>
<td>83</td>
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</table>

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<th>Letter</th>
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<th>No.</th>
<th>I/</th>
<th>Live/</th>
<th>Dead/</th>
<th>Dead/</th>
<th>Living</th>
<th>Dead/</th>
<th>Dead/</th>
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<td>I</td>
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<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<td>7</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
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<td>4</td>
<td>6</td>
<td>7</td>
<td>5</td>
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<td>19</td>
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<th>Loss</th>
<th>Route</th>
<th>100 and 200 mg/kg Phenylbutazone</th>
<th>Results of Gross Examination after Treatment with</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Table 10</td>
</tr>
</tbody>
</table>
B-MACROSCOPIC EXAMINATION OF FETAL SKELETON

Some foetuses obtained from the control females were prepared according to Dawson's method and their skeletons were macroscopically examined. All bones of the axial and appendicular skeleton were recognized as previously described in Chapter III (page 52).

No major skeletal defects were observed among foetuses subjected to phenylbutazone during development. However, some foetuses which were morphologically normal had some of their small bones missing. The caudal vertebrae were in most cases markedly reduced and in few cases they were missing. The astragalus bone was in some foetuses missing, however, in rare cases both the astragalus and calcaneous were absent.

The three foetuses which had posteriorly flexed fore-limbs (fig. 64), had identical skeletal preparations, (fig. 68). The abnormality in their fore-limbs was not due to skeletal defects, the bones were complete and normal in shape. The caudal vertebrae of the foetuses were reduced to 5-6 vertebrae. The calcaneous bone in both limbs was markedly smaller than normal while the astragalus bone was completely absent.
The malformed foetus (fig. 65) showed complete absence of the tarsus bones. The caudal vertebrae were reduced to four vertebrae. The fore-limbs had very short humerus, radius and ulna, metacarpus bones and phalanges.

The malformed foetus (fig. 66) had the caudal vertebrae reduced to four vertebrae. Bones of the fore-limbs and hind limbs were normal except the tarsus bones in the hind limbs were absent (fig. 69).

The anomaly in the hind limb of the foetus shown in (fig. 67) was not due to skeletal defect. The two tarsus bones were missing in both limbs. The caudal vertebrae were reduced to five vertebrae.
C-MICROSCOPIC EXAMINATION

1- Foetal Liver

Microscopic examination of the serial transverse sections of foetuses from mothers treated by phenylbutazone during the different periods of gestation, showed that the drug had caused degenerative changes in the liver. The foetal liver cells showed different phases of pathological lesions in the form of cloudy swelling, hydropic degeneration, fatty degeneration, focal necrosis, complete necrosis and patchy fibrosis. The degenerative changes appeared either singly or in combination. They were previously described in chapter (III) except patchy fibrosis, which was not observed in the liver of foetuses affected by chloramphenicol.

Patchy fibrosis:

Areas of necrosis were over-shadowed by replacement of some of the parenchyma cells by fibrous tissue. This material was disposed in thin bands, thus the tissue became divided into disordered trabeculae with no specific length or direction (figs. 70 and 71). The connective tissue which occurred as bands between the islands appeared vascular, in varying degrees fibrous with fibroblastic proliferation
and irregularly infiltrated with lymphocytes, plasma cells, polymorphs and eosinophiles (fig. 71). The surviving liver cells showed different phases of division to form new cells (fig. 72).

The incidence of these pathologic changes in the liver of foetuses examined from each identically treated group was represented in table (11). The code number of each foetus examined was recorded under every lesion observed.
2- Foetal Kidney

Microscopic examination of transverse sections of foetuses from mothers treated by phenylbutazone during the different periods of embryonic development, showed degenerative changes in the kidneys. These changes mainly affected the convoluted tubules and the ascending limbs of Henl's loops. The cells lining these tubules showed cloudy swelling, cloudy swelling passing to focal necrosis or complete necrosis. (figs. 73, 74, 75 and 76). In most cases these lesions appeared in combination. They were previously described in chapter (III).

In few cases, cloudy swelling and necrosis of the tubules was accompanied with congestion of the glomeruli and capsular haemorrhages.

**Congestion and haemorrhages:**

The glomeruli were enlarged to fill completely the intracapsular space (fig. 77). The epithelial cells lining the Bowman's capsules were not swollen, while the intracapsular space contained extravasated blood cells. The straight vessels of the medulla were engorged with red blood cells. Haemorrhages appeared in the capsular and cortical areas of the kidney.
(fig. 78).

These pathologic changes were usually accompanied with cloudy swelling and necrosis.

The results of microscopic examination of foetuses from mothers treated by the drug during the different periods of embryonic development; early from the 1st to the 6th day, mid—-from the 7th to the 12th day, and late from the 13th to the 18th day of gestation, orally by 100 and 200 mg per kg body weight, were demonstrated in table (12). In this table the code number of each foetus examined was recorded under every lesion observed.
Table 12
Results of maternal administration of Phenylbutazone on the foetal kidney

<table>
<thead>
<tr>
<th>Dose</th>
<th>Period of treatment</th>
<th>Fetuses examined</th>
<th>Cloudy swelling</th>
<th>Necrosis</th>
<th>Congestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/Kg 5. W.</td>
<td>Ist - 6th</td>
<td>$S_1$  $S_2$</td>
<td>$S_1$  $S_2$</td>
<td>$S_1$</td>
<td>$-$  $-$</td>
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<tr>
<td></td>
<td>7th -12th</td>
<td>$T_1$  $T_2$</td>
<td>$T_1$  $T_2$</td>
<td>$T_1$</td>
<td>$T_2$  $-$</td>
</tr>
<tr>
<td></td>
<td>13th-18th</td>
<td>$U_1$  $U_2$</td>
<td>$U_1$  $U_2$</td>
<td>$U_1$</td>
<td>$U_2$  $U_1$</td>
</tr>
<tr>
<td>200 mg/Kg 3. W.</td>
<td>Ist - 6th</td>
<td>$V_1$  $V_2$</td>
<td>$V_1$  $V_2$</td>
<td>$V_1$</td>
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<td>$W_1$  $W_2$</td>
<td>$-$  $-$</td>
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<tr>
<td></td>
<td>13th-18th</td>
<td>$X_1$  $X_2$</td>
<td>$-$  $-$</td>
<td>$X_1$</td>
<td>$X_2$  $X_2$</td>
</tr>
</tbody>
</table>
3. Foetal Spinal Cord

The external configuration of the foetal spinal cord was first studied in serial transverse sections of 2 foetuses extracted from the control females. No spontaneous malformations were observed in any specimen.

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

Foetuses maternally treated from the 1st to the 6th day of gestation by either the lower or higher doses; 100 or 200 mg per kg, showed normal external configuration of the spinal cord throughout the cervical, thoracic and lumbar regions.

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

Foetuses from mothers treated from the 7th to the 12th day of gestation by the lower dose (100 mg/kg) showed normal structures of the spinal cord in the different regions. However, two foetuses examined among those treated by the higher dose showed affected spinal cord.

The two halves of the spinal cord were completely separated from the dorsal side till the site of the central canal (figs. 79 and 80). This
effect was markedly observed in the cervical region.

The vertebral arch, in foetuses subjected to the higher dose, showed deficiency in ossification specially in the basiventral region (fig. 79).

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

Foetuses from mothers treated during the last period of pregnancy, from the 13th to the 18th day, by 100 mg per kg showed normal structures of the spinal cord throughout the different regions. However, the spinal cord and the surrounding tissues were markedly affected in the foetuses treated with the higher dose (200 mg per kg).

In one foetus, the white matter showed deficiency from the ventral surface in both halves. The two halves of the spinal cord appeared asymmetric as a result of the deficiency of the white matter from one lateral side. The dorsal fissure appeared more deep than normal, and contained plenty of connective tissue. The dura mater was disrupted around the cord in different regions. The central canal showed incomplete lining (fig. 81).
The second foetus had the cervical and thoracic parts of the spinal cord with both the gray and white matter reduced at the ventral side. Invagination in one lateral side of the cervical and thoracic spinal cord was the cause of its asymmetrical appearance (figs. 82 and 83).

The vertebral arch was seen markedly destructed in these affected regions, and haemorrhages were detected inside the vertebral space. In the anterior thoracic region the vertebral arch, seemed to be the cause of the invagination as it appeared pressing on the cord (fig. 82). However, it was found that in the posterior thoracic region, the cord showed large subdural invagination in the same side while the vertebral arch showed deficiency in this region (fig. 83).
DISCUSSION

Choice of the drug:

Tuchmann-Duplessis (1965) suspected phenylbutazone, among other drugs, to be teratogenic on the ground of results obtained by animal experiments. Schardein (1965) investigated the effects of aspirin and phenylbutazone on the rabbit blastocyst. He did not observe malformations but foetal wastage. Larsen and Bredahl (1966) treated female rabbits with phenylbutazone for the first twenty days after mating. Two doses were given to two groups of females, 30 mg per kg and 60 mg per kg subcutaneously. In the first group, among 32 foetuses, they observed 2 of the young had the right eyes slightly opened, and another 2 had umbilical hernias. In the second group the same authors observed, among 35 foetuses, one had umbilical hernia with intestinal loops and a lobe of the liver in it, one had no tail and only 3 sacral segments, and one had short tail with 3 caudal segments. They claimed also that phenylbutazone does not reduce fertility.

Phenylbutazone, like chloramphenicol, exerts severe haematological complications with aplastic anemia as the extreme irreversible toxic effect.
Phenylbutazone and salicylates inhibit the biosynthesis of mucopolysaccharides. It has been demonstrated that sodium salicylate, methyl salicylate, and acetyl salicylic acid can affect embryonic development in mice and rats (Larsson, Boström and Ericson, 1965; Larsson, Ericson and Boström, 1965; Trasler, 1965; Warkany and Takacs, 1959). Larsson and Boström (1965) correlated between the varying teratogenic potency of the three salicylates and their ability to inhibit the synthesis of acid mucopolysaccharide. Woodbury (1970) stated that phenylbutazone uncouples oxidative phosphorylation and also inhibits the ATP dependent biosynthesis of mucopolysaccharides sulphates in cartilage.

Selection of doses:

The maximum therapeutic dose of phenylbutazone in men is about 17 mg per kg daily administered in divided doses. The lethal dose for the mouse is not stated in the available literature. The higher dose tested by Larsen and Bredahl (1966), subcutaneously in rabbits, was 60 mg per kg. Bhatia, Zaidi and Sigh (1965) demonstrated that no ulcer could be produced in mice after 30 days of administration of
100 mg per kg, phenylbutazone. Thus two doses were tested in the present study, 100 and 200 mg per kg body weight. Higher doses than 200 mg/kg caused vaginal bleeding when given to the pregnant females.

Route of administration:

Phenylbutazone in clinical application is preferably given by mouth because it may cause abscess formation when given by injection, also it enters the bloodstream more rapidly when given orally (Burns et al., 1953). Kuzell et al., (1952) claimed that intramuscular injection of the 20 per cent solution of the sodium salt is somewhat painful, but did not lead to abscess formation. In the present study when some animals were treated by intramuscular injection of 2.5 to 5 per cent solution, severe tissue reaction occurred that ended with abscess formation in most cases. Intraperitoneal injections of the same concentrations caused sudden death of the animals within few minutes. Consequently, the oral route was adopted to study the effect of this drug on the embryonic development of the mouse.

Treatment during early gestation:

The females administered phenylbutazone
from the 1st to the 6th day of gestation were the most susceptible for abortion. Among the ten females administered 200 mg/kg phenylbutazone daily 3 were aborted, and among 10 females administered 100 mg/kg one was aborted. Most cases of abortions were observed within 3-4 days after the drug administration. The average number of foetuses per litter in the two groups treated with 100 and 200 mg/kg was 7.7 and 6.0 respectively. The percentage of dead foetuses to the total number of foetuses was 2.9 and 7.1 per cent for the low and high doses respectively. All the foetuses obtained from the females treated by the low dose showed no morphological anomalies. However, 3 foetuses from one litter in the group subjected to the high dose of phenylbutazone were abnormal (Fig. 64). This litter comprised, one normal living foetus, two normal dead foetuses, two abnormal living foetuses, and one abnormal dead foetus.

The incidence of abortion among females treated during early development was also reported by Warkany (1963). He stated that the effects of drugs had been found to vary according to their
time of administration. The same drug may cause embryonic death before organogenesis, malformations during organogenesis and foetal distress if administered late in pregnancy.

Concerning the anomalies observed in the present study, it should be stated that the three abnormal foetuses were obtained in one litter. These anomalies might result from general effects of the treatment persisting to affect the embryo later.

**Treatment during mid-gestation:**

The females treated from the 7th to the 12th day of gestation were relatively less susceptible to abortion. Only by the administration of the high dose two females among 10 were aborted. The average number of foetuses per litter was markedly decreased among this group from 8.2 in the controls to 6.2 and 6.4 in the groups subjected to treatment with 100 and 200 mg/kg respectively. The average number of foetuses per litter, in rabbit, recorded by Larsen and Bredahl (1966), was not affected by the administration of 60 mg/kg of phenylbutazone for the first 20 days of gestation.
In the present study the percentages of dead foetuses obtained to the total number of foetuses were 1.6 and 9.8 for the low and high doses respectively. One of the females gave 6 normal living foetuses, two normal dead foetuses, and three abnormal dead foetuses. The abnormal foetuses were markedly smaller in size.

Treatment during late gestation: The third group of females treated from the 13th to the 18th day of gestation were the least susceptible to the administration of phenylbutazone in the doses 100 and 200 mg/kg body weight daily. No cases of abortions were observed, and no dead or morphologically abnormal foetuses were obtained. The average number of foetuses per litter was 7.8 and 8.3 for the low and high doses respectively.

Examination of the foetal skeleton of the morphologically normal foetuses showed that phenylbutazone has no major effect on the embryonic development of the skeleton. The long bones were all present and were shorter than normal in the affected limbs. Some caudal vertebrae, the astragalus, and the calcaneous bones were frequently missing.
The morphological abnormalities obtained may be due to indirect effect of the drug on the foetuses. The incidence of minor malformations in the limbs among foetuses obtained from females treated from the 1st to the 6th day of gestation (fig. 64), is difficult to explain as a direct toxic effect of the drug on the foetus during this early period. The anomalies observed are due to impairment of development during late gestation. In the monkey, dog, rabbit and rat this drug is metabolized in three to four hours (Tuchmann-Duplessis, 1965). Consequently it is probable that phenylbutazone has some effects on the mother which can persist to affect the foetuses later.

The malformed foetuses obtained from each group of animals were from one mother. This supports the abovementioned explanation that the drug indirectly affect the foetuses. It is probable that malformed foetuses were obtained from a female susceptible to this drug.
Effects of maternal administration of phenylbutazone on the foetal liver

Bean (1960) reported some cases of liver and kidney enlargement among patients administered 60 mg per day of phenylbutazone. Microscopic examination revealed widespread lymphocytic infiltration in the two organs.

In the present study, phenylbutazone was found to affect the foetal liver cells with varying severity. The pathological changes in the foetal liver cells ranged from cloudy swelling to diffuse necrosis. Fibrosis was also observed in few cases. Congestion of the central veins and the sinusoids was relatively common.

Maternal treatment during early gestation with 100 mg per kg mainly showed cloudy swelling and hydropic degeneration in the liver of foetuses examined. Congestion of the central veins and the sinusoids was observed in all the foetuses examined. Foetuses treated during the same period with 200 mg per kg had more degenerative and necrotic effects in the liver since cloudy swelling and hydropic degeneration had passed to diffuse necrosis in wide
areas. Bile pigments retention and lymphocytic infiltration appeared in 50 per cent of the foetuses examined. Congestion of the central veins and the sinusoids was observed in all the foetuses examined.

Maternal treatment during mid-gestation by 100 mg per kg, caused hydropic degeneration in combination with diffuse necrosis. Bile pigment retention and lymphocytic infiltration appeared in combination with necrosis in one foetus. However, the incidence of congestion in the central veins and the sinusoids was less in this group than in the first group.

Treatment by 200 mg per kg resulted in less incidence of cloudy swelling and hydropic degeneration, since more severe degenerative changes, as fatty degeneration and necrosis occurred. Peripheral areas of complete necrosis beside bile pigment retention and lymphocytic infiltration were seen in half the cases examined. Again the incidence of congestion was more than in those treated by 100 mg per kg.

Treatment during late gestation with either 100 or 200 mg per kg caused fatty degeneration and
focal necrosis in the liver cells of all the foetuses examined. Peripheral and central areas of complete necrosis beside bile pigments and lymphocytic infiltration appeared accompanying half the cases of necrosis. Fibroblastic proliferation accompanied by fibrosis appeared in the necrotic area of half of the foetuses examined. The incidence of congestion in the central veins and sinusoids was higher in this group of foetuses than the second group.
Effect of maternal administration of phenylbutazone on the foetal kidney.

Treatment of the pregnant females by 100 and 200 mg/kg phenylbutazone, orally, during the different periods of embryonic development was found to affect the foetal kidneys. Degenerative changes in the convoluted tubules and the ascending limbs of Henl's loops, such as cloudy swelling and necrosis were commonly found in all the examined foetuses (figs. 73 & 76).

In some cases the drug caused congestion of the glomeruli and straight vessels of the medulla with haemorrhages in the capsular and cortical areas (figs. 77 & 78).

The incidence of these degenerative changes was higher in foetuses of mothers treated by the high dose (200 mg/kg) than those treated by the lower dose (100 mg/kg), at any period of embryonic development.

Foetuses of the 2nd and 3rd groups showed the same incidence of degenerative changes. However, congestion of the glomeruli and straight vessels of the medulla and the capsular haemorrhages of the kidney appeared only in foetuses of the third group.
The lower dose of phenylbutazone (100 mg/kg) was enough to cause the degenerative pathological changes of the foetal kidney when administered to the pregnant at any period of embryonic development.

Effect of maternal administration of phenylbutazone on the foetal spinal cord

Maternal administration of 100 and 200 mg per kg during early gestation did not affect the structures of the foetal spinal cord.

Maternal administration of 100 mg per kg phenylbutazone during mid-gestation did not affect the structures of the spinal cord of the foetuses examined. However, maternal administration of 200 mg per kg affected the development of the foetal spinal cord. The two halves of the cord were completely separated from the dorsal side till the central canal but they were still attached ventrally (figs. 79 and 80). It seems that the drug, in this case, affected the closure of the neural tube which normally takes place during this period.
Maternal administration of 100 and 200 mg per kg during late gestation did not affect the development of the foetal spinal cord. However, the drug in this period caused degenerative effects on the tissues of the foetal spinal cord. Thus, the white matter and the gray matter were reduced at different areas of the cord and the outer surfaces appeared crenated (fig. 81). The drug severely affected the dura mater which appeared disrupted possibly due to demyelination.

Haemorrhages from the dorsal and ventral spinal arteries seem to be the cause for the deformity observed in the foetal spinal cord in the foetuses maternally subjected to treatment with 200 mg per kg phenylbutazone. Subdural haemorrhages pressed upon the surface of the spinal cord in some areas and resulted in the presence of large invaginations (fig. 83). Haemorrhages observed in the vertebral space were extravasated from the vertebral arch through the destroyed periosteum.
ATLAS WITH EXPLANATION
Fig. 63: A photograph of a side view of a foetus from the control group subjected to the same treatment except the drug (phenylbutazone), from the 1st to the 18th day of gestation.

Fig. 64: A photograph of a side view of an abnormal foetus after maternal administration of 200 mg/kg phenylbutazone orally, from the 7th to the 12th day of gestation. The fore-limbs are abnormally flexed posteriorly.
Fig. 65: A photograph of a side view of an abnormal foetus after maternal administration of 200 mg/kg phenylbutazone orally, from the 7th to the 12th day of gestation. The foetus has a markedly small size and the fore-limbs atrophied.

Fig. 66: A photograph of a side view of an abnormal foetus after maternal administration of 200 mg/kg phenylbutazone orally, from the 7th to the 12th day of gestation. The foetus has a markedly small size, specially posteriorly, and the body axis abnormally flexed.

Fig. 67: A photograph of a side view of an abnormal foetus after maternal administration of 200 mg/kg phenylbutazone orally, from the 7th to the 12th day of gestation. The foetus has a markedly small size and malformed hind limb.
Fig. 68: A photograph of a side view of an alizarin red skeletal preparation of an abnormal foetus (fig. 64) after maternal administration of 200 mg/kg phenylbutazone orally, from the 1st to the 6th day of gestation. The foetus showed absence of astragalus bone and reduced calcaneous. There are only 5 caudal segments.

X 4

The arrow denotes to the reduced calcaneous bone

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Fig. 69: A photograph of a side view of an alizarin red skeletal preparation of an abnormal foetus (fig. 66), after maternal administration of 200 mg/kg phenylbutazone orally, from the 7th to the 12th day of gestation. The foetus had the two tarsus bones absent and the caudal vertebrae reduced to 5 segments.

X 4

The arrow denotes to the place of the tarsus bones
Fig. 70: A photomicrograph of section of liver of a foetus maternally treated by 200 mg/Kg phenylbutazone during late gestation. It shows necrotic liver cells and in some regions they are replaced by fibrous tissue. Haematoxylin and eosin  X 540

Fig. 71: A photomicrograph of the same above section showing the connective tissue between the islands more vascular, in varying degrees fibrous and irregularly infiltrated with lymphocytes. Haematoxylin and eosin  X 1080

- The arrows are denoting to fibroblasts
Fig. 72: A photomicrograph of the same previous section highly magnified showing the still surviving liver cells at different phases of division to form new cells.

Haematoxylin and eosin  X 1350
- The arrows are denoting to the dividing cells
Fig. 72
Fig. 73: A photomicrograph of transverse section of kidney of a foetus maternally treated by 100 mg/kg phenylbutazone during early gestation. It shows cloudy swelling of the convoluted tubules and the ascending limbs of Henl's loops.

Haematoxylin and eosin  X 405

Fig. 74: A photomicrograph of transverse section of kidney of a foetus maternally treated by 200 mg/kg of phenylbutazone during late gestation. It shows necrosis of the convoluted tubules and the ascending limbs of Henl's loops since the lining cells appear as epithelial casts inside the lumina of the affected tubules.

Gomori stain  X 1080

- The arrows are denoting to epithelial casts inside the lumina of the affected tubules
Fig. 75: A photomicrograph of a transverse section of kidney of a foetus maternally treated by 100 mg/kg of phenylbutazone during late gestation. It shows necrosis of the lining cells of the convoluted tubules and the ascending limbs of Henl's loops.

Gomori stain       X 405

Fig. 76: A photomicrograph of a transverse section of kidney of a foetus maternally treated by 200 mg/kg phenylbutazone during early gestation. It shows necrosis of the lining cells of the convoluted tubules and the ascending limbs of Henl's loops.

Haematoxylin and eosin   X 405
Fig. 77: A photomicrograph of a transverse section of kidney of a foetus maternally treated by 100 mg/kg phenylbutazone during late gestation. It shows enlargement of the glomerulus to fill completely the intracapsular space.

Gomori stain X 1015
- The arrows are denoting to the swollen glomerulus

Fig. 78: A photomicrograph of a transverse section through kidney of a foetus maternally treated by 200 mg/kg phenylbutazone during late gestation. It shows haemorrhages in the capsular and cortical areas.

Gomori stain X 270
- The arrows are denoting to the areas of haemorrhages
Fig. 79: A photomicrograph of transverse section through the cervical sp.c. of a foetus maternally treated by 200 mg/Kg phenylbutazone during mid-gestation. It shows the two halves separated from the dorsal side till the region of the central canal. The vertebral arch shows lack of ossification. Haematoxylin and eosin.

X 45
Fig. 80: A photomicrograph of transverse section through the cervical sp.c. of a foetus maternally treated by 200 mg/kg phenylbutazone during mid-gestation. It shows the two halves separated from the dorsal side till the region of the central canal. The duramater around the cord is also disrupted.

Haematoxylin and eosin X55.

- The arrows are denoting to the disrupted duramater.

Fig. 81: A photomicrograph of transverse section of a foetus maternally treated by 200 mg/kg of phenylbutazone during late gestation. It shows deficiency from the ventral and lateral white matter. The wall of the central canal is disrupted and the dorsal fissure is more deep and contains plenty of connective tissue than normal. The duramater is highly disrupted.

Gomori stain X55

- The arrows are denoting to the disrupted duramater.
Fig. 82: A photomicrograph of a transverse section through the thoracic sp.c. of a foetus maternally treated by 200 mg/kg of phenylbutazone during late gestation. It shows asymmetry of the cord due to the compression of the destructed vertebral arch. Haemolysed blood is seen inside the vertebral canal.

Gomori stain X65

1- Subdural haemorrhages.
2- Haemorrhages inside the vertebral canal.
3- The destructed areas of the vertebral arch.

Fig. 83: A photomicrograph of T.S. through the posterior thoracic sp.c. the same above foetus. It shows asymmetry of the cord as a result of one lateral subdural invagination. The vertebral arch shows deficiency opposite to the region of the affected cord.

Gomori stain X80

1- Subdural invagination.
2- The area of the destructed vertebral canal.