to treatment with penicillin as infections due to sensitive staphylococci. If these observations are confirmed, it suggests that penicillin is still adequate for the treatment of most forms of staphylococcal sepsis seen in out-patients.

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INFLUENCE OF CORTISONE ON TERATOGENIC EFFECTS OF HYPERVITAMINOSIS-A

BY

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The important part which environmental factors may play in influencing the occurrence of congenital malformations is becoming recognized as a result of animal experiments in which the dams have been subjected during pregnancy to the effects of a wide variety of teratogenic agents. Amongst the substances whose teratogenic importance is now well established is vitamin A: both hypovitaminosis-A and hypervitaminosis-A in the mother have been shown to result in the birth of young with anomalies of the brain and its coverings. Congenital hydrocephalus has been reported in the offspring of animals fed on a vitamin-A-deficient diet (rabbits—Millen, Woollam, and Lamming, 1954; rats—Rokkones, 1955). In rats, maternal hypervitaminosis-A is known to produce anencephaly, exencephaly, and hydrocephalus in the young (Cohlan, 1953; Giroud and Martinet, 1954).

The investigation reported in the present communication was undertaken to explore the effect of cortisone, administered during pregnancy, upon the incidence of deformities of the brain and calvaria produced by hypervitaminosis-A.

Material and Methods

Female white rats of the Wistar strain were used in the experiments. Each of these rats had previously given birth to a normal litter. At the beginning of the experiment the rats were given 2,000 I.U. of vitamin D and 50 mg. of vitamin E by mouth to ensure that they had an adequate supply of these vitamins.

Daily vaginal smears were taken at 9 a.m. and conception was determined by the presence of spermatozoa. The day on which spermatozoa were found was regarded as Day 1 of the pregnancy. The pregnant rats were divided into three groups, each group consisting of 12 animals.

Group 1 received 60,000 I.U. of vitamin A acetate daily from the 8th to the 13th day, inclusive, of pregnancy.

Group 2 received 20 mg. of cortisone acetate daily from the 9th to the 12th day, inclusive, of pregnancy.

Group 3 received 60,000 I.U. of vitamin A acetate daily from the 8th to the 13th day, inclusive, and 20 mg. of cortisone acetate from the 9th to the 12th day, inclusive, of pregnancy.

Vitamin A was given by intubation. A rubber No. 8 French catheter was passed into the stomach and the dose of vitamin A dissolved in 1 ml. of arachis oil was introduced. The procedure was repeated on the appropriate days. Cortisone acetate was given by subcutaneous injection.

The rats were killed on the 20th day of pregnancy and the young removed from the uterus. The foetuses were inspected for abnormalities and preserved in 10% formalin for further examination. Various deformities were encountered in the young, but only those affecting the brain and the calvaria are considered in this communication.

Findings

The results of the experiments are shown in the Table. It will be noted from this that the number of young in group 3 is much smaller than in the other two groups.

Incidence of Deformities of the Brain and Calvaria Found in the Young

Group	No. of Rats	Total No. of Young	No. of Young with Deformity of Brain and Calvaria	Percentage of Young with Deformity of Brain and Calvaria
1	12	77	6	7.8
2	12	73	0	0
3	12	41	15	36.6

This was due to the resorption of all the young in three animals, and of many of the young of another four animals. Total resorption also occurred in two of the rats in group 2.

Varying degrees of malformation were encountered and are shown in the illustration. In some animals the deformity was in the nature of a small meningocele associated with a marked acrocephaly, whilst in others there appeared to be a complete extroversion of the cerebral hemispheres, so that the chorioid plexuses were exposed on the surface of the brain. In the latter animals the amniotic fluid was invariably tinged with blood pigment.

Discussion

The most significant finding which emerges from this experiment is that cortisone given to hypervitaminotic rats on the 9th to 12th days produces a great increase in the number of young, surviving on the 20th day, which show malformations of the brain and calvaria (36.6%).

It is well known that hypervitaminosis-A in rats results in malformations of the brain and calvaria in their young.

As has been stated earlier, anencephaly and exencephaly have been reported by Cohlan (1953) and by Giroud and Martinet (1954). Cohlan (1954) obtained young with a congenital cranial anomaly in 48.7% of the foetuses by giving 35,000 I.U. of vitamin A daily from the 2nd or 4th day to the 16th day of pregnancy. Giroud and Martinet (1956) observed a 6% incidence in the young of rats which had received 20,000 I.U. of vitamin A daily for 12 days from the 2nd or 4th day of pregnancy. The incidence of 7.8% young with gross deformities of the brain and skull obtained in group 1 of the present experiment (rats which received 60,000 I.U. of vitamin A daily from the 8th to the 13th day) is in complete agreement with these earlier reports.

Cortisone alone has not been shown to have a teratogenic action. The animals which received 20 mg. of cortisone daily from the 9th to the 12th day (group 2 of the experi-

The various degrees of deformity of the brain and calvaria (actual size).

ment) produced no young with malformations of the brain. Fraser, Fainstat, and Kalter (1953), however, found an increased incidence of cleft-palate following the administration of cortisone to mice of a genetic strain in which there was already a low incidence of cleft-palate.

The explanation of the striking increase in the incidence of abnormalities of the brain and calvaria when hypervitaminosis-A and cortisone are combined awaits further experiment. The results of the work reported here suggest strongly that cortisone potentiates the teratogenic effect of the hypervitaminosis-A. It may well be that the increased incidence of cleft-palate observed by Fraser *et al.* in mice genetically susceptible to the condition was also due to the cortisone potentiating the expression of the inherent genetic weakness.

It has been suggested that all environmental teratogenic activity is mediated through the influence of teratogenic agents on genetic weaknesses which, by themselves, do not result in the appearance of congenital malformations (Fraser, 1953). The malformations of the brain and calvaria described above have not been reported in the offspring of normal rats of the strain used in this experiment. Even if the hypothesis of an inherent genetic tendency to the deformity be admitted, it is clear that the effect of cortisone in potentiating the teratogenic action of vitamin A must be of considerable significance.

It will be noted that more resorptions of litters occurred in the rats receiving both vitamin A and cortisone. The probable explanation for this is suggested by the consideration of the possible outcomes of pregnancy. Pregnancy must result in the birth of normal or malformed young or in the death and resorption of the foetus. If a teratogenic agent is at work there will be a shift in the direction of total failure of the pregnancy—that is, the death and resorption of the young. Hence with a diminution in the number of normal young and an increase in the number of malformed young one would expect to find also an increase in the mortality of embryos during pregnancy.

It may well be argued that, because of the large doses of the teratogenic agents employed, experiments with animals such as those reported here have little bearing on the possible outcomes of human pregnancy. But, in evaluating the strength of this argument it is necessary to compare the incidence of the naturally occurring malformation in man with its incidence as produced experimentally in laboratory animals. The frequency of anencephaly and associated anomalies in man has been stated to be about 0.25% of total births (Böök and Fraccaro, 1956). The incidence recorded in the present experiment, 36.6%, is 150 times as great as the incidence in man. It appears, therefore, not wholly illogical to suppose that during the early months of pregnancy the situation occurs occasionally where the circulating amounts of cortisone and vitamin A may be of such proportions as to influence unfavourably both the development and the survival of the foetus. Such a possibility is at least worthy of further consideration in the light of the findings of this investigation.

Summary

The effect of cortisone upon the teratogenic action of hypervitaminosis-A has been studied in rats.

The incidence of gross malformations of the brain and calvaria in the young of mothers who received both vitamin A and cortisone was greatly increased (36.6%), compared with those from animals which received vitamin A alone (7.8%) or cortisone alone (0%).

These findings are discussed in relation to earlier work upon the teratogenic effects of hypervitaminosis-A and of cortisone.

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EFFECT OF CORTISONE ON THE INCIDENCE OF CLEFT-PALATE INDUCED BY EXPERIMENTAL HYPERVITAMINOSIS-A

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Cleft-palate may be produced experimentally in the rat by the administration of massive amounts of vitamin A to the dam during the early and middle periods of pregnancy (Cohan, 1953; Giroud and Martinet, 1954). Fraser *et al.* (1954) have demonstrated that cortisone greatly increases the incidence of cleft-palate in strains of mice which show a genetic predisposition to the spontaneous occurrence of the deformity.

It appeared, therefore, that both hypervitaminosis-A and cortisone may be implicated in the production of cleft-palate in experimental animals. An investigation was therefore carried out in order to determine whether the incidence of cleft-palate produced by hypervitaminosis-A during pregnancy was influenced by giving cortisone.

Materials and Methods

Female white rats of the Wistar strain were used in the experiment. Each of these rats had previously given birth to a normal litter. At the beginning of the experiments the rats were each given 2,000 I.U. of vitamin D and 50 mg. of vitamin E by mouth to ensure that an adequate supply of these vitamins was available. Vaginal smears were taken daily at 9 a.m., and conception was determined by the presence of spermatozoa. The day on which spermatozoa were found was regarded as Day 1 of the pregnancy.

The pregnant rats were divided into five groups, each group consisting of 12 animals.

Group 1 received 60,000 I.U. of vitamin A acetate, by subcutaneous injection, daily from the 8th to the 13th day, inclusive, of pregnancy.

Group 2 received 20 mg. of cortisone acetate, by subcutaneous injection, daily from the 9th to the 12th day, inclusive, of pregnancy.

Group 3 received 60,000 I.U. of vitamin A acetate, by subcutaneous injection, daily from the 8th to the 13th day, inclusive, of pregnancy, and 20 mg. of cortisone acetate, by subcutaneous injection, daily from the 9th to the 12th day, inclusive, of pregnancy.

Group 4 received 60,000 I.U. of vitamin A acetate, by oral intubation, daily from the 8th to the 13th day, inclusive, of pregnancy.

Group 5 received 60,000 I.U. of vitamin A acetate, by oral intubation, daily from the 8th to the 13th day, inclusive, of pregnancy, and 20 mg. of cortisone acetate, by subcutaneous injection, daily from the 9th to the 12th day, inclusive, of pregnancy.