by a biological assay procedure. The appropriate test compound was dissolved in propylene glycol in a series of concentrations which were administered orally to day-old male white Pekin ducklings. Each animal received 0.1 ml of solution daily for 5 consecutive days. Surviving animals were killed by decapitation on the 8th day and their livers were removed, weighed, and fixed in formalin. Frozen sections stained with hematoxylin and eosin were subjected to histologic evaluation. The criteria of toxicity were reduction in growth and liver size and the extent of bile duct hyperplasia characteristic of the field syndrome (*1*), which has proved to be a consistent and reproducible response to these toxic compounds.

The doses were selected to provide a range in intensity of the response from minimal to severe bile-duct hyperplasia, without extensive mortality. The range of 5-day total doses was 2.0 to 15.7 μg for aflatoxin B_1 and 50 to 200 μg for the derivative. The results of the assays are shown in Table 1.

These data indicate that both compounds have toxic properties manifested by depression in growth, reduction of liver weight, and histopathologic lesions in the liver. The intensity of the toxic response is related to dose in all instances. Clearly, however, the biological potency of aflatoxin B_2 is markedly reduced by comparison with aflatoxin B_1. It may be presumed, therefore, that the point of unsaturation in the B_1 compound,—absent in the B_2—is an important contributing factor to the potency of aflatoxin B_1 (*6*).

SEA BONG CHANG, M. M. ABDEL KADER
EMILY L. WICK, GERALD N. WOGAN
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge

References and Notes

1. R. Allcroft and R. B. A. Carnaghan, *Chem. Ind. (London)* **1963**, 50 (1963).
2. T. Asao, G. Buchi, M. M. Abdel Kader, S. B. Chang, E. L. Wick, G. N. Wogan, *J. Am. Chem. Soc.* **85**, 1706 (1963).
3. P. C. Spensley, *Endeavour* **22**, 75 (1963).
4. D. A. van Dorp, A. S. M. van der Zijden, R. K. Beerthuis, S. Sparreboem, W. O. Ord, K. de Jong, R. Kenning, *Rec. Trav. Chim.*, in press.
5. R. D. Hartley, B. F. Nesbitt, J. O'Kelly, *Nature* **198**, 1056 (1963).
6. Contribution No. 564 from the Department of Nutrition and Food Science, Massachusets Institute of Technology, Cambridge. We thank Profs. G. Buchi and P. M. Newberne for help in the chemical and histopathological aspects, respectively, of this work. Supported by the National Cancer Institute.

26 August 1963

Behavior of Adult Rats Is Modified by the Experiences Their Mothers Had as Infants

Abstract. *Some rat pups were handled for 20 days in infancy, while others were not. When the rats reached adulthood the females were bred. Some of the offspring were left with their natural mothers, others were fostered to mothers of the same background (handled/nonhandled) as that of their natural mothers, while still others were fostered to mothers with a different background from that of their natural mothers. The offspring were weaned and weighed at 21 days; at 50 days, activity and defecation scores were obtained in the open field. The weights at weaning and the defecation scores at 50 days were significantly influenced by the experience in infancy of the "postnatal" mother, whether she was the natural mother or a foster mother. The natural mother and the foster mother jointly affected the open-field activity of the offspring.*

Handling rats in infancy has marked effects upon their subsequent behavioral and physiological processes (*1*). To date no one has investigated whether the handling of female rats in infancy affects their offspring. Modifications of the offspring's characteristics could occur during their fetal period, as a result of physiological changes induced in the mother by the handling she had received in infancy, or they could occur after birth as a result of either physiological changes (which could, for example, modify milk supply) or behavioral changes induced in the mother by the handling she had received in infancy. We now report results of such an investigation (*2*).

About 45 litters of Purdue-Wistar rats were handled in infancy. Handling consisted of removing a complete litter from the home cage (leaving the mother in the cage), placing the pups on shavings in a can for 3 minutes, and then returning the pups to their home cage. This was done once a day from day 1 through day 20 of life. About 45 other litters were not disturbed during this time. Once these litters were born the shavings in their cages were never changed; food and water were supplied without opening the cages.

At 21 days the handled and nonhandled litters were weaned, and the females were placed in specially designated cages. When mature, the females were bred to a random sample of colony males; the males were systematically moved from one cage to another and were exposed equally often to handled and nonhandled females.

When pregnant, the females were placed in stainless-steel maternity cages. The day after birth all litters were sexed, and those containing more than eight pups were reduced to four of each sex when possible, but never to less than two of one sex. No litter containing less than seven pups was used. The litters were then returned to their natural mothers. At this time (i) some litters were left with their natural mothers, (ii) other litters were fostered to mothers that had had the same experience (handled or not handled) in infancy as the natural mothers, and (iii) still other litters were fostered to mothers that in infancy had received the treatment opposite to that of the natural mothers. Fostering was done by moving the mothers from one cage to another, leaving the pups in the cage in which they had been born. In most instances fostering took place between litters born on the same day; in six cases the foster mother had given birth 1 day earlier than the natural mother, and in one case the foster mother had given birth 3 days earlier than the natural mother. Except for fostering, the litters were not disturbed. At 21 days the pups from 55 litters were weaned, weighed, sexed, earpunched, and placed in laboratory cages with littermates of the same sex.

Starting at 50 days of age, the animals were given 4 days of open-field testing. The field was 45 inches (115 cm) square, painted flat black, with walls 18 inches (46 cm) high. The floor was marked off in 9-inch (23-cm) squares by thin white lines. A rat was placed in one corner of the field, and its behavior was observed for 3 minutes. Total numbers of squares entered and boluses defecated were recorded. Two males and two females from each of 47 litters were tested in the open field. Testing was completed at 53 days, at

which time the animals were again weighed.

The body weights at 21 days are summarized in Table 1. The weights of all animals within a litter were averaged to give one mean litter weight, thus yielding a Between Litter Error Mean Square based upon 49 degrees of freedom. To determine whether fostering had any effect, the two nonfostered groups (A and D) were compared with the two groups in which litters were fostered to mothers with the same infantile experiences as the natural mothers (groups B and E). No significant effects were found. Therefore, the fostering variable was ignored in assessing the prenatal and postnatal contributions of the mothers toward the body weight of the offspring. The analysis of these data found that the postnatal factor was significant ($F = 5.08$, $p < .05$). Young reared by mothers that had been handled in infancy (groups C, D, and E) weighed significantly more than pups reared by mothers that had not been handled in infancy (groups A, B, and F). The only significant difference among the body weights in adulthood was between males and females.

The data for open-field behavior are summarized in Table 1. In the analysis of variance the scores of the 4 animals within each litter were combined to give one litter score, thus yielding a Between Litter Error Mean Square based upon 41 degrees of freedom. To evaluate the sex variable and all interactions with this variable, the male and female means were obtained separately for each litter, and the Between Litter $\times$ Sex Error Mean Square (df = 41) was used in the denominator of these F tests.

When the two nonfostered groups (A and D) were compared with the two groups in which fostering was done between mothers with the same experience in infancy (B and E), a significant interaction ($F = 4.58$, $p < .05$) was obtained between the presence or absence of fostering and the mother's experience in infancy; those young which were born of and reared by nonhandled mothers and which were not fostered (group A), were significantly more active than the other three groups. Therefore, the four fostered groups were used to evaluate the prenatal and postnatal contributions of the mothers toward the activity pattern of the offspring. A significant interaction ($F = 4.81$, $p < .05$) was found between the prenatal and postnatal mothers. Young born of mothers that had not been handled in infancy, and fostered to handled mothers (group C), were significantly more active than young from the other three groups (B, E, and F); the next most active group was the complement of this—rats born of handled mothers and reared by nonhandled mothers (group F). The sex variable was significant ($F = 6.87$, $p < .05$) but did not interact significantly with any of the maternal variables.

Table 1. Mean body weight, in grams, at 21 days, mean number of squares entered, and mean number of boluses defecated during 4 days of open-field testing. The N per mean is given in parentheses.

Group	Experience of natural mothers in infancy	Postnatal fostering of pups	21-days body wt.	Squares entered		Boluses	
				Male	Female	Male	Female
A	Not handled	Not fostered	35.87 (69)	75.69 (16)	118.62 (16)	7.12 (16)	4.19 (16)
B	Not handled	Fostered to nonhandled mother	36.93 (62)	47.00 (16)	67.94 (16)	6.37 (16)	4.37 (16)
C	Not handled	Fostered to handled mother	41.29 (62)	90.56 (16)	97.75 (16)	12.31 (16)	5.56 (16)
D	Handled	Not fostered	40.81 (70)	53.06 (16)	58.69 (16)	10.87 (16)	7.81 (16)
E	Handled	Fostered to handled mother	38.87 (56)	50.64 (14)	70.93 (14)	10.50 (14)	12.21 (14)
F	Handled	Fostered to nonhandled mother	38.24 (101)	53.69 (16)	85.94 (16)	6.87 (16)	5.37 (16)

The test evaluating the fostering effect found that offspring born of and raised by mothers that had been handled in infancy (groups D and E) defecated significantly more ($F = 7.69$, $p < .01$) than offspring born of and raised by nonhandled control mothers (groups A and B); the procedure of fostering did not have any significant effect. The test separating the prenatal and the postnatal factors found that the postnatal mother was the significant contributor: young raised by mothers that had been handled in infancy (groups C, D, and E) defecated more ($F = 6.62$, $p < .05$) than young raised by mothers that had not been handled in infancy (A, B, and F). Again the sex factor was significant ($F = 9.15$, $p < .01$). Though sex did not interact significantly with any of the maternal variables, the Sex $\times$ Prenatal Mother interaction approached significance ($F = 3.49$, $p < .07$).

The results clearly establish that the experiences which the mother received while an infant were profound enough to modify her offsprings' body weight at weaning and open-field behavior in adulthood. These modifications were mediated through both the prenatal mother-fetus relationship and the postnatal mother-young interaction. The generality of this phenomenon has been confirmed by Grota (*3*), who extra-uterized fetuses (that is, removed the fetuses from the uterus but left them in the body cavity) of rat mothers which had been handled or not handled in infancy. Grota found that, for handled mothers, fetuses reared in the uterus had a higher survival rate between birth (by means of cesarean delivery) and weaning than those reared in the body cavity; extra-uterization of fetuses in nonhandled mothers did not affect postnatal survival rate. In addition, Grota found that both the prenatal and postnatal mother contributed significantly to the weaning weights of pups delivered by cesarean section.

VICTOR H. DENENBERG*
ARTHUR E. WHIMBEY†
Department of Psychology, Purdue University, Lafayette, Indiana

References and Notes

1. V. H. Denenberg, *J. Comp. Physiol. Psychol.* **55**, 813 (1962); ——— and G. G. Karas, *Psychol. Rept.* **7**, 313 (1960); *J. Comp. Physiol. Psychol.* **54**, 685 (1961); S. Levine, *J. Personality* **25**, 70 (195➡ *Science* **135**, 795 (1962); J. R. C. Morton, V. H. Denenberg, M. X. Zarrow, *Endocrinology* **72**, 439 (1963); J. T. Tapp and H. Markowitz, *Science* **140**, 486 (1963). For a general review of much of this work, see V. H. Denenberg, in *The Behaviour of Domestic Animals*, E. S. E. Hafez, Ed. (Bailliere, Tindall and Cox, London, 1962), pp. 109–138.
2. Research was supported in part by a grant from the National Science Foundation.
3. L. J. Grota, thesis, Purdue University (1963).
* On sabbatical leave, 1963–64, at Subdepartment of Animal Behaviour, Cambridge University, Cambridge, England.
† Cooperative fellow of the National Science Foundation.

30 August 1963