Erythrocytes from man, sheep, and goat, modified by O antigens from enterobacteriaceae and heterogenetic (staphylococcal) antigen, are readily agglutinated by the corresponding antibodies, and treatment of these erythrocytes with proteolytic enzymes only slightly enhances the agglutination reaction. (2) In contrast, similarly treated erythrocytes from alligator and ox are not agglutinated or agglutinated only in low titer; enzyme treatment markedly enhances the agglutination reaction. (3) Erythrocytes from all these animal species modified by Vi antigen are readily agglutinated by Vi antiserum in high titer. (4) Poorly agglutinable ox erythrocytes modified by O antigen are lysed in the presence of O antibody and guinea pig complement. (5) Vi antigen present together with O antigen on latex particles and erythrocytes significantly inhibits agglutination by O antibodies. (6) Erythrocytes modified by both O and Vi antigens effectively remove O antibodies.

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Effect of Adrenalectomy and Adrenocorticoid Replacement on Lactational Performance in Mice.*† (27436)

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The necessity of adrenocorticoids for maintenance of lactation has been demonstrated in rats(1,2,3), guinea pigs(4,5), and dogs (6). Studies by Gaunt(7,8,9) and Cowie (10,11,12,13) have been directed toward determining levels of various adrenocorticoids required for maintenance of normal lactation in adrenalectomized rats. A similar approach in determining adrenocortical requirements for lactation in mice has been lacking. This study was undertaken to determine levels of hydrocortisone and desoxycorticosterone required to support normal lactation in mice.

Materials and methods. Albino mice of Webster-Swiss strain were maintained on Purina lab chow pellets in an animal room with constant temperature of 78 ± 1°F. Females were bred in community pens with 6 females to one male per pen. When a female was observed to be in late pregnancy she was transferred to an individual litter cage. With the exception of normal control group, dams were adrenalectomized on day 4 of lactation and litter was reduced to 6 pups. In addition to a normal control group various treatments after adrenalectomy included 7 experimental groups as listed in Table I. Carrier consisted of 5 mg gum arabic per 100 mg adrenocorticosteroid brought to total volume with physiological saline.

Criteria of lactational performance included litter weights on days 4, 14, and 20 of lactation, mortality rates of pups on days 14 and 20 of lactation and in Groups a, g, and h

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TABLE I. Effect of Adrenalectomy (Day 4 of Lactation) and Corticoid Replacement on Lactational Performance in Mice.

Treatment group	No. of dams Day 4	Wt of dams, g Day 4	Wt of dams, g Day 14	Wt of dams, g Day 20	Litter wt, g Day 4	Litter wt, g Day 14	Litter wt, g Day 20	Milk production, g Day 14	Pup mortality, % Day 14	Pup mortality, % Day 20
a. Normal control	26	35.9 ± .7*	35.1 ± .9	34.9 ± .7	12.8 ±.3	35.4 ± 1.2	47.2 ± 1.4	2.29 ±.18	4	8
ADRX day 4 lactation										
b. No treatment	11	34.6 ± 1.7	35.3 ± 2.5 (4)§	38.3 ± 2.2 (3)	12.6 ±.9	21.3 ± 6.3‡ (4)	34.7 ±12.7† (3)		74	80
c. One inj. 100 μg HCA; 1% NaCl water	11	34.0 ± 1.5	34.4 ± 1.2 (9)	33.7 ± 1.5 (6)	13.2 ±.8	20.0 ± 2.3‡ (9)	19.7 ± 6.4‡ (6)		38	74
d. 100 μg DOCA	16	33.9 ± .9	34.1 ± 1.0(12)	35.7 ± 1.3(12)	12.2 ±.6	21.5 ± 2.4‡(12)	24.0 ± 3.8‡(12)		40	50
e. 100 μg HCA; 1% NaCl water	16	34.3 ± .8	32.7 ± .8(15)	33.4 ± 1.2(15)	12.7 ±.5	30.6 ± 1.1‡(15)	46.2 ± 2.6 (15)		13	16
f. 100 μg Pred.; 1% NaCl water	4	34.5 ± 2.3	35.0 ± 3.1	35.3 ± 2.5	13.2 ±.8	33.5 ± 1.3	42.8 ± 2.5		13	17
g. 100 μg HCA; 50 μg DOCA	16	35.1 ± .9	32.6 ± .8†	32.6 ± .8†	13.9 ±.5	35.3 ± 2.4	47.6 ± 4.1	1.83 ±.16	11	15
h. 250 μg HCA	14	34.0 ± .7	30.3 ± .8‡	29.6 ± 1.1‡	12.4 ±.4	31.2 ± 1.6†	42.1 ± 3.1	1.51 ±.22†	2	4

* Mean ± stand. error. † Significantly different from normal control ($P < .05$). ‡ Significantly different from normal control ($P < .01$).
§ Numbers in parentheses indicate No. of surviving litters. ADRX = Adrenalectomized. HCA = Hydrocortisone acetate. DOCA = Desoxycorticosterone acetate. Pred. = Prednisone.

milk production of dam after 10-hr isolation period on day 14 of lactation. This was determined by isolating dam and pups for 10 hr, weighing dam and pups before reuniting them, injecting one I.U. of oxytocin intraperitoneally into dam when pups began to suckle, allowing a suckling period of 30 min and weighing pups and dam again at end of this period. Differences in body weights of pups and dam before and after nursing were averaged to obtain estimate of milk production. Body weights were obtained to nearest 0.01 g on an O'Haus Centrogram Triple-Beam Balance (capacity 311 g).

Lactators were sacrificed on day 20 of lactation or on day when all pups in litter were dead. Animals showing adrenal fragments upon autopsy were eliminated from experiment.

Results. Eight of 11 adrenalectomized dams receiving no treatment were incapable of producing enough milk to support pups to day 20 of lactation (Table I). Only 4 dams had live pups by day 14 of lactation. Litter weights on days 14 and 20 were significantly less than normal ($P < .01$) and mortality of pups was 74% by day 14 and 80% by day 20.

Dams given one injection of 100 μg HCA immediately after adrenalectomy and maintained on 1% NaCl drinking water were unable to support lactation. Litter weights were significantly less than normal on days 14 and 20 of lactation ($P < .01$). Pup mortality in this group was only 38% on day 14 but 74% by day 20.

Treatment of 100 μg DOCA per day maintained low level of lactation. Litter weights were significantly less than normal on days 14 and 20 ($P < .01$), but pup mortality reached only 50% by day 20.

Injection of 100 μg HCA or Pred per day for glucocorticoid action and 1% NaCl drinking water for maintenance of electrolyte balance resulted in maintenance of normal level of lactation as judged by litter weights on days 14 and 20. However, pup mortality rates in these groups were slightly higher than in normal control.

When 100 μg HCA and 50 μg DOCA per day were administered simultaneously, litter weights on days 14 and 20 and milk produc-

tion on day 14 were not significantly different from normal. Mortality rate of pups by day 20 was slightly higher than normal, however. Significant losses in body weight of dams on days 14 and 20 resulted from this treatment ($P<.05$).

HCA at 250 μg per day in adrenalectomized rats supported maintenance of lactation which resulted in only 4% pup mortality by day 20. However, milk production and litter weight on day 14 were significantly less than normal ($P<.05$). This treatment caused significant reduction in body weight of dams on days 14 and 20 ($P<.01$).

Discussion. The inability of adrenalectomized mice to maintain lactation concurs with results reported in rats(9).

Discrepancies in the literature concerning ability of DOCA to support lactation in adrenalectomized rats(9,10) have not been clarified in present experiment. DOCA at 100 μg per day in mice was not able to support normal lactation but was better than 1% NaCl drinking water in this respect. Whether or not higher dosage would support lactation is not known.

Glucocorticoids, HCA or Pred, at level of 100 μg per day enabled maintenance of normal lactation when 1% NaCl drinking water was included as part of treatment. Group maintained on 1% NaCl drinking water only was unable to support lactation, indicating great importance of glucocorticoids in enabling mammary gland secretory cells to receive milk precursors for milk secretion.

Group receiving both HCA and DOCA was able to support normal lactation, indicating that 50 μg DOCA per day is sufficient to maintain favorable electrolyte balance in mice. However, body weight losses of dams in this group suggest that DOCA level used was slightly too low or HCA level was slightly too high.

Ability of adrenalectomized mice on 250 μg HCA only per day to support such a high rate of live pups to day 20 was surprising. This high level of glucocorticoid must have had ability to maintain favorable electrolyte balance in some way. Loss in body weight of dams on this treatment was expected since glucocorticoid activity of HCA was probably breaking down body protein at a high rate.

Data presented show importance of using several criteria for evaluating lactational performance in experimental animals. All criteria must be considered, as exemplified in group receiving 250 μg HCA per day. Although mortality of pups was lower than normal, weight of litters and dams and milk production on day 14 were not as high as normal control group indicating that this treatment did not support lactation equal to normal.

Summary. Hormone substitution therapy experiments involving adrenalectomized lactating Webster-Swiss albino mice suggest that normal daily adrenocorticosteroid secretion of lactating mice approximates 100 μg HCA per day or its equivalent in glucocorticoid activity and 50 μg DOCA per day or its equivalent in mineralocorticoid activity.

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