SUMMARY

Reports of receptor sites for 1,25-dihydroxy-vitamin D₃ in most of the tissues of the body suggest that vitamin D may have a more generalised function rather than merely in the hitherto known target organs i.e. the intestine and the bone. Recent clinical studies (including a few by the author) have indicated a beneficial effect of vitamin D supplementation during pregnancy on the fetal and neonatal growth. This experimental study was conducted to elucidate the effects of vitamin D supplementation during pregnancy on the skeletal and soft tissue growth in the rat pups.

Throughout pregnancy and lactation female rats were fed on a commercial diet containing adequate amounts of vitamin D, calcium and phosphorus (1800 IU of vitamin D₃, 1% calcium and 0.6% phosphorus). Moderate supplements of vitamin D₃ were administered to such rats or their pups in four series of experiments. Plasma calcium levels and histological study of the epiphyseal cartilage indicated that neither the control groups of pups were deficient in vitamin D nor were the supplemented groups hyper-vitaminotic.

GROUP A STUDIES: In this series, three different doses of vitamin D₃ were administered as a single intramuscular injection on 10-12th day of pregnancy as follows:

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<th>Group</th>
<th>A I</th>
<th>Control</th>
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<tr>
<td>Group</td>
<td>A II</td>
<td>Vitamin D₃ 3,000 IU</td>
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<tr>
<td>Group</td>
<td>A III</td>
<td>Vitamin D₃ 7,500 IU</td>
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<tr>
<td>Group</td>
<td>A IV</td>
<td>Vitamin D₃ 15,000 IU</td>
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The pups were sacrificed at 28th day of age. Vitamin D status of the pups was known from the histological studies of the upper ends of tibiae and from plasma calcium levels. Estimations of dry bone weight and bone ash in the tibiae were used as indices of skeletal growth. Soft tissue growth was studied in the liver, brain and gastrocnemius muscle. In these tissues, the pattern of cellular growth was investigated by the estimation of tissue weight, protein content, RNA content, DNA content, protein/DNA ratio and RNA/DNA ratio. The maternal food intake was estimated at d14-16 of lactation.

**GROUP B STUDIES**: In this series of experiments, the mothers were supplemented with three different doses of vitamin D₃ as a single intramuscular injection on the third day of lactation as follows:

- **Group B I** Control
- **Group B II** Vitamin D₃ 3,000 IU
- **Group B III** Vitamin D₃ 7,500 IU
- **Group B IV** Vitamin D₃ 15,000 IU

At d 28 the pups were sacrificed and the skeletal as well as soft tissue growth was studied as in Group A series of experiments.

**GROUP C STUDIES**: In this series, the mothers in the experimental group (group C II) were given 7500 IU of vitamin D₃ as a single intramuscular injection on 10-12th day of pregnancy and the pups were sacrificed at d1, d10 and d 20. The pattern of cellular growth was studied in the liver and the brain and
compared with group C I (controls).

**GROUP D STUDIES**: In this series, two doses of vitamin D\textsubscript{3} (500 IU and 1000 IU) were administered directly to the pups at d10 of age and their body weights were compared with non-supplemented controls at d20 and d28.

The pups in group A II and A III (whose mothers received 3,000 IU and 7,500 IU of vitamin D\textsubscript{3} respectively, in pregnancy) weighed slightly more than controls (group A I) at d1 of age but the difference was not statistically significant. However, at d10, d20 and d28, mean weights of the pups in group A II and A III were 24% and 31% more than control, respectively.

At d28 mean weight of the liver in group A III pups was significantly more than in controls. Hepatic protein content, RNA content, cell number (DNA content), cell size (protein/DNA ratio) and protein synthetic capacity (RNA/DNA ratio) were also significantly greater in group A III than in controls. In group A II pups, many of the indices of cellular growth were more than controls but the difference did not reach the significant level in most of the indices.

In gastrocnemius muscle the weight and all the indices of cellular growth were significantly more in both groups A II and A III than in controls.

Brain weight and its protein content, RNA content and RNA/DNA ratio were significantly more in groups A II as well as group A III than in controls.
At d28, the mean weights of the liver, brain and gastrocnemius muscle were 20%, 10% and 35% more than controls respectively.

In groups A II and A III the dry bone weight and bone ash weight of the tibiae were significantly more than in controls. However, bone/body weight and ash weight/dry bone weight ratios in these two supplemented groups were not significantly different from controls.

Group A IV pups (whose mothers received 15,000 IU of vitamin D₃ in pregnancy) did not show any evidence of growth acceleration in total body weight, in the skeleton or the soft tissues.

Mean daily food intakes of the mothers in groups A II & A III, measured at d14-16, were significantly more than in group A I. Inspite of this, the mean body weights of the mothers in groups AII & A III at d28 were not significantly different from group A I (nor were at d1). This observation indicated greater lactational performance of the mothers in groups A II & A III than in group A I. From the results described so far, it was concluded that vitamin D supplementation in pregnancy promoted neonatal growth at least in part by increasing the lactational performance of the mothers.

Supplementation of vitamin D₃ in early lactation (group B studies) also produced a significant increase in the weight of the pup in groups B II & B III at d20 & d28. However, as compared to (control) group B I, the increase in the weights
of the pups in groups B II & B III was only 16%, whereas 24% and 31% increase in weight of the pups was observed in groups A II & A III respectively. The weight of dry tibiae and ash weight as well as weights of the liver, brain and gastrocnemius were also more in groups B II & B III than in group B I but the difference in weights was not as much as observed in group A studies. Maternal food intakes in groups B II & B III, measured at d14-16, were also more than in controls but the difference did not reach a significant level. There was no difference between different groups as regards the maternal weight at d1 & d28.

Like groups A IV, group B IV pups also did not show any evidence of growth acceleration.

Thus neonatal growth of the pups could be increased by administration of vitamin D₃ supplements not only in pregnancy (group A pups) but also in lactation (group B pups). However, the effect was more pronounced in case of the former mode of vitamin D supplementation. Besides this difference, there were other qualitative differences between groups A II & A III on one hand and groups B II & B III on the other. In the former groups, vitamin D₃ administration increased the soft tissue growth by producing greater cellular hyperplasia and cellular hypertrophy. An increase the protein synthetic capacity was also observed. In groups B II & B III, on the other hand,
the increase in neonatal growth could only be attributed to greater cellular hyperplasia. These results suggested an anabolic effect on the fetal tissues when vitamin D₃ supplement was administered in pregnancy. This possibility was further supported by the observations in group C studies.

Study of the pattern of cellular growth in the liver and brain at d1, d10 and d20 revealed the usual pattern of cellular growth in the control pups (group C1). In these tissues, there was an initial phase of cellular proliferation followed by a phase of cellular hypertrophy. The proliferative phase (increase in DNA content) lasted mainly up to d10 in the brain but continued even beyond d20 in the liver. In group C II pups (whose mothers received 7,500 IU of vitamin D₃ in pregnancy) both the phases could be observed but in an accentuated form. In addition, the intrauterine anabolic effect of vitamin D₃ was evident in the brain (which unlike the liver undergoes a greater proportion of total growth during gestation period). Even at d1, the mean weight of the brain in group C II was significantly more than in group C I.

Administration of vitamin D₃ directly to the pups (group D studies) at d10 of age did not produce any improvement in the neonatal growth.

To conclude, the results of this study have demonstrated that in the rats on normal intakes of vitamin D, calcium and phosphorus, administration of a limited supplement of vitamin D₃ during pregnancy produced a beneficial effect on the fetal and
neonatal growth. The increase in growth involved both the skeletal and soft tissues. The accelerated neonatal growth was partly due to an improvement in the lactational performance of the mothers. In addition, certain evidences suggest an anabolic action of vitamin D on the offspring which began during gestation and extended into neonatal period.