lymph node, thymus and bone marrow of preleukemic mice. Alterations were observed in the cytoarchitecture of the reticular cell networks that contained many particles. The possible importance of the occurrence of C-type particles in lymphoid germinal centers was discussed in terms of the pathogenesis of spontaneous leukemia.

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Received September 13, 1967. P.S.E.B.M., 1967, v126.

Prenatal Glucocorticoid Administration and the Development of the Epinephrine-Forming Enzyme.* (32557)

LAWRENCE N. PARKER AND ERNEST P. NOBLE

Department of Psychiatry, Stanford University School of Medicine, Palo Alto, Calif.

The conversion of norepinephrine to epinephrine is catalyzed by the enzyme phenylethanolamine-N-methyl transferase (PNMT). The enzyme is highly localized in the adrenal medulla but lesser activity is detectable in heart and brain(1). Animals with a larger adrenal cortex and greater proximity of the cortex and medulla have a higher ratio of epinephrine to norepinephrine(2). Recent studies have shown that PNMT activity is controlled by the pituitary *via* the adrenal cortex. PNMT activity and epinephrine content are decreased following hypophysectomy and are restored by treatment with ACTH or glucocorticoids(3). The present report describes the effects of prenatal injection of physiological(4,5) and higher doses of glucocorticoids on PNMT activity and epi-

* This study was supported in part by an NIMH research grant MH-11935, by a USPHS career development award MH-6563 (to E.P.N.) and by a grant from National Assn. of Mental Health.

nephrine content of offspring at various stages of postnatal development.

Materials and methods. Pregnant Sprague-Dawley rats, weighing 250-300 g, were injected i.p. with saline or with various doses (1, 10 or 100 μg) of dexamethasone† from days 12-21 of gestation. Uninjected groups of pregnant rats were also used. At birth the mother and litter were housed together in individual cages and the young were weaned at 21 days. At appropriate times after birth the offspring were killed by decapitation and the adrenals, heart, and brain removed and immediately frozen. PNMT was assayed by the method of Wurtman *et al*(4) and epinephrine by the method of von Euler and Lishajko(6).

Results. Adrenals of 21-day-old animals treated prenatally with 10 and 100 μg of dexamethasone show approximately a 2-fold increase in both PNMT activity and epinephrine content as compared with rats left untreated or injected with saline (Table I). Epinephrine content is substantially elevated in the adrenals of 45-day-old animals treated prenatally with dexamethasone relative to the

TABLE I. Effects of Maternally-Administered Dexamethasone on Adrenal Weight, Phenyl-Ethanolamine-N-Methyl Transferase (PNMT) Activity and Epinephrine Content of Offspring.*

Age (days)	Treatment	Adrenal wt (mg)	PNMT activity†	Epinephrine content (μg per adrenal pair)
21	Untreated	8.4 ± 1.2	.96 ± .12	2.31 ± .47
	Saline	10.2 ± .7	.69 ± .10	2.12 ± .34
	1 μg dexamethasone	10.8 ± .8	.85 ± .17	2.49 ± .14
	10 " "	14.9 ± .6	1.57 ± .21[b]	4.43 ± .28[a]
	100 " "	12.1 ± .8	1.68 ± .20[a]	4.68 ± .38[a]
45	Untreated	24.9 ± 1.9	3.17 ± .35	3.8 ± .7 [c]
	Saline	29.0 ± 1.5	3.86 ± .58	6.0 ± .1
	1 μg dexamethasone	28.0 ± 1.0	4.18 ± .53	15.3 ±1.8 [b]
	10 " "	35.5 ± .5	3.45 ± .09	15.4 ±2.8 [a]
	100 " "	26.1 ± 1.6	3.65 ± .58	16.1 ± .9 [a]

* Pregnant rats were injected with saline or dexamethasone from days 12 to 21 of gestation, or left untreated. An average of 8 animals drawn from 2 or more litters was used for each point. Data are expressed as mean ± standard error.

† nmoles N-methyl phenylethanolamine produced per adrenal pair per hr.

[a] Differs from group treated with saline $p < .005$

[b] Differs from group treated with saline $p < .001$

[c] Differs from group treated with saline $p < .01$

saline-treated group. Also, the animals prenatally treated with saline have a higher adrenal epinephrine content than the uninjected group at 45 days. There are no pronounced differences in adrenal weight within the 21- or 45-day-old groups.

At one day of age, PNMT activity of heart per unit weight of tissue is increased 3-fold in the animals which had received 100 μg of dexamethasone prenatally, as compared with the saline treated controls (Table II). The increase present at 1 and 3 days is diminished at 7 days and absent at 21 days. PNMT activity of whole heart shows a small but significant increase in the steroid-treated animals at 1 day which disappears in the 3, 7, and 21-day-old animals. In saline-treated animals PNMT activity per whole heart at 3 days is 4 times that found at 21 days. There are no differences in brain PNMT activity at 1 day between the groups injected prenatally with 100 μg of dexamethasone and saline. The PNMT activity per unit weight of brain in 1-day-old saline-treated animals is 0.15% that of the adrenals.

Discussion. In the rat, the main para-aortic chromaffin body (Organ of Zuckerkandl) contains large amounts of norepinephrine and involutes one week after birth. However, when newborn rats are treated with hydrocortisone, the para-aortic body persists and is found to contain large amounts of epinephrine(7). The overall age-related de-

† 9α-fluoro-16 α-methyl -Δ^1-dehydrocortisol, obtained as dexamethasone-21-phosphate (Merck, Sharp & Dohme).

TABLE II. Effects of Maternally-Administered Dexamethasone on Heart Phenyl-Ethanolamine-N-Methyl Transferase (PNMT) Activity of Offspring.*

Age (days)	Treatment	PNMT activity† Per gram tissue	Per whole heart
1	Saline	260 ± 22	11.9 ± 1.3
	Dexamethasone	766 ±100[a]	16.3 ± 2.2[c]
3	Saline	646 ± 26	47.8 ± 6.8
	Dexamethasone	876 ± 54[b]	40.0 ± 2.6
7	Saline	430 ± 68	52.0 ± 7.4
	Dexamethasone	564 ± 18	55.4 ± 3.6
21	Saline	56 ± 8	12.0 ± 1.8
	Dexamethasone	46 ± 6	10.2 ± 1.1

* Pregnant rats were injected with saline or 100 μg dexamethasone from days 12 to 21 of gestation. An average of 6 animals drawn from 2 litters was used for each point. Data are expressed as mean ± standard error.

† μμmoles N-methyl phenylethanolamine produced per hour.

[a] Differs from group treated with saline $p < .0005$

[b] Differs from group treated with saline $p < .005$

[c] Differs from group treated with saline $p < .05$

crease in PNMT activity in the hearts of saline-treated animals and the increase in enzyme activity following dexamethasone treatment suggest similarity between the heart and para-aortic bodies in these respects. In contrast, brain PNMT activity is unresponsive to effects of steroids under the present conditions, while adrenal PNMT activity and epinephrine are markedly altered.

It has recently been found that PNMT activity arises in rat adrenal glands at 17.5 days *in utero*, at which time if hypophysectomy is performed by fetal decapitation, decreased PNMT activity and epinephrine content are observed in the adrenal glands of the neonates at birth(8). If ACTH or cortisol acetate is administered at 19.5 days to hypophysectomized fetuses, epinephrine and PNMT levels in the adrenals of the newborns are substantially elevated(8).

Several factors may contribute to the increase in adrenal PNMT activity observed in 21-day-old rats treated prenatally with dexamethasone. Among these are 1) proliferation of adrenal chromaffin cell types containing PNMT, 2) increased concentration of activator or decreased concentration of inhibitor of PNMT and 3) increased rate of synthesis or decreased rate of breakdown of enzyme protein.

In steroid-treated organ cultures of chromaffin cells derived from neonatal rabbits it has been found that epinephrine storage granules arise within cells previously concerned with norepinephrine storage(9). The scarcity of mitotic figures observed suggests that the appearance of epinephrine does not follow cell division and differentiation but results instead from a change in metabolic activity of preexisting chromaffin cells.

Studies on the characteristics of PNMT have revealed no activators of this enzyme in adult adrenals(1). Dexamethasone itself added *in vitro* to adrenal homogenates does not stimulate PNMT activity(3). Furthermore, results of investigations on the age-related increase in PNMT activity of rat adrenals are incompatible with the notion that the elevation of PNMT activity is related either to the appearance of an activator or disappearance of an inhibitor(8).

While PNMT activity in both fetal and adult rat adrenals decreases following hypophysectomy, treatment with steroids raises enzyme activity to normal levels. However, if hypophysectomized adult rats are pretreated with actinomycin D or puromycin, dexamethasone does not restore PNMT activity(3). These results imply that the effects of corticosteroids are mediated by increased amount of enzyme, either due to increased synthesis or decreased destruction of enzyme protein.

The rise in PNMT activity in the various treated groups at 21 days parallels the increase in adrenal epinephrine content, but this relationship is not observed in the 45-day-old animals. These observations suggest that in the steroid-treated animals, control mechanisms arise between 21 and 45 days which largely obliterate the initial differences in the PNMT activity within the various experimental groups. The relatively short half-life of adrenal PNMT activity (2-4 days (3)) as compared to the half-life of epinephrine in the adrenal gland (30-82 days(5)), makes it likely that the effects of steroids on PNMT activity may be abolished at 45 days, while increased content of epinephrine still persists.

Although the pituitary-adrenal axis has been implicated in the development of PNMT activity in the fetus(8), the mechanism of action of dexamethasone in causing elevation in enzyme activity in the 21-day-old animal as well as the control of PNMT activity by 45 days requires elucidation. Furthermore additional work is necessary to describe factors other than PNMT that contribute to the observed increase in adrenal epinephrine levels. These might include the activity of catecholamine-synthesizing and degradative enzymes, and the binding characteristics of endogenous epinephrine. Such studies are presently underway in this laboratory.

Epinephrine is involved in a wide variety of biological functions. The finding that treatment of pregnant rats with steroids leads to enhanced levels of epinephrine in the progeny suggests an animal model for further studies of the physiological and psychological (10) role of high endogenous levels of this catecholamine in mammals.

Summary. The administration of the synthetic glucocorticoid dexamethasone to pregnant rats raised the activity of the adrenal epinephrine-forming enzyme (PNMT) in the 21-day-old offspring. Adrenal epinephrine content was also elevated at 21 and 45 days. Heart PNMT activity was raised in the immediate postnatal period in the progeny of steroid-treated mothers, although enzyme activity returned to control values at 21 days. Brain PNMT activity was unaffected by steroid treatment.

We gratefully acknowledge the skillful technical assistance of Miss Judith Davis and Mrs. Susan Goulian.

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Received September 18, 1967. P.S.E.B.M., 1967, v126.

Disturbance of Cholesterol Metabolism in Alloxan Diabetes and Its Prevention by Glucose-cycloacetoacetate. (32558)

M. C. NATH, KUMUD S. DESHMUKH, AND NUSRAT F. SIDDIQUI
Department of Biochemistry, Nagpur University, India

Best(1) is of the opinion that the incidence of atherosclerosis is high in diabetes. Root and Wilson(2) have reported that a close correlation exists between diabetes and atherosclerosis. Our experimental work supports such a view in that continued injection of acetoacetate not only produces hyperglycemia (3) but also tends to lead to thrombosis(4).

The present investigation is based on the report of several workers that cholesterol and other lipids tend to be high in diabetes (5-8) and our own finding that the onset of diabetes caused by alloxan can be prevented by glucose-cycloacetoacetate, GCA(9-12). GCA is a crystalline compound formed by condensing glucose with acetoacetate. The purpose was to determine the C/P ratio (total cholesterol/lipid phosphorus) in plasma and tissues of alloxan diabetic rats and to see whether GCA can effectively maintain this ratio within normal limits. Furthermore, Nath and Brahmankar(13) have shown that GCA can increase elimination of fecal bile acids of animals fed a high saturated fat diet. Siperstein *et al*(14) have reported that excess cholesterol is metabolized and eliminated as