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Prenatal Exposure to Prednisone in Humans and Animals Retards Intrauterine Growth

Abstract. *Prednisone treatment for infertility and subsequent pregnancy maintenance in humans resulted in a significant decrease in the birth weight of full-term infants and a marked increase in the percentage of newborn infants weighing 2500 grams or less, that is, "light for dates" in comparison to control offspring. A parallel experiment with mice indicated that the reduction of birth weight was caused by exposure to corticosteroids rather than to maternal disease or malfunction.*

Since their synthesis nearly three decades ago, corticosteroids have been the treatment of choice for such life-threatening disorders as lupus erythmatosus, Addison's disease, and asthma to less serious complaints such as dermatitis and tennis elbow (*1*). Serious threats to maternal well-being, at times, necessitate corticosteroid therapy during pregnancy (*2–4*). Moreover, adrenocortical hormones have been used expressly to induce ovulation and support pregnancy in women suffering from infertility. Specifically, steroid therapy is administered when the suspected etiology of the disorder is a mild abnormal elevation of adrenal androgen levels (*5*). Thus, both the incidental and deliberate treatment of pregnancy with corticosteroids have resulted in the exposure of large numbers of fetuses to augmented adrenal hormone levels.

The paucity of reports on the possible effects on the human fetus of these powerful substances is surprising. Those reports which do exist are based on small numbers of individuals and fall into one of two categories. In one type of study, subjects received relatively large amounts of corticosteroids for the alleviation of severe symptomatology unrelated to pregnancy (*2–4*). In the second type, the offspring were evaluated during clinical trials in which low doses of corticosteroids were administered over long periods for infertility and subsequent maintenance of pregnancy (*5, 6*). In both types of experiments, little risk of exposure was noted for either mother or offspring, although somewhat higher frequencies of stillbirth and spontaneous abortion as well as isolated instances of cleft palate occasionally were reported (*4, 6*).

These reports of negligible effects on the human fetus are in marked contrast to data derived from animals in which exposure to corticosteroids during gestation apparently produces deleterious effects. Such effects as resorption, stillbirth, reduced weight and length, cleft palate, and decreased thymus weight have been observed in chick embryos and mouse, rat, and rabbit fetuses exposed to corticoids (*2, 7, 8*). However, the dosages administered in most of these investigations were proportionally higher than those used for therapeutic treatment in humans. To our knowledge, no studies have been conducted with animals subjected to long-term treatment with low doses of corticosteroids analogous to the treatment used frequently for human infertility.

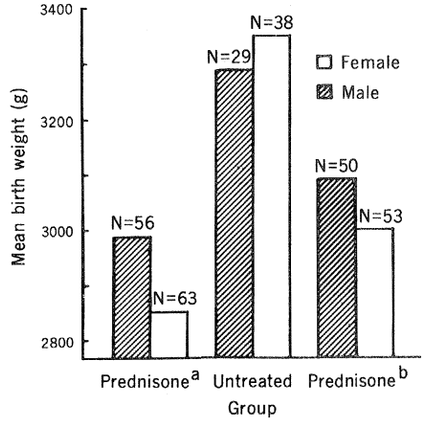

Fig. 1. Mean birth weight of male and female human infants exposed or not exposed prenatally to prednisone. (*Prednisone*[a]) based on data from all prednisone-exposed offspring. (*Prednisone*[b]) based on data from only those subjects that did not qualify as "light for dates" (weighing 2500 g or less at birth); only one subject in the untreated group was "light for dates."

Since no study of human offspring exposed to low dosages of corticosteroids throughout gestation could be found, we studied a large sample of offspring of women who were treated with corticosteroids for infertility and for maintenance of pregnancy. In any study of the offspring of women treated with drugs during pregnancy, the most serious confounding variable is the maternal disease or malfunction which necessitated the initiation of therapeutic intervention. This makes it difficult, if not impossible, to extricate the effects of the treatment from those of the maternal complaint. Therefore, we undertook a simultaneous study of laboratory animals treated with corticosteroids in dosages proportional to those given humans. Thus, if similar effects were obtained from the human and animal subjects, any observed consequences could be more confidently ascribed to the treatment. Furthermore, by terminating pregnancy in mice at various intervals during adrenocortical hormone administration, we could make a time-related assessment of drug exposure.

The full-term offspring of all women who received prednisone (*9*) during pregnancy ($N = 119$) at a private southern California infertility clinic between 1955 and 1975, served as the experimental subjects. The women in this group received 10 mg of prednisone per day for infertility and treatment was continued throughout pregnancy. No other steroid hormone therapy was administered to women in this group during these pregnancies. The comparison group was composed of the offspring of 67 women from the same clinic population who did not require prednisone or any other hormonal therapy during pregnancy. Thus, mothers of offspring from both the experimental and comparison groups were comparable in that they were subject to problems of infertility sometime during their reproductive lives.

Length of gestation in most cases was calculated from temperature charts and

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was therefore measured from the day of conception. In all cases, the deliveries were full-term (38 ± 2 weeks). In the few cases for which the last menstrual period was the landmark, gestation was calculated from 2 weeks after this date so that all gestation lengths would be comparable. Birth weight was obtained from both hospital and doctors' records recorded at the time of delivery.

A factorial analysis of variance revealed that offspring exposed prenatally to prednisone weighed significantly less than control offspring ($P < .0001$). No difference was found between the birth weight of male and female neonates (see Fig. 1).

As mentioned above, we undertook an experiment with animals to add support to the assumption that the lowered birth weight of offspring exposed prenatally to prednisone was mediated by the hormonal intervention and not by the clinical condition for which it was given. In addition, this experiment permitted a determination of the time course of the effect of prednisone on the fetus.

Male and female Rockland-Swiss mice (*10*) were maintained on a cycle of 12 hours of light and 12 hours of darkness and provided with unlimited access to food and water. After mating had occurred, as evidenced by the presence of copulatory plugs, the females were separated and housed in groups of four or five in 28 by 18 by 13 cm translucent polypropylene cages until day 12 of pregnancy, at which time they were housed singly. The day a copulatory plug was found was designated as day 0 of pregnancy. Animals were assigned to one of four groups on day 13 of pregnancy (*11*): one group received no treatment; a second group received 100 μg of prednisone; those in the third group received 400 μg of prednisone; the fourth group received vehicle only. Based upon body weight and surface area, the 100-μg dose was calculated to be proportional to that which the women received. Prednisone was suspended in 0.05 ml of steroid suspending vehicle (*12*). Each animal received one subcutaneous injection per day between 0900 and 1000 hours commencing on day 13 of pregnancy.

Each group was then divided into four subgroups. Those in the first subgroup were killed by cervical dislocation between 1900 and 2000 hours on day 14 of gestation after having received two injections of prednisone. Mice of a second subgroup were killed on day 16 after having received four prednisone injections, and animals of the third subgroup were killed on day 18 after six injections. The fetuses were removed, cleaned, sexed (*13*), and weighed. Pregnant animals of the fourth subgroup were allowed to deliver on day 19 after having received seven injections. Their young were weighed between 0900 and 1000 hours on the day of birth.

Table 1. Mean weights of 14-, 16-, and 18-day-old mouse fetuses and 1-day-old pups exposed to 100 or 400 μg of prednisone. Control animals either were exposed to the vehicle only or left undisturbed during gestation.

Group	Weight (g)			
	Day 14*†	Day 16‡	Day 18‡	Day 19 (term)‡
Control				
Male	0.28 (N = 63)	0.74 (N = 32)	1.33 (N = 17)	1.53 (N = 73)
Female		0.73 (N = 28)	1.32 (N = 14)	1.53 (N = 63)
Prednisone (100 μg)				
Male	0.27 (N = 49)	0.67 (N = 27)	1.25 (N = 34)	1.37 (N = 53)
Female		0.69 (N = 30)	1.26 (N = 27)	1.35 (N = 38)
Prednisone (400 μg)				
Male	0.27 (N = 47)	0.68 (N = 29)	0.89 (N = 16)	0.95 (N = 43)
Female		0.67 (N = 26)	0.87 (N = 18)	0.95 (N = 36)

*No significant differences among groups. †Fetuses at day 14 of gestation could not be assessed with regard to sex. ‡Significant differences among groups ($P < .0001$ in all cases). No differences were found between males and females.

Separate analyses of variance were performed to compare weights at each of the three fetal ages (days 14, 16, and 18) and at term (Table 1). No significant differences in weight were found between offspring of the two control groups, that is, the groups of mice that received only the vehicle or no treatment. Thus, the data for these groups were combined for each pregnancy-termination day and at term. Significant differences were not observed among any of the groups on day 14. However, 16-day-old fetuses exposed to prednisone (either 100 or 400 μg) for only 4 days weighed significantly less than did controls ($P < .00001$). Both the 18-day-old fetuses and the full-term pups exposed to either dose of the hormone also weighed less than did offspring of control mothers ($P < .00001$). Further, no significant differences in weight between males and females were obtained (*13*). Multiple comparison procedures (Duncan's test) revealed that on days 16 and 18 and at term animals exposed to 100 μg of prednisone weighed significantly less than did controls ($P < .05$). Finally, while not differing significantly on day 16, by day 18 and at term the fetuses and pups exposed to 400 μg of prednisone weighed significantly less than those exposed to 100 μg ($P < .001$).

The results of these experiments indicate that the growth of offspring exposed to prednisone during gestation is significantly retarded and results in diminshed full-term birth weight. Three explanations can be offered to describe the mechanism by which prednisone affects fetal weight. First, since corticosteroids readily cross the placental barrier (*14*), the lowered weights observed may be the outcome of a direct effect upon the fetus. It is interesting that children treated with corticosteroids for the alleviation of asthmatic symptons show evidence of severe growth retardation and suppression (*15*). Second, the effects may be mediated by placental abnormalities resulting from prednisone exposure. This possibility is supported by data demonstrating that adrenocorticosteroid-exposed placentas show decreased growth (*16*) and, in particular, glycogen depletion (*8*) in comparison to nonexposed placental tissue. Finally, it also is possible that a direct effect of exogenous corticoids on maternal physiology leads to a subsequent indirect influence on placental or fetal growth, or both. These explanations should not be considered mutually exclusive since any combination of the three could theoretically operate in concert.

In 1967 it was suggested that babies born at or below 2500 g after a full-term pregnancy be designated "light for dates" (*17*) to distinguish them from low-birth-weight infants who were premature (*18, 19*). Full-term babies born at these diminished weights have been identified as at-risk for the neonatal period as well as for later mental and physical development (*17, 20, 21*). The incidence of "light for dates" babies with no congenital anomalies has been estimated in various populations to lie between 1.04 and 2.0 percent (*18, 20, 22*). Analysis of "light for dates" births in our sample reveals the incidence to be 1.52 percent (1/67) in the control group and 13.92 percent (16/119) in the prednisone-exposed off-

spring, a statistically significant difference (χ^2, $P < .01$).

In response to the discovery of the significant number of "light for dates" births in the prednisone-exposed group of human infants, a second analysis of variance was performed to determine if the significant difference between the experimental and control groups would persist with the extremely low-weight subjects removed. The analysis, with "light for dates" subjects eliminated from both prednisone and control groups, showed that the hormone-exposed subjects were still significantly lighter than controls ($P < .0001$) (see Fig. 1). This finding indicates that, although only 16 prednisone-exposed subjects were born at weights which fit the strictly defined criterion for "light for dates," the majority of the experimental subjects were stillborn at weights lighter than would be expected. To illustrate the magnitude of intrauterine growth retardation in the prednisone-exposed offspring, it was determined that with "light for dates" subjects removed from both conditions, 60 percent of the remaining hormone-exposed individuals fell below the 25th percentile of the unexposed group.

The retardation of intrauterine growth after exposure to prednisone indicates that exogenous adrenocortical hormones can have a marked effect on fetal development. We therefore suggest that physicians be alerted to the possible at-risk status of babies born to prednisone-treated mothers, regardless of apparently normal birth weights and lengths of gestation.

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12. Steroid suspending vehicle was donated by the National Cancer Institute.
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Hepatic Fibrosis in Schistosomiasis: Egg Granulomas Secrete Fibroblast Stimulating Factor in vitro

Abstract. *Cytosol extracts and culture supernatants of isolated egg granulomas obtained from livers of mice with* Schistosoma mansoni *infection stimulated fibroblasts to incorporate tritiated thymidine and to proliferate in vitro. This finding suggests that hepatic granulomas may play a role in regulating hepatic fibrosis in* Schistosoma mansoni *infections.*

Hepatic fibrosis may be initiated by a variety of insults, including toxins and infection, and can result in portal hypertension and gastrointestinal hemorrhage. In infections due to the trematode worms *Schistosoma mansoni* and *S. japonicum*, hepatic fibrosis is the major cause of morbidity and mortality. The development of hepatic fibrosis in schistosomiasis is preceded by formation of granulomas surrounding the trematode eggs which become trapped in the portal venules of the liver. The development of these granulomas is similar in humans and mice infected with *S. mansoni*. In mice the granulomatous reaction has been shown to represent a delayed hypersensitivity response to soluble egg antigens (*1*). Here we report results of our investigations that suggest that products of schistosomal granulomas can stimulate fibroblasts in vitro and therefore may play a role in the development of hepatic fibrosis in schistosomiasis.

Lymphocytes elaborate soluble substances in vitro that are capable of inhibiting (*2*) or enhancing (*3*) fibroblast proliferation, and of stimulating collagen synthesis in vitro (*2*, *3*). Macrophages also influence fibroblast growth and protein synthesis (*4*, *5*). Since lymphocytes and macrophages constitute the predominant cell population in the prefibrotic granuloma, we investigated whether these granulomas might also regulate fibroblast activation in vitro. To do so, we prepared isolated schistosomal granulomas by a modification of the technique described by Pellegrino and Brener (*6*). This technique separates granulomas from liver homogenates by repeated sedimentation at 1*g*; free hepatic parenchymal cells, cell stroma, and cell debris remain in the supernatant and are discarded.

Outbred white mice (National Institutes of Health general purpose) were infected subcutaneously with approximately 500 cercariae of *S. mansoni* [Puerto Rico I strain (*7*)] obtained by passage through the snail vector (*Biomphilaria glabrata*). Seven weeks later, livers from individual mice were homogenized in a Waring blender for 1 to 2 minutes in Hanks buffer (Hanks balanced salt solution) and granulomas

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