equivalents/g fresh weight (12 extracts). In addition, analysis of HPLC fractions covering a wide range of polarity did not show an accumulation of RIA-positive material in any particular fraction. The difficulty of determining the exact titer of ecdysteroids in the fresh fungus may be explained by the assumption that ecdysone is present in a conjugated form or in a precursor form. It may be speculated that these precursor forms are then converted into ecdysteroids inside the larvae, where they finally determine the reproductive mode.

1 Supported by the Swiss National Science Foundation, grants No. 3.218-0.77 to D.F.W. and 3.714-0.80 to B.L. We thank Xiang-Xiong Zhu for help with the RIA and Mrs G. Rhyner for technical assistance. Gifts of ecdysone antiserum from Dr J.D. O'Connor and of 23,24-[$^3H_2$]-ecdysone from Dr J. Koolman are also gratefully acknowledged.
2 Reprint requests to D.F.W., Institute for Animal Production, ETH-Zürich, CH-8092 Zürich (Switzerland).
3 Ulrich, H., Z. indukt. Abstamm.- VererbLehre *71* (1936) 1.
4 Camenzind, R., Revue suisse Zool. *69* (1962) 377.
5 Went, D.F., Int. J. Invertebr. Reprod. *1* (1979) 21.
6 Went, D.F., J. Insect Physiol. *24* (1978) 53.
7 Treiblmayr, K., Pohlhammer, K., Rieske, E., and Adam, H., Mikroskopie *38* (1981) 97.
8 Laverdure, A.-M., C.r. Acad. Sci. Paris *269* (1969) 82.
9 Laverdure, A.-M., Gen. comp. Endocr. *17* (1971) 467.
10 Beckemeyer, E.F., and Lea, A.O., Science *209* (1980) 819.
11 Redfern, C.P.F., J. Insect Physiol. *28* (1982) 97.
12 Went, D.F., Experientia *31* (1975) 1033.
13 Zhu, X.X., Gfeller, H., and Lanzrein, B., J. Insect Physiol. *29* (1983) 225.
14 Lafont, R., Pennetier, J.L., Andrianjafintrimo, M., Claret, J., Modde, J.F., and Blais, C., J. Chromat. *236* (1982) 137.
15 Borst, D.W., and O'Connor, J.D., Steroids *24* (1974) 637.
16 Horn, D.H.S., Wilkie, J.S., Sage, B., and O'Connor, J.D., J. Insect Physiol. *22* (1976) 901.
17 Garen, A., Kauvar, L., and Lepesant, J.-A., Proc. natl Acad. Sci. USA *74* (1977) 5099.
18 Kraminsky, G.P., Clark, W.C., Estelle, M.A., Gietz, R.D., Sage, B.A., O'Connor, J.D., and Hodgetts, R.B., Proc. natl Acad. Sci. USA *77* (1980) 4175.
19 Richards, G., Molec. cell. Endocr. *21* (1981) 181.
20 Wentworth, S.L., Roberts, B., and O'Connor, J.D., J. Insect Physiol. *27* (1981) 435.
21 Hsiao, T.H., and Hsiao, C., J. Insect Physiol. *23* (1977) 89.
22 Kaiser, P., Zool. Jb. Anat. *87* (1970) 377.
23 Went, D.F., Wilhelm Roux Arch. *170* (1972) 13.

0014-4754/84/090998-03$1.50 + 0.20/0


## Persistent inverse maternal effect on corticosterone production in vitro

P.R.W. Wood and J.G.M. Shire[1]

*Institute of Genetics, University of Glasgow, Glasgow (Scotland), and Department of Biology, University of Essex, Colchester CO4 3SQ (England), 18 July 1983*

*Summary.* Adrenal cells from C57BL/Tb mice produced more steroid than those from DBA/2J mice. Reciprocal differences between both backcross and F1 hybrids showed a persistent maternal effect. Mothers with high output produce offspring with reduced hormone production when adult. Corticosterone output thus depends on maternal phenotype as well as on the genotype of the isolated cells.
*Key words.* Adrenals; corticosterone; maternal effects; stress; inbred strains; mice.

C57BL/10J mice show a much greater rise in plasma corticosterone following stress than DBA/2J mice and have 7-fold greater stores of esterified cholesterol in their adrenals[2]. We have used enzymically dissociated suspensions of adrenal cells to investigate the synthesis of corticosterone so that factors intrinsic to the steroidogenic cells could be separated from genetic differences acting elsewhere[3]. To reveal their biosynthetic potential adrenal cells isolated from individual mice of the C57BL/Tb and DBA/2J strains and their hybrids were maximally stimulated with ACTH. All the mice were young adult males, as developmental changes in the juxtamedullary X-zone complicate adrenal structure in females[4], and were given the same standard diet[5,6].

Cells suspensions were made from the paired adrenals of individual mice by a modification of the method of Barofsky et al.[7]. Cells were dissociated enzymically first with 0.35% trypsin in Krebs-Ringer bicarbonate buffer containing 0.2% glucose (1 ml KRBG per adrenal pair) and then with 0.04% chromatographically purified collagenase in KRBG containing 4% bovine serum albumin (0.25 ml KRBG-BSA per adrenal pair). Both enzyme incubations also contained 12.5 µg/ml deoxyribonuclease and 50 µg/ml ribonuclease. The cells were mechanically dispersed by repeatedly drawing them into a Pasteur pipette, washed and aliquots counted and assayed for viability. Steroid production was stimulated by incubating about $3 \cdot 10^5$ viable cells in 0.605 ml of KRBG-BSA containing 17 ng (2.5 m units/ml) of porcine ACTH. All 3 incubation steps were carried out at 37 °C for 1 h in an atmosphere of 95% $O_2$ and 5% $CO_2$. All glassware was siliconized. Steroid production was stopped by adding 0.2 ml of cold 39% ethanol to each incubation and steroids were extracted with methylene chloride. Corticosterone was measured fluorimetrically in duplicate samples for each incubation[8]. The identity of corticosterone was established by chromatography in 3 thin-layer systems, before and after acetylation.

Cells from C57 mice produced significantly more ($p = 0.02$, 2-tailed Kolmogorov-Smirnov test) corticosterone that those from DBA males (table). Differences in overall mean between the backcross to DBA and the backcross to C57 were expected on the basis of segregation at a locus resembling *ald*, which controls cholesterol ester levels in crosses with AKR mice[9]. The striking reciprocal differences in all 3 hybrid generations were completely unexpected. The significant ($p = 0.02$, 2-tailed K-S test) difference between the reciprocal F1 males could have been caused by differences in either their sex-chromosome complement or by other differences in their parents[10]. Measurements on adrenal cells from individual mice showed significant differences ($p = 0.04$, 2-tailed K-S test) between the backcrosses of reciprocal F1 females to males of the C57 strain. Only maternal effects differed between these 2 backcrosses. Such effects were the only difference between the reciprocal backcrosses to DBA, whose adrenal cells also differed in their output of corticosterone. The maternal effect on the backcrosses cannot have been due to differences in their nuclear genotype for reciprocal F1 females have identical sets of autosomes and X-chromosomes. The difference between the 2 C57 backcrosses was 28% of their joint mean. The maternal effect could thus account for most or all of the difference be-

Corticosterone production by adrenal cells from individual C57, DBA and hybrid mice

| Stock | Number of mice | Corticosterone (pg/h/cell) ± SE | Chromosome origin X | Y | Autosomes | Paternal influence | Maternal influence |
|---|---|---|---|---|---|---|---|
| DBA | 9 | 2.18 ± 0.21 | DBA | DBA | DBA | DBA | DBA |
| C57 | 21 | 3.37 ± 0.19 | C57 | C57 | C57 | C57 | C57 |
| (DBA × C57)F1 | 16 | 3.83 ± 0.44 | DBA | C57 | Heterozygous | C57 | DBA |
| (C57 × DBA)F1 | 19 | 2.76 ± 0.14 | C57 | DBA | Heterozygous | DBA | C57 |
| (D × C)F1 × C57 | 23 | 2.69 ± 0.18 | DBA&C57 | C57 | Segregating | C57 | DBA × C57 |
| (C × D)F1 × C57 | 30 | 3.55 ± 0.28 | DBA&C57 | C57 | Segregating | C57 | C57 × DBA |
| (D × C)F1 × DBA | 31 | 1.85 ± 0.08 | DBA&C57 | DBA | Segregating | DBA | DBA × C57 |
| (C × D)F1 × DBA | 21 | 2.08 ± 0.10 | DBA&C57 | DBA | Segregating | DBA | C57 × DBA |

tween the reciprocal F1 males, which was 32% of their joint mean.

Offspring had a relatively low mean rate of hormone production when their mother came from a stock with a high production rate (e.g., C57 mothers and C × D offspring, D × C mothers and (D × C) × C57 backcross offspring). When the mother came from a stock with a low rate of corticosteroid production, such as DBA or C × D, then the offspring, D × C (C × D) × C57 mice respectively, had relatively high rates of production. Support for the existence of differences between reciprocal F1 females is provided by the differences in the rate and form of X-zone involution, which is related to adrenocortical activity[4], between reciprocal DBA × C57BL F1 hybrids[11]. Our suggestion of reciprocal differences in adrenocortical function in males is strengthened by the finding of significant reciprocal differences ($p = 0.02$, 2-tailed t-test) in the adrenal stores of esterified cholesterol in DBA/2J × C57BL/10J F1 hybrids[9] and in the adrenal lipid/cortex ration in DBA/2J × C57BL/6J F1 males[12]. In both males and females the adrenals of DBA/1J × C57BL/10J F1 mice were larger than those of the corresponding reciprocal F1 hybrids[13]. The character whose pattern of inheritance shows the inverse maternal effect is expressed in vitro by cells isolated from adults. The cortical cells must therefore have been influenced, directly or indirectly, by the adrenocortical function of their mother. The functional and metabolic characteristics of liver cells and individual nerve cells in adult mammals can be preprogrammed, or imprinted, by androgenic steroids acting neonatally or before birth[14]. Cross-fostering experiments will be needed to identify the time of action of the maternal effect described here but corticosteroids can be transmitted to offspring both in milk and transplacentally[15]. Both hyper- and hypofunction of the mother's adrenals have been shown to affect fetal adrenal activity[16], possibly by a modification of negative feedback sensitivity similar to that caused by neonatal administration of cortisol[17]. Alternatively the maternal effect could be more indirect, acting on the young through differences in maternal behavior, since handling weanlings has been shown to alter adrenocortical function profoundly[18]. However, crossfostering between the C57BL/10Bg and DBA/1Bg strains affected the body weight of the pups but not their behavior[19].

The inverse maternal effect may have effects on other systems for maternal stress, or the prenatal administration of hormones to the mother, has been shown to affect the behavior[20], amphetamine responsiveness[21], immune system[22] and plasma testosterone levels of offspring[23]. Such an effect in C57 mice, which have marked stress responses[2], might underlie, at least in part, the relative androgen deficiency of these mice[24]. Maternal effects on reproductive success lasted over 2 generations[25]. Comparison of individuals from different maternal environments, whether a consequence of genetic differences between parents or a result of animals being bred in differing environments[5,12], will introduce important interactions into investigations of homeostatic responses or adaptive behavior. This could give rise to increased phenotypic variation when measurements on individuals from different generations, or born at different times of year, are compared. In a family affected with dexamethasone-suppressible hyperaldosteronism the severity of symptoms shown by a boy, his mother and grandmother alternated from generation to generation[26]. This family, taken with other reports[27,28] of altered endocrine states in the children of hyperendocrine mothers, suggests that long-lasting maternal effects may also exist in man. Such effects would be important in modulating an individual's susceptibility or resistance to diseases associated with, or triggered by, stress. These include psychoses, hypertension and infectious and autoimmune disease[29]. Until the roles of nuclear and non-nuclear inheritance, and their interactions with environmental events, are fully understood the etiology of such diseases will be incomplete. The radical nature of many maternal effects suggest that this mode of inheritance may possess a real adaptive advantage in increasing phenotypic ranges and, particularly in animals with short lifecycles, preadapting offspring to the prevailing environment.

1 We thank Margaret MacKendrick for her help and the Medical Research Council for a research studentship for PRWW.

2 Levine, S., and Treiman, D., Endocrinology *75* (1964) 142.
3 Lindberg, M., Shire, J.G.M., Doering, C.H., Kessler, S., and Clayton, R.B., Endocrinology 90 (1972) 81.
4 Shire, J.G.M., and Spickett, S.G., Gen. comp. Endocr. *11* (1968) 355.
5 Shire, J.G.M., Horm. Metab. Res. *12* (1980) 117.
6 Miller, K.K., Teratology *15* (1977) 249.
7 Barofsky, A., Feinstein, M., and Halkerston, I., Exp. Cell Res. *79* (1973) 263.
8 Butte, J.C., and Noble, E.P., Acta endocr. *61* (1969) 678.
9 Doering, C.H., Shire, J.G.M., Kessler, K., and Clayton, R.B., Biochem. Genet. *8* (1973) 101.
10 Shrenker, P., and Maxson, S.C., Behav. Genet. *12* (1982) 429.
11 Daughaday, W., Cancer Res. *1* (1941) 883.
12 Wahlsten, D., and Anisman, H., Physiol. Behav. *16* (1975) 401.
13 Meckler, R.J., and Collins, R.L., J. Endocr. *31* (1965) 95.
14 De Moor, P., Verhoeren, P.G., and Heyns, W., Differentiation *1* (1973) 241.
15 Zarrow, M.X., Philpott, J.E., and Denenberg, V.H., Nature *226* (1970) 1058.
16 Klepac, R., and Milkovic, K., Biol. Neonate *36* (1979) 154.
17 Erskine, M.S., Geller, E., and Yuwiler, A., Acta endocr. *96* (1981) 252.
18 Levine, S., and Mullins, R.F., Science *152* (1966) 1585.
19 Maxson, S.C., and Trattner, A., Behav. Genet. *11* (1981) 153.
20 Joffe, J.M., in: Early Influences, p. 107. Ed. G. Gottlieb. Academic Press, New York 1978.
21 Jori, A., and Rutczynski, M., Psychopharmacology *59* (1978) 199.
22 Raveche, E.S., Tjio, J.H., and Steinberg, A.D., Arth. Rheum. *23* (1980) 48.
23 Ward, I.L., and Weisz, J., Science *207* (1980) 328.
24. Shire, J.G.M., Symp. zool. Soc. Lond. *47* (1981) 547.
25 Christian, J.J., and Lemunyan, C.B., Endocrinology *63* (1958) 517.
26 Ganguly, A., Grim, C.E., Bergstein, J., Brown, R.D., and Weinberger, M.H., J. clin. Endocr. Metab. *53* (1981) 1040.
27 Man, E.G., Shaver, B.A., and Cooke, R.E., Am. J. Obstet. Gynec. *75* (1958) 728.
28 Herbst, A.L., N. Engl. J. Med. *287* (1972) 1259.
29 Elliott, G.R., and Eisdorfer, C., Stress and Human Health. Springer Publ., New York 1982.

0014-4754/84/091000-02$1.50 + 0.20/0