gravity has an active and direct role in influencing larval biology in at least *B. stolonifera*, but that fundamental differences in responses to environmental cues exist between it and its congener *B. neritina*.

ANTHONY PIRES*
ROBERT M. WOOLLACOTT
Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

References and Notes

1. Although geotaxis in a restricted sense means an active oriented response to gravity, the term is sometimes used to describe any light-independent vertical movement in the water column, including responses to environmental cues such as temperature gradients or geomagnetic fields. For convenience we shall adopt the broader definition of geotaxis and use "gravity response" to signify geotaxis in the restricted sense. Thus, barokinesis, or modulation of swimming rate by changes in hydrostatic pressure, coupled with an active or passive vertical orientation is considered an apparent geotaxis although it is mediated only indirectly by gravity.
2. Ctenphore cydippids [A. Agassiz, *Am. Acad. Arts Sci. Mem.* **10**, 357 (1874)]; veligers of gastropods [F.-S. Chia, R. Koss, L. R. Bickell, *Cell Tissue Res.* **214**, 67 (1981)]; pediveligers of bivalves [S. M. Cragg and J. A. Nott, *J. Exp. Mar. Biol. Ecol.* **27**, 23 (1977)]; decapod megalops [C. W. Prentiss, *Bull. Mus. Comp. Zool. Harv. Univ.* **36**, 167 (1901)]; larvae of inarticulate brachiopods [S. Chaung, *Am. Zool.* **17**, 39 (1977)]; and tadpoles of ascidians [R. M. Eakin and A. Kuda, *Z. Zellforsch. Mikrosk. Anat.* **112**, 287 (1971)].
3. Swimming of bivalve veligers or pediveligers [B. L. Bayne, *Nature (London)* **198**, 406 (1963); S. M. Cragg, *J. Mar. Biol. Assoc. U.K.* **60**, 551 (1980); ——— and L. L. D. Gruffydd, in *Proceedings of the Ninth European Marine Biology Symposium*, H. Barnes, Ed. (Univ. of Aberdeen Press, Aberdeen, 1975), pp. 43–57]; crab larvae [A. C. Hardy and R. Brainbridge, *Nature (London)* **167**, 354 (1951); D. Wheeler and C. E. Epifanio, *Mar. Biol.* **46**, 167 (1978); S. D. Sulkin, *J. Exp. Mar. Biol. Ecol.* **13**, 73 (1973)]; ascidian tadpoles [D. J. Crisp and A. F. A. A. Ghobashy, in *Proceedings of the Fourth European Marine Biology Symposium*, D. J. Crisp, Ed. (Cambridge Univ. Press, Cambridge, 1971), pp. 443–465].
4. R. L. Zimmer and R. M. Woollacott, in *Biology of Bryozoans*, R. M. Woollacott and R. L. Zimmer, Eds. (Academic Press, New York, 1977), p. 57.
5. See J. S. Ryland, in *ibid.*, p. 411.
6. Colonies of *B. neritina* were collected in the vicinity of Los Angeles, Calif., in April 1982 and shipped to the Museum of Comparative Zoology where they were maintained at 12°C in tubs of aerated seawater from off Nahant, Mass. Colonies of *B. stolonifera* were collected from Woods Hole and Onset, Mass., in August 1982 and maintained at 20°C in tubs of aerated seawater from the collection sites. Larvae were obtained by illuminating the colonies with a 500 W photolamp; colonies were kept in darkness at all other times. Experiments on *B. neritina* larvae were conducted at 12°C in the seawater from off Nahant and those on *B. stolonifera* larvae were conducted at 20°C in water from the collection sites.
7. W. F. Lynch, *Biol. Bull. (Woods Hole, Mass.)* **92**, 115 (1947).
8. K. Banse, *Prog. Oceanogr.* **2**, 53 (1964).
9. We are grateful to C. B. Calloway and E. Arbas for helpful discussions about experimental design and to L. Brooks for advice on appropriate statistical tests. C. Phillips prepared Fig. 1. K. J. Carle, B. K. Holldobler, J. J. McCarthy, and N. A. Welschmeyer provided important criticisms of the manuscript. Supported by ONR contract N00014-78-C-0064 with Harvard University.
* Present address: Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

7 September 1982; revised 11 November 1982

Social Stress and Atherosclerosis in Normocholesterolemic Monkeys

Abstract. *Socially stressed adult male cynomolgus monkeys (*Macaca fascicularis*) fed a low fat, low cholesterol diet developed more extensive coronary artery atherosclerosis than unstressed controls. Groups did not differ in serum lipids, blood pressure, serum glucose, or ponderosity. These results suggest that psychosocial factors may influence atherogenesis in the absence of elevated serum lipids. Psychosocial factors thus may help explain the presence of coronary artery disease (occasionally severe) in people with low or normal serum lipids and normal values for the other "traditional" risk factors.*

The initiation and progression of coronary artery atherosclerosis is often associated with increased concentrations of lipids in the serum (*1*). Despite this association, many individuals develop severe atherosclerotic lesions while having low serum lipid concentrations, and others develop far more atherosclerosis than would be expected on the basis of a modest elevation of serum lipids (*2*). Work with animal models suggests that some of this variability may be explained by the influence of hypertension and immunologic injury to arteries (*3, 4*). Yet, much additional variability in atherosclerosis lesion extent remains unexplained, suggesting the existence of other pathogenetic mechanisms among normocholesterolemic individuals. In recent years, psychosocial variables have been linked increasingly to ischemic heart disease in human beings (*5*) and psychosocial manipulations have been shown to exacerbate atherosclerosis in cholesterol-fed cynomolgus monkeys, rabbits, and swine (*6–8*). At present, though, it is unclear whether psychosocial manipulations are capable of promoting atherogenesis in normocholesterolemic animals and, by implication, in human beings with low or normal serum cholesterol concentrations. The purpose of the present investigation was to provide an initial test of this hypothesis. Our results demonstrate that socially stressed monkeys fed a low fat, low cholesterol diet developed more extensive intimal lesions in the coronary arteries than control animals living under unstressed conditions. Moreover, the differences in lesion extent observed here were not associated with elevations or group differences in serum lipids, blood pressure, serum glucose, or ponderosity.

The experimental animals were 30 male, cynomolgus monkeys (*Macaca fascicularis*), imported as adults from Malaysia and the Philippine Islands. They were assigned to two experimental conditions (designated the "stressed" and "unstressed" conditions), and within each condition ($N = 15$), the monkeys were divided randomly into three, five-member groups. During the study, all groups were housed separately in identical pens measuring 2.0 by 3.2 by 2.5 m. The experiment lasted 21 months, after which all animals were killed and necropsied.

Throughout the study the monkeys were fed a "prudent" diet, modeled on the current recommendations of the American Heart Association; this diet contained almost no cholesterol (0.05 mg of cholesterol per calorie) and was low in saturated fats (*9*). Blood samples for determination of total serum cholesterol and high-density lipoprotein cholesterol (HDLC) concentrations were taken approximately once per month over the course of the study. Other physiologic variables associated with atherosclerosis were measured at regular intervals; these variables included systolic and diastolic blood pressure (bimonthly), fasting serum glucose concentration (semiannually) and ponderosity (the ratio of body weight to body length) (semiannually). All monkeys were sampled in the morning, under ketamine restraint and following a suitable fast (*10*).

To create a significantly stressful social environment, we periodically altered group memberships in the stressed condition by redistributing animals among the three affected groups. The monkeys were redistributed once every 12 weeks in the first year of the study and once every 4 weeks in the following 9 months. Unlike the stressed animals, group memberships among monkeys assigned to the unstressed, or control condition, remained constant throughout the 21-month experiment.

Reorganization of groups was selected as a means of inducing stress in the present study because previous reports had indicated that introduction of strangers fosters a high degree of social instability in macaques (*11*). In an attempt to further enhance competition and social uncertainty, an ovariectomized female

Table 1. Behavioral characteristics of the stressed and unstressed groups.

Group	Rate of aggression*	Percentage with severe aggression	Rate of submission	Percentage with severe submission	Percentage of time in affiliation
Stressed (N = 15)	5.16	30.0	6.59	27.0	21.0
Unstressed (N = 14)	6.69	20.0	7.10	19.0	26.0
Probability†	N.S.	< .05	N.S.	< .05	< .05

*Median rates of performance per hour per monkey, as determined by focal samples. †Tests of significance by Mann-Whitney U test (two-tailed).

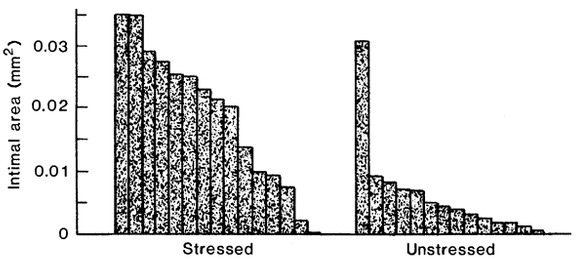

Fig. 1. Area occupied by intimal lesion in stressed and unstressed monkeys; bars represent individual animals. Early mortality resulted in the loss of one animal from the unstressed condition.

with an estrogen-containing capsule implanted under the skin was also placed into each of the stressed groups for the last 2 weeks of each 4-week reorganization during the final 9 months of the study. Finally, to document behavioral effects associated with these experimental manipulations, we recorded the frequencies of aggressive, submissive, and affiliative acts. These data were obtained by means of a focal sampling technique in which each animal was observed for 195 15-minute periods (*10*).

At the time of necropsy, the heart was removed and the coronary arteries were perfused with 10 percent neutral buffered Formalin at a pressure of 100 mmHg. Fifteen tissue blocks (each 3 mm in length) were then cut perpendicularly to the long axis of the coronary arteries. Five of these were serial blocks taken from the left circumflex, five from the left anterior descending, and five from the right coronary artery. Two sections were cut from each block and stained with either hematoxylin and eosin or Verhoeff Van Gieson stains. Upon projection of the Verhoeff Van Gieson–stained sections (at ×40 magnification), two measures of atherosclerosis were calculated with the use of a Zeiss MOP III Image Analyzer. The first measurement was of the total area occupied by intima or intimal lesion (termed intimal area). The second measure, intimal thickness, was calculated as the maximum distance obtaining at any one point between the internal elastic lamina and the lumen of the artery.

For the present analysis, the extent of coronary artery atherosclerosis in each monkey was expressed as a mean intimal area (in square millimeters) and mean intimal thickness (in millimeters) of 15 sections of coronary artery. The area measures for all monkeys are depicted in Fig. 1. Substantial differences in coronary artery atherosclerosis were observed between the stressed and unstressed conditions, with stressed animals having larger intimal areas relative to controls ($P < .002$, Mann-Whitney U test) (*12*). Maximum intimal thickness was highly correlated with intimal area ($\rho = .73$, $P < .001$) and was also significantly greater in stressed than control animals ($P < .02$, Mann-Whitney U test).

The arterial lesions of each monkey were also graded for extent of change. One of three grades was assigned to each coronary artery upon the consensus of five investigators who did not know the animals' experimental conditions. The grades recorded were 0 for no intimal changes; 1 for intimal changes present but characterized by fatty streaks only (that is, foam cells); and 2 for fatty streaks that had progressed to small plaques with smooth muscle cell proliferation. Among animals in the stressed condition, 11 of 15 monkeys received grade 2 on one or more coronary arteries, whereas only 4 of 14 unstressed animals had lesions of similar extent. This difference in the distribution of relatively advanced lesions (that is, plaque) between the stressed and unstressed conditions was statistically significant ($\chi^2 = 4.16$, $P < .05$).

Animals in the two conditions also differed behaviorally. Stressed and unstressed monkeys had similar rates of aggressive and submissive behaviors (expressed as incidents per hour) over the course of the experiment (Table 1). Yet the proportions of all agonistic encounters involving both direct, contact aggression (for example, biting, grabbing, slapping) and extreme forms of submission (for example, fleeing, cowering, grimacing) were significantly greater in the periodically reorganized groups (Table 1). Likewise, stressed animals spent significantly less time huddling affiliatively (that is, in passive body contact). These findings suggest that the redistribution of group members fostered a high degree of tension among animals in the stressed condition, promoting a greater proportion of overt fights and interfering with the development of social bonds.

The effects of the psychosocial manipulation on coronary artery atherosclerosis apparently are not due to concomitant differences among other physiologic variables commonly associated with atherosclerosis. Comparisons of the two conditions (by t-tests) revealed no significant differences between the stressed and unstressed monkeys on measures of total serum cholesterol [157 ± 20 mg/dl (mean ± standard error) and 146 ± 22 mg/dl, respectively] or HDLC concentrations (57 ± 11 mg/dl; 59 ± 13 mg/dl), systolic (89 ± 7 mmHg; 88 ± 9 mmHg) or diastolic (56 ± 6 mmHg; 53 ± 6 mmHg) blood pressure, fasting blood glucose concentration (59 ± 4 mg/dl; 58 ± 6 mg/dl), or ponderosity (1.79 ± 0.26 kg/cm; 1.84 ± 0.15 kg/cm).

The present study provides evidence that psychosocial stress promotes atherogenesis in normocholesterolemic, normotensive monkeys, thus identifying a possible variable in the pathogenesis of atherosclerosis among some "low risk" individuals in human populations. Though it is unclear what mechanisms may mediate behavioral influences on the development of atherosclerosis, we believe the present results may be attributable, in part, to effects of repeated arterial injury. For example, there is a close resemblance between the histological characteristics of lesions reported here (intimal smooth muscle cell proliferation with intra- and extracellular lipid accumulation) and those seen after repeated mechanical injury to arteries among normocholesterolemic rabbits (*13*). Also, an experimental stressor (electric tail shock) has been shown to result in endothelial injury in normocholesterolemic rats (*14*). Albeit speculative, several investigators have suggested that such arterial injury may result, in turn, from hormonal (for example, cortisol, catecholamine) and hemodynamic alterations commonly observed in response to laboratory and social stressors (*15*).

Reorganization of group memberships in the present experiment resulted in proportionately more displays of intensely aggressive behavior among animals assigned to the stressed condition.

In a prior investigation involving hypercholesterolemic cynomolgus monkeys, periodic group reorganization also led to increased contact aggression and to greater atherosclerosis (*16*). It is interesting that a high "potential for hostility" represents, among humans, a central component of the type A (coronary-prone) behavior pattern (*17*). Moreover, independent of its association with type A behavior, hostility has been found associated with extent of angiographically documented coronary artery atherosclerosis (*18*). Although these findings reflect only descriptive behavioral similarities, it is noteworthy that aspects of the present data are consistent with studies of psychosocial factors among human beings.

JAY R. KAPLAN
Arteriosclerosis Research Center,
Bowman Gray School of Medicine,
Wake Forest University,
Winston-Salem, North Carolina 27103
STEPHEN B. MANUCK
Department of Psychology,
University of Pittsburgh,
Pittsburgh, Pennsylvania 15260
THOMAS B. CLARKSON
FRANCES M. LUSSO
Arteriosclerosis Research Center,
Bowman Gray School of Medicine
DAVID M. TAUB
Yemassee Primate Center,
Yemassee, South Carolina 29945
ERIC W. MILLER
Arteriosclerosis Research Center,
Bowman Gray School of Medicine

References and Notes

1. National Heart, Lung, and Blood Institute, *NIH Pub. No. 81-2034* (1981).
2. L. Solberg, S. Enger, I. Hjermann, A. Helgeland, I. Holme, P. Leren, J. Strong, in *Atherosclerosis V*, A. Gotto, L. Smith, B. Allen, Eds. (Springer-Verlag, New York, 1980), p. 57; L. Carlson, in *Metabolic Risk Factors in Ischemic Cardiovascular Disease*, L. Carlson and B. Pernow, Eds. (Raven, New York, 1982), p. 1.
3. H. McGill, M. Frank, J. Geer, *Arch. Pathol.* **71**, 96 (1961).
4. C. Minick, G. Murphy, W. Campbell, Jr., *J. Exp. Med.* **124**, 635 (1966); T. Clarkson and N. Alexander, *J. Clin. Invest.* **65**, 15 (1980).
5. C. Jenkins, *Annu. Rev. Med.* **29**, 543 (1978).
6. J. Kaplan, S. Manuck, T. Clarkson, F. Lusso, D. Taub, *Arteriosclerosis* **2**, 359 (1982).
7. R. M. Nerem, M. J. Levesque, J. F. Cornhill, *Science* **208**, 1475 (1980).
8. H. Ratcliffe, H. Luginbuhl, W. Schnarr, K. Chacko, *J. Comp. Physiol. Psychol.* **68**, 385 (1969).
9. Each 100 g of diet contained 8.0 g of casein, 8.0 g of lactalbumin, 35.0 g of wheat flour, 6.0 g of dextrin, 5.0 g of sucrose, 4.5 g of applesauce, 7.0 g of lard, 1.2 g of safflower oil, 3.0 g of beef tallow, 0.37 g of dried egg yolk, 15.37 g of alphacel, 4.0 g of Hegsted salt mixture, and 2.56 g of vitamin mixture.
10. For reliabilities of measurement and methods for collecting and analyzing behavioral, pathologic, and physiologic data, see (*6*).
11. I. Bernstein, T. Gordon, R. Rose, *Folia Primatol.* **21**, 90 (1974).
12. All tests of significance were two-tailed.
13. S. Moore, *Lab. Invest.* **29**, 478 (1973).
14. D. Gordon, J. Guyton, M. Karnovsky, *ibid.* **45**, 14 (1981).
15. J. Herd, in *Perspectives on Behavioral Medicine*, S. Weiss, J. Herd, B. Fox, Eds. (Academic Press, New York, 1981), p. 55; R. B. Williams, Jr., J. D. Lane, C. M. Kuhn, W. Melosh, A. D. White, S. M. Schanberg, *Science* **218**, 483 (1982).
16. See J. Kaplan *et al.* (*6*). In the previous experiment exacerbated atherosclerosis was observed only in the stressed animals that were socially dominant. Because hierarchical relationships in the stressed group were somewhat less stable in the current investigation, here we were unable to identify similarly well-differentiated dominant and subordinate animals. Nevertheless, 2 of 15 monkeys housed in the stressed condition clearly retained dominant positions throughout the experiment; as in the previous study, these had the most extensive coronary artery atherosclerosis (see Fig. 1).
17. M. Friedman, *Pathogenesis of Coronary Artery Disease* (McGraw-Hill, New York, 1969).
18. R. Williams, T. Haney, K. Lee, Yi-Hong Kong, J. Blumenthal, R. Whalen, *Psychosom. Med.* **42**, 539 (1980).
19. Supported in part by grants from the National Heart, Lung, and Blood Institute (HL 14164 and RO1 HL 26561) and R. J. Reynolds Industries, Inc.

15 February 1983

Parthenogenesis in the Endemic Australian Lizard *Heteronotia binoei* (Gekkonidae)

Abstract. *Chromosome variation in the gekkonid lizard* Heteronotia binoei *reveals that this endemic Australian vertebrate reproduces by parthenogenesis. Triploid parthenogenetic females are distributed throughout central and western Australia and are all heterozygotes for one or more pericentric inversions that also distinguish the extant bisexual diploid cytotypes. These data on karyotype provide strong evidence that the various clones have arisen through multiple hybridization events between bisexual ancestors.*

In vertebrates, all-female populations that reproduce by parthenogenesis have now been reported in several American and European taxa (*1*). A newly discovered parthenogenetic biotype of the endemic lizard *Heteronotia binoei* Gray, which is distributed throughout most of the Australian continent, also exists as diploid bisexual populations for much of its range (Fig. 1A). A cytogenetic analysis of diploid and triploid forms provides evidence for a hybrid origin of the parthenogenetic biotype and multiple hybridization events between the bisexual ancestors appear to have been important in generating the considerable clonal diversity found in *H. binoei*.

Karyological analysis of diploid *H. binoei* (2n = 2x = 42) by both standard Giemsa-stained preparations and G- and C-banding has revealed two major cytotypes, SM6 and A6, distinguished by a pericentric inversion associated with chromosome 6 (Fig. 2A). Within the SM6 cytotype a further pericentric rearrangement (SM6-2) on chromosome 4 was polymorphic in lizards in north-central Australia and absent in those on the western Australian coast (SM6-1). A distal nucleolar organizing satellite on chromosome 6 was found in eastern and southern A6 populations and all SM6 populations. This did not occur in A6 populations from central and western Australia, although the former did maintain an active distal nucleolar organizing region (*2*). The A6 and SM6-2 cytotypes overlap broadly in north-central Australia (Fig. 1A). However, there are no known sympatric localities, and no diploids heterozygous for the inversion on chromosome 6 have been found.

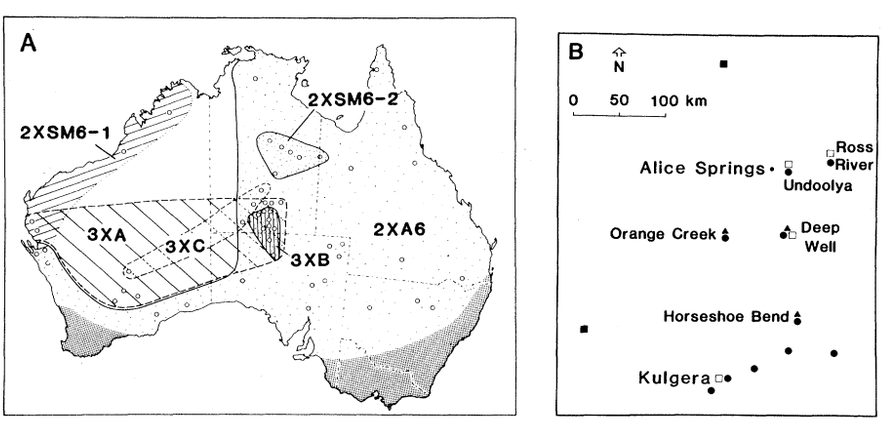

Fig. 1. (A) Distribution of the triploid and diploid cytotypes of *Heteronotia binoei* throughout Australia. Circles represent sampling points and the shaded regions are where *H. binoei* is absent. The boundaries are shown to emphasize the extensive overlap, but more sampling in western Australia is required before the distribution limits of the various cytotypes can be determined. (B) Detailed distribution data for the central Australian region. Symbols: □, diploid A6; ●, triploid clone A; ▲, triploid clone B; and ■, triploid clone C.