1. Nemeth, A. M., *J. Biol. Chem.*, 1954, v208, 773.
2. Weber, G., Cantero, A., *Cancer Research*, 1955, v15, 679.
3. Cori, G. T., Cori, C. F., *J. Biol. Chem.*, 1952, v199, 661.
4. Hogeboom, G. H., Schneider, W. C., Palade, G. E., *ibid.*, 1948, v172, 619.
5. Ashmore, J., Hastings, A. B., Nesbett, F. B., *Proc. Nat. Acad. Sci. U. S.*, 1954, v40, 673.
6. Fishman, W., Bernfeld, P., in Colowick, S. P., and Kaplan N. D., *Methods in Enzymology*, vI, 262 Academic Press (1955).
7. Hastings, A. B., Teng, C. T., Nesbett, F. B., Sinex, F. M., *J. Biol. Chem.*, 1952, v194, 69.
8. Nelson, N., *ibid.*, 1944, v153, 375.
9. Friedemann, T. E., Haugen, G. E., *ibid.*, 1943, v147, 415.
10. Good, C. A., Kramer, H., Somogyi, M., *ibid.*, 1933, v100, 485.
11. Renold, A. E., Hastings, A. B., Nesbett, F. B., Ashmore, J., *ibid.*, 1955, v213, 135.
12. Ashmore, J., Weber, G., *Vitamins and Hormones*, 1959, v27, 91.
13. de Duve, C., Beaufay, H., *Bull. soc. chim. biol.*, 1951, v33, 421.

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Teratogenic Action of a Hypocaloric Diet and Small Doses of Cortisone.* (25895)

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Cortisone acetate produced cleft palate in offspring of treated pregnant mice(1), in various frequencies depending on genetic and certain non-genetic variables involved(2,3,4). Supplemental treatment with riboflavin, folic acid, protein, or carbohydrate did not influence the incidence of the defect(5). The following study was undertaken to investigate the effect on fetal development of restricted daily food intake and smaller doses of cortisone than have usually been used in the past.

Materials and methods. Mice were young adult B6AF1 females, from the cross C57BL/6J ♀ x A/J ♂. They were bred to A/J males and put into individual cages upon observation of a vaginal plug, a sign to indicate that ⅓ day had passed since conception. Females were weighed at this time and divided into 5 groups of approximately similar weight composition. The groups were treated as follows: Group 1, 0.5 mg, Group 2, 1 mg, cortisone acetate/day for 4 consecutive days, beginning 11⅓ days after conception (called 0.5 and 1 mg cortisone, respectively). Group 3, given a pellet of 2.5 g food/day for 5 consecutive days, beginning 10⅓ days after conception (called restricted diet). Group 4, 0.5 mg cortisone + restricted diet. Group 5, 1 mg cortisone + restricted diet. Cortisone (Merck) was administered intramuscularly in the thigh. The food, Purina Lab Chow, was fed *ad lib.* at all times, except to females when on restricted diet. Fresh tap water was available always. Females were killed at 17⅓ days after conception, *i.e.*, about 2 days prepartum, resorption sites counted, and young removed, dried, weighed to nearest hundredth of g, and examined.

Results. Fig. 1 shows mean weights of 5 groups of females during pregnancy. Females treated only with cortisone continued gaining weight throughout treatment period, but mice fed the restricted diet lost weight early in treatment period, then became stabilized in weight, until *ad lib.* feeding was resumed, and animals given both treatments concurrently lost more weight and for a longer period than those of Group 3. The Fig. also shows that females given the restricted diet attained at time of sacrifice about the same weight as those given cortisone alone, but that animals given combined treatments did not catch up (mean weight in g, at 17⅓ days after concep-

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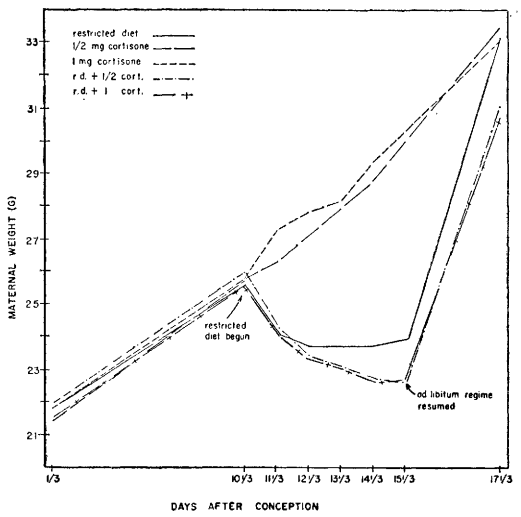

FIG. 1.

tion, for groups 1-3 = 33.2 ± .5; for groups 4 and 5 = 31.1 ± .6; $P < .01$).

Table I contains various relevant data. In view of the inverse association (3,4,6) between frequency of cortisone-induced cleft palate and maternal weight at conception, and the results to be presented, it should be noted that mean maternal weights at ⅓ day after conception of the 5 groups were greatly alike and that decrease in mean litter size was roughly proportionate to increase in resorption rate.

Number and frequency of abnormal young are found in Table II. It is seen here that 0.5 mg cortisone produced a minute incidence of young with cleft palate, namely 1.4%, whereas 1.0 mg cortisone produced 12% cleft palate. A quite unexpected result (Table II) is that 5.6% of young had cleft palate after their mothers were fed the restricted diet during pregnancy. Further, this Table shows that the restricted diet plus either dose of cortisone greatly increased frequency of cleft palate over that produced by that dose of cortisone or by the restricted diet, alone, to 36.8 and 50.7% respectively.

It is also seen from Table II that percentage of litters containing abnormal young increased with greater incidence of cleft palate, indicating that the increased rate of the anomaly was not due to greater numbers of abnormal offspring per defective litter, but that the malformation occurred in more litters. Finally, Table II shows that there occurred for most groups a decrease in mean fetal weight with increasing cleft palate frequency.

Discussion. Several points of interest have emerged from this experiment. First, it was found that simple underfeeding of a whole diet at a certain time in pregnancy is in itself teratogenic. A past study (7) showed that administration of 2.5 mg of cortisone/day for 4 consecutive days (11⅓-14⅓ days after conception) to pregnant mice caused food con-

TABLE II. Fetal Weight and Incidence of Cleft Palate (CP).

Treatment*	Fraction with CP	% CP	% abnormal litters	Fetal wt (g)†
.5 mg cortisone	2/144‡	1.4	10.5	.86 ±.01
1.0 " "	17/142	12.0	35.0	.74 "
Restricted diet (R.D.)	9/161	5.6	21.7	.72 "
R.D. + .5 mg cort.	42/114	36.8	82.4	.67 "
R.D. + 1.0 mg cort.	69/136‡	50.7	90.9	.67 "

* See *Materials and methods* for details.
† Mean ± stand. error.
‡ Less 2 and 1 young, respectively, with cleft lip and palate, a condition that occurs spontaneously in a low incidence in mice of this genetic composition.

TABLE I. Maternal Weight, Litter Size, and Percent Resorbed.

Treatment†	Females: No.	Females: Wt* (g) at Conception	Females: Wt* (g) at Sacrifice	Offspring: No.	Offspring: Litter size*	Offspring: % resorbed
.5 mg cortisone	19	21.4 ± .5	33.4 ± 1.2	146	7.7 ± .4	4.6
1.0 " "	20	21.8 ± .4	33.1 ± .8	142	7.1 ± .4	4.7
Restricted diet (R.D.)	23	21.8 ± .5	33.2 ± .6	161	7.0 ± .3	8.0
R.D. + .5 mg cort.	17	21.9 ± .7	31.1 ± 1.0	114	6.7 ± .5	16.8
" + 1.0 " "	22	21.6 ± .5	31.0 ± .9	137	6.2 ± .4	18.4

* Mean ± stand. error.
† See *Materials and methods* for details.

sumption to be elevated from the control mean for these 4 days of 5.8 g food/day to 6.6 g/day for the same 4 days, an increase of 14%. The amount fed in this study, 2.5 g/day, is 43% of control value, and 38% of experimental value. It can be taken, therefore, that the mice fed the restricted diet in the present study consumed about 40% of an *ad lib.* intake.

Starvation for 24 hours on 9th day of pregnancy was found to produce exencephaly and costal and vertebral defects in the 129 strain of mice(8), and for 72 hours from 9th to 12th days of pregnancy to produce cleft palate in mice of a different genetic constitution(9). But these results were not preparation for the finding that a hypocaloric diet would be teratogenic.

The second point of interest is that 1.0 mg cortisone produced so much greater an incidence of cleft palate than 0.5 mg cortisone (1.39% *vs.* 11.97%; $\chi^2 = 11.3$, $P<.001$). Apparently a threshold of sensitivity is crossed in this experimental setup by a dose lying somewhere between these amounts of the hormone.

Third, it is of great interest that a synergistic effect resulted from simultaneous treatment with the restricted diet and doses of cortisone. In Table II, however, it can be noted that the lesser amount of cortisone plus restricted diet had a larger relative effect than the greater dose of cortisone plus restricted diet. In the first case the combined treatment resulted in a percentage of cleft palate 5.3 times that of the sum of the individual treatments whereas with the second combination this was 2.9 times the sum of the treatments alone. It seems therefore that the smaller dose of cortisone, although producing by itself a negligible incidence of the defect, sensitizes the maternal-fetal system to a relatively greater extent to a concomitant embryopathogenic circumstance than does the larger dose.

Summary. Two relatively small doses of cortisone acetate administered to pregnant mice caused 1.4 and 12.0% cleft palate, respectively, in the offspring. A hypocaloric diet, consisting of about 40% of *ad lib.* daily intake, given during middle 3rd of pregnancy, produced 5.6% cleft palate. The doses of cortisone plus the restricted diet caused 36.8 and 50.7% cleft palate, respectively. These results are discussed.

1. Fraser, F. C., Fainstat, T. D., *Pediatrics,* 1951, v8, 527.
2. Kalter, H., *Genetics,* 1954, v39, 185.
3. ———, *Am. J. Physiol.,* 1956, v185, 65.
4. ———, *J. Exp. Zool.,* 1957, v134, 449.
5. ———, *Plastic Reconstr. Surg.,* 1959, v24, 498.
6. ———, *Genetics,* 1957, v42, 380.
7. ———, *Canad. J. Biochem. Physiol.,* 1955, v33, 767.
8. Runner, M. N., Miller, J. R., *Anat. Rec.,* 1956, v124, 437.
9. Kalter, H., *Genetics,* 1954, v39, 975.

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