Bull. Environm. Contam. Toxicol. 29, 146–152 (1982)

Variations with Age in the Adrenocortical Responses of Mallard Ducks (*Anas platyrhynchos*) Consuming Petroleum-contaminated Food

J. Gorsline and W. N. Holmes

Department of Biological Sciences and The Marine Science Institute, University of California, Santa Barbara, CA 93106

There is good evidence that adrenocortical function may become severely impaired in birds consuming petroleum-contaminated food; as a result, contaminated birds may be less able to withstand the effects of some environmental stresses (HOLMES et al. 1979, HOLMES & GORSLINE 1980, GORSLINE & HOLMES 1981, HARVEY et al. 1981, RATTNER & EASTIN 1981, HOLMES et al. 1982). Most of these studies have been conducted on ducks and in each instance declines in peripheral plasma corticosterone concentrations have characterized their responses to the ingested petroleum. Indeed, in some cases declines have been reported to occur during the first two days of exposure to contaminated food and levels of contamination as low as 0.15 ml crude oil per 100 g dry food have been found to be effective in this regard (GORSLINE & HOLMES 1981, RATTNER & EASTIN 1981). More recently, studies *in vivo* and *in vitro* have confirmed that the petroleum-induced decreases in plasma corticosterone concentration reflect diminished adrenocortical activities, due primarily to a suppression of corticotropic responsiveness among cells in the inner zone of the adrenal gland (GORSLINE & HOLMES 1982).

When ingested, different crude oils seem to produce qualitatively similar effects on plasma hormone concentrations, although the magnitude of the responses evoked in laboratory-maintained ducks have varied considerably (HOLMES & GORSLINE 1980, GORSLINE & HOLMES 1981, RATTNER 1981, HARVEY et al. 1981, RATTNER & EASTIN 1981, HOLMES et al. 1982). Many factors may have been responsible for this variability. For example, the chemical composition of the crude oil and the environmental conditions under which the birds were maintained could each have contributed to these quantitative differences in response. Also, the magnitude of the perceived response may vary with the time of day when blood samples were taken and hormone concentrations were compared (GORSLINE & HOLMES 1981).

It is of particular interest that the extent of the reported decreases in adrenocortical function, even in response to ingesting a particular crude oil, may also differ between birds exposed to contaminated food for only a few days and those that have consumed the same food for several months (GORSLINE & HOLMES 1981, RATTNER & EASTIN 1981). In these instances, it is impossible to determine the exact reason for the differences in evoked change; they may have been due primarily to the duration of exposure or they could have reflected modifications in response due to ageing. To partially resolve this problem, we have examined the peripheral plasma corticosterone concentrations in mallard ducks of different age-classes exposed for the same short period to food contaminated with the same concentration of a specific crude oil. At the same time, the effects of this crude oil on hepatic mixed function oxidase activity have been used to assess the levels of petroleum contaminants that appear to have become distributed systemically in these birds.

0007-4861/82/0029-0146 $01.40

MATERIALS AND METHODS

Animal maintenance

Male mallard ducks *(Anas platyrhynchos)*, hatched from eggs purchased commercially (Whistling Wings, Hanover, IL), were raised and maintained at 20°C and exposed to a constant photoperiod of 6 h light and 18 h darkness. All birds were housed individually in wire mesh cages within full view of each other.

Experimental protocol

On day 1 of the 10-day experimental period, birds in three different age-classes were given an *ad libitum* supply of mash (Big Feeder Layer Kracketts, Perris, CA) containing 3 ml South Louisiana crude oil (American Petroleum Institute, Reference oil II) per 100 g dry food. Samples of contaminated food were prepared daily and all birds were fed at the beginning of the light phase (08.00 h). Food remaining from the previous day was removed and weighed to estimate daily food and petroleum consumption. At the same time, control birds in each age-class were treated in exactly the same manner except they were given *ad libitum* supplies of uncontaminated food.

On the eleventh day, control and experimental birds were weighed and sacrificed by decapitation; this was done within two min of capture and at the time of day corresponding to the primary diurnal peak in plasma corticosterone concentration, i.e. during the first hour of the light phase (GORSLINE & HOLMES 1981). Blood samples were collected in heparinized beakers, immediately centrifuged at 12,300g for 5 min at 5°C and the plasma was frozen at -20°C. The liver was removed, rinsed in ice-cold sucrose, blotted dry, weighed and frozen at -20°C.

Corticosterone analysis

Plasma samples were thawed at room temperature, extracted with 10 vol of ether and corticosterone concentrations were determined by radioimmune assay (NISHIDA et al. 1976, GORSLINE & HOLMES 1981). The corticosterone antiserum was produced in rabbits immunized with corticosterone-21-thyroglobulin (Miles Laboratories, Inc, Elkhart, IN). The assay was sensitive to approximately 16 pg corticosterone per assay tube and the intra- and inter-assay variations were 5.5% and 10.3%, respectively.

Estimation of hepatic mixed function oxidase activity

Microsomal fractions were prepared from liver homogenates by centrifugation of the post-mitochondrial supernatant at 105,000g for 60 min. Protein concentrations of microsomal preparations were determined by the Biuret method using bovine serum albumin in 0.25M sucrose as a standard (GORNALL et al. 1949). Hepatic MFO activities of microsomal preparations were assayed in terms of their naphthalene-metabolizing properties *in vitro* (NILSSON et al. 1976, GORSLINE et al. 1981). Specific activity was expressed in nmol naphthalene metabolized per min of incubation per mg microsomal protein. Total hepatic MFO activity was estimated from the product of the specific activity (nmol naphthalene per min per mg protein), the microsomal protein concentration (mg per g liver) and the relative liver weight (g per 100 g body weight) and was expressed in nmol naphthalene metabolized per min per 100 g body weight.

Statistics

Mean values were expressed ± standard error of the mean (SEM). The analysis of variance (ANOVA) followed by Duncan's multiple range test was used to identify differences with age among the groups of control and experimental birds. Mean values derived from the control and experimental groups of birds within each age-class were compared by the Student "t" test.

RESULTS

Body weights and daily intakes (Table 1)

The birds in each age-group, whether consuming either uncontaminated or contaminated food, showed no significant changes in body weight during the ten-day experimental period. Also, no significant differences were detected between the rates of food consumption by birds in the various age-groups and similar volumes of crude oil were ingested each day by those birds consuming petroleum-contaminated food.

TABLE 1

The effects of consuming petroleum-contaminated food on body weights and daily intakes of food and crude oil by three age-classes of male mallard ducks. The birds had consumed food contaminated with 3% (v/w) South Louisiana crude oil for ten days.

Treatment (n)	Body Weight (g)		Daily Intake (g or ml•kg^{-1} body weight)	
	Initial	Terminal	Food	Crude Oil
3-4 months				
Uncontaminated (10)	1013 ±25.6	1032 ±30.4	71.4 ±2.93	0
Contaminated (14)	1030 ±34.2	1119 ±21.4	80.4 ±7.95	2.48
6-7 months				
Uncontaminated (10)	1003 ±31.6	1026 ±29.6	75.6 ±1.98	0
Contaminated (10)	1009 ±25.4	968 ±27.4	82.5 ±8.13	2.47
8-9 months				
Uncontaminated (10)	999 ±29.2	1058 ±35.2	72.4 ±2.45	0
Contaminated (10)	1031 ±30.4	1046 ±24.2	81.9 ±6.40	2.46

Hepatic naphthalene-metabolizing activities (Table 2)

Relative liver weights among the contaminated birds of the two youngest age-groups were similar to those of the uncontaminated birds of the same age, but birds in the oldest group (8-9 months) showed a significant increase in their liver weight after exposure to similarly contaminated food. Microsomal protein concentrations, however, were the same among the contaminated and the uncontaminated birds in each age-class. Significant increases in both the specific and the total hepatic enzyme activity were observed among the birds in each age-group that had consumed the petroleum-contaminated food.

TABLE 2

The effects of consuming petroleum-contaminated food on liver composition and hepatic naphthalene-metabolizing activity in three age-classes of male mallard ducks. The birds had consumed food contaminated with 3% (v/w) South Louisiana crude oil for ten days.

	Liver Composition		Naphthalene Metabolism[1]	
Treatment (n)	Relative Weight ($g \cdot 100g^{-1}$ BW)	Microsomal Protein ($mg \cdot g^{-1}$ liver)	Specific Activity	Total Hepatic Activity
3-4 months				
Uncontaminated (10)	2.25 ±0.137	21.4 ±2.84	0.35 ±0.115	13.5 ±1.82
Contaminated (14)	2.15 ±0.105	21.1 ±2.68	1.80*** ±0.286	59.5*** ±4.78
6-7 months				
Uncontaminated (10)	1.82 ±0.134	20.8 ±2.59	0.29 ±0.054	9.8 ±1.50
Contaminated (10)	2.05 ±0.119	25.6 ±2.59	1.10*** ±0.148	53.3*** ±4.97
8-9 months				
Uncontaminated (10)	1.79 ±0.088	19.8 ±2.64	0.36 ±0.030	12.0 ±1.72
Contaminated (10)	2.46*** ±0.139	24.1 ±2.16	1.00*** ±0.079	58.6*** ±3.21

[1]Specific activity expressed in nmol naphthalene metabolized per min per mg microsomal protein and total hepatic activity expressed in nmol naphthalene metabolized per min per 100g body weight.
*** = $p<0.001$ with respect to corresponding control values.

Adrenal weights and plasma corticosterone concentrations (Table 3)

The relative weights of contaminated birds in each age-group were similar to those found in the corresponding age-group of uncontaminated birds. Also, no changes in peripheral plasma corticosterone concentration occurred with ageing among the control birds. Significant decreases in plasma corticosterone concentrations occurred in each of the groups of contaminated birds, but the magnitude of this decline was greatest in the youngest birds and least in the oldest; the youngest birds suffered over a 10-fold decrease in plasma hormone concentration, while birds of intermediate age showed approximately a 3.6-fold decrease and the oldest birds showed only a 1.8-fold decline.

TABLE 3

The effects of consuming petroleum-contaminated food on the relative adrenal weights and peripheral plasma corticosterone concentrations in three age-classes of male mallard ducks. The birds had consumed food contaminated with 3% (v/w) South Louisiana crude oil for ten days.

Treatment (n)	Adrenal Weight ($mg \cdot 100g^{-1}$ BW)	Plasma Corticosterone ($ng \cdot ml^{-1}$)
3-4 months		
Uncontaminated (10)	7.23	13.5
	±0.413	±2.82
Contaminated (14)	8.19	1.20***a
	±0.356	±0.43
6-7 months		
Uncontaminated (10)	8.20	21.9
	±0.424	±1.395
Contaminated (10)	7.56	6.08***a
	±0.366	±0.622
8-9 months		
Uncontaminated	7.76	20.10
	±0.340	±2.516
Contaminated	8.77	11.17***a
	±0.383	±1.147

*** = $p<0.001$ with respect to corresponding control value and means followed by the same letter are significantly different from each other with $p<0.05$.

DISCUSSION

The similar levels of total hepatic MFO activity induced by the similar volumes of crude oil consumed by the individuals in each age-class strongly suggests that the declines in plasma corticosterone concentration occurred in response to similar levels of systemic contamination. Furthermore, since the decreases were larger in the younger birds than in the older birds, age seems to have been an important factor determining the degree of hypoadrenocorticalism developed following their exposure to contaminated food.

There is reason to believe that resting levels of adrenocortical activity and the responsiveness of the adrenal cortex to ACTH may change with age. For example, the corticosterone secretory rates in ducks decrease significantly as the birds grow older (HOLMES & KELLY 1976). Also, in response to a given level of corticotropic stimulation, the mammalian adrenal cortex seems to secrete less corticosteroid in older than in younger animals (RIEGLE & NELLOR 1967, HESS 1970, PRITCHETT et al. 1979). It is possible, therefore, that such age-related changes in adrenocortical responsiveness to ACTH may have determined the degree of hypoadrenocorticalism induced in birds consuming petroleum-contaminated food. Indeed, it is the corticotropic responsiveness of the adrenal cortex that appears to be most sensitive to the presence of many circulating contaminants, particularly hydrocarbons (HOLMES & GORSLINE 1980, GORSLINE & HOLMES 1982).

Other specific changes with age, such as sexual maturation, may have also contributed to the different responses observed in the older and younger birds. Although the testes remained regressed in birds up to 7 months of age, the constraints of the short photoperiod were not completely sustained in the oldest birds and some testicular differentiation had started to occur. Changes in plasma androgen concentrations, therefore, may have futher influenced the response of the oldest birds to the petroleum contaminants.

Whatever the mechanism, the age of the bird appears to be an important factor in determining the degree of hypoadrenocorticalism that develops following exposure to petroleum-contaminated food. Even in the oldest birds, however, the magnitude of the apparent decreases in adrenocortical activity would probably still have been sufficient to render them vulnerable to the effects of environmental stressors.

ACKNOWLEDGEMENTS

This research was supported by funds awarded to W.N. Holmes by the National Science Foundation, Washington, D.C. (Grant No. PCM 80-07865).

REFERENCES

GORNALL, A.G., BARDAWILL, C.J., AND M.M. DAVID: J. Biol. Chem. 177, 751 (1949).

GORSLINE, J., and W.N. HOLMES: Arch. Environ. Contam. Toxicol. 10, 765 (1981).

GORSLINE, J., and W.N. HOLMES: Arch. Environ. Contam. Toxicol. 11, in press (1982).

GORSLINE, J., HOLMES, W.N., and J. CRONSHAW: Environ. Res. 24, 377 (1981).

HARVEY S., KLANDORF, H., and J.G. PHILLIPS: Gen. Comp. Endocrinol. 45, 372 (1981).
HESS, G.D., and G.D. RIEGLE: J. Geront. 25, 354 (1970).
HOLMES, W.N., and J. GORSLINE: In, Endocrinology 1980, "Proceedings of the VIth International Congress of Endocrinology, Canberra, Aust. Acad. Sci., p.311 (1980).
HOLMES, W.N., and M.E. KELLEY: Pflugers Arch. 365, 145 (1980).
HOLMES, W.N., CAVANAUGH, K.P., and J. GORSLINE: In Proceedings Ninth International Symposium on Comparative Endocrinology, Hong Kong, University of Hong Kong Press, in press (1982).
HOLMES, W.N., GORSLINE, J., and J. CRONSHAW: Environ. Res. 20, 425 (1979).
NILSSON, R., PETERSON, E., and G. DALLNER: Anal. Biochem. 70, 209 (1976).
NISHIDA, S., MATSUMURA, S., HORINO, M., OYAMA, H., and A. TENKY: Endocrinol. Jpn. 23, 465 (1976).
PRITCHETT, J.F., SARTIN, J.L., MARPLE, W.L., HARPER, W.L., and M.L. TILL: Hormone Res. 10, 96 (1979).
RATTNER, B.A.: Toxicol. Lett. 8, 337 (1981).
RATTNER, B.A., and W.C. EASTIN: Comp. Biochem. Physiol. 68, 103 (1981).
RIEGLE, G.D., and J.E. NELLOR: J. Geront. 22, 83 (1967).

Accepted June 19, 1982