assessment cue. Molting, common in many animals, can cause such variation (*19*).

RICK STEGER
ROY L. CALDWELL
Department of Zoology, University of California, Berkeley 94720

References and Notes

1. E. Curio, *The Ethology of Predation* (Springer-Verlag, New York, 1976); W. Wickler, *Mimicry in Plants and Animals* (McGraw-Hill, New York, 1968).
2. D. Otte, *Annu. Rev. Ecol. Syst.* **5**, 385 (1974).
3. R. Dawkins and J. R. Krebs, in *Behavioural Ecology: An Evolutionary Approach*, J. R. Krebs and N. B. Davies, Eds. (Sinauer, Sunderland, Mass., 1978), chapter 10.
4. J. Maynard Smith, *Proc. R. Soc. London* **205**, 475 (1979).
5. H. Dingle, *Anim. Behav.* **17**, 567 (1969); _____ and R. L. Caldwell, *Behaviour* **33**, 115 (1969).
6. M. Reaka, *J. Morphol.* **146**, 55 (1975).
7. G. A. Parker, *J. Theor. Biol.* **47**, 223 (1974).
8. We use the *G*-test of independence, the recommended statistical test for two-by-two tables obtained from a model 2 experimental design, or Fisher's exact test when appropriate [R. R. Sokal and F. J. Rohlf, *Biometry*, (Freeman, San Francisco, ed. 2, 1981)].
9. We report data only from contests on the day of molting since they most clearly are instances of bluffing.
10. The frequencies of all acts were indistinguishable from control values by day 10.
11. Lunge may be part of the aggressive front of some new molts, but we chose conservatively to concentrate on the major component, meral spread.
12. R. L. Caldwell and J. Dingle, *Behaviour* **69**, 255 (1979).
13. Intruders cannot detect newly molted residents visually since new molts easily fall within the wide range of intermolt color variations in *G. bredini*.
14. This is a conservative estimate. When size discrepancies exist between residents and intruders, meral spread is used more frequently. Also, large individuals molt less often. Both factors would lower the ratio of bluffs to honest displays.
15. Gonodactylids use chemical cues to detect and identify individual residents (*17*).
16. Two new molts died in day 1 contests; both had hidden but were located and attacked by probing intruders.
17. R. L. Caldwell, *Anim. Behav.* **27**, 194 (1979).
18. J. G. Van Rhijn and R. Vodegal, *J. Theor. Biol.* **85**, 623 (1980).
19. Supported by an NSF predoctoral fellowship to R.S. and NSF grant BNS 78-27363 to R.L.C.

7 September 1982; revised 28 January 1983

Coronary Artery Spasm Induced in Atherosclerotic Miniature Swine

Abstract. *Angiographically demonstrable coronary artery spasm could be provoked repeatedly by giving intracoronary or intravenous injections of histamine to miniature swine with experimentally induced atherosclerotic lesions of the coronary artery. The spasm induced in this way subsided either spontaneously or after the administration of nitroglycerin and was prevented by a calcium antagonist or an agent that blocks histamine H_1 receptors. This model, which suggests that atherosclerotic changes may be one of the primary factors in the occurrence of coronary artery spasm, should facilitate studies on the pathogenesis of this condition.*

Coronary artery spasm causes not only variant angina but also certain forms of effort angina, acute myocardial infarction, and sudden death (*1*). Because the pathogenesis of coronary artery spasm is unknown, its prevention and treatment remain important clinical problems. However, the induction of coronary artery spasm in an animal model has not been successful. In the presence of severe stenosis, drastic coronary flow reduction may occur with minimal reduction in the stenotic area, probably not only as a result of physiologic changes in arterial tone but also because of minimal platelet aggregation. We therefore believe that an experimental model of coronary artery spasm should fulfill the following criteria: (i) no significant, angiographically demonstrable stenosis before induction of the spasm; (ii) transient and reproducible provocation of total or near total obstruction that can be documented by coronary arteriography; and (iii) regional myocardial ischemia in the spastic coronary territory.

We previously reported that experimentally induced atherosclerotic lesions in the canine coronary artery constrict more extensively with ergonovine than do non-atherosclerotic lesions in the same dog (*2*). We have now succeeded in provoking coronary artery spasm associated with ischemic electrocardiographic changes in miniature swine.

Fifteen male miniature swine (4 to 6 months of age) were fed on a diet containing 2 percent cholesterol after they were subjected to endothelial balloon-denudation of the left circumflex coronary artery. Such treatment promotes the development of selective coronary atherosclerotic lesions (*2, 3*). Before the denudation, and 1 and 3 months after the operation, selective coronary arteriography was repeated after intracoronary or intravenous administration of various vasoconstrictive agents, such as ergonovine (*4*), phenylephrine (*5*), histamine (*6*), and serotonin (*7*), all compounds that seem to be potent agents of coronary artery spasm in humans.

Coronary artery spasm in these experiments was defined as the transient excess vasoconstriction that subsides either spontaneously or after the administration of nitroglycerin and that is characterized by a decrease of over 75 percent in coronary diameter compared with that after the intravenous administration of nitroglycerin (20 μg/kg). Significant ischemic electrocardiographic changes were defined as more than 0.1 mV ST-segment elevation or depression from the control level. At the end of the experiments, intact and denuded portions of the left coronary arteries were examined histologically.

Coronary artery spasm was provoked repeatedly by intracoronary or intravenous administration of histamine, with or without cimetidine (a histamine-H_2 receptor blocking agent), in doses of 100 to 400 μg or 10 to 100 μg/kg, respectively (Fig. 1). The other three drugs were ineffective, except in one animal in which serotonin as well as histamine provoked the spasm. The spasm occurred only in the denuded portion of the left circumflex coronary artery, although the portion was angiographically normal before the spasm. Spasm was induced in none of 15 pigs before the endothelium was denuded, in five of nine pigs after 1 month, and in five of six pigs after 3 months (Table 1). Significant electrocar-

Table 1. Number of pigs in which coronary artery spasm was induced before and after endothelial denudation of the left circumflex coronary artery and before and after the intracoronary (i.c.) or intravenous (i.v.) administration of various drugs. The doses of each drug were as follows: histamine, i.c. 100 or 400 μg and i.v. 10 or 100 μg/kg; cimetidine, i.v. 60 mg/kg; serotonin, i.c. 60 μg and i.v. 30 μg/kg; phenylephrine, i.c. 20 μg and i.v. 3 μg/kg; ergonovine, i.v. 0.2 or 0.4 mg.

Group	Route of administration	Before denudation	After denudation	
			1 month	3 months
Total		0/15	5/9	5/6
Histamine	i.c.	0/1	1/2	2/2
Histamine	i.c.	0/3	5/6	3/3
Histamine plus cimetidine	i.v.	0/7	2/7	2/4
Serotonin	i.c. or i.v.	0/3	1/4	0/4
Phenylephrine	i.c. or i.v.	0/3	0/4	0/5
Ergonovine	i.v.	0/15	0/8	0/5

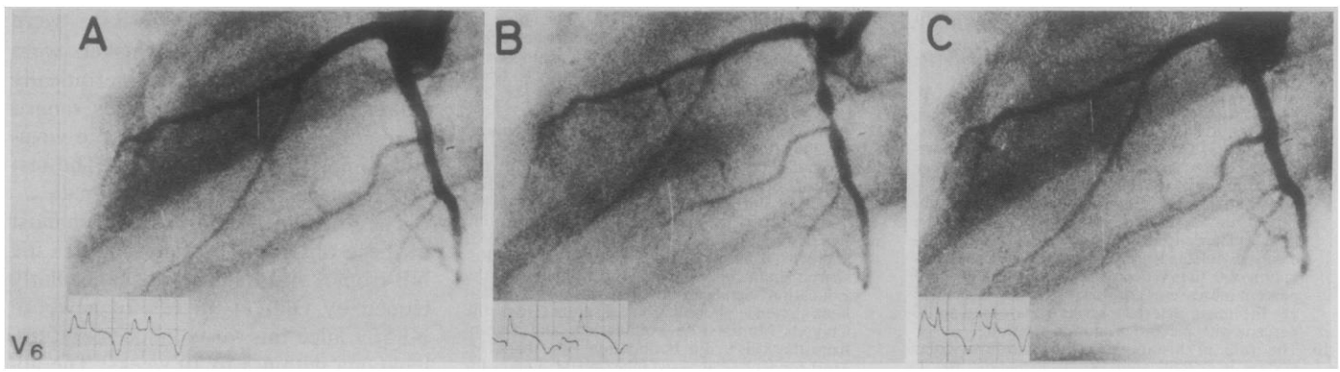

Fig. 1. Coronary arteriograms in miniature swine fed an atherogenic diet. (A) Before and (B and C) after intracoronary administration of histamine (200 μg) in the presence of cimetidine administered intravenously in doses of 60 mg/kg. Coronary artery spasm, with associated electrocardiographic ST-segment elevation in the precordial lead, was provoked at multiple sites in the circumflex branch of the left coronary artery where skip-lesions of coronary atherosclerosis were observed histologically in accordance with the sites of the spasm.

diographic changes occurred simultaneously with the coronary artery spasm. The configuration of the spasm varied from a single- or multifocal type to a diffuse spastic type, although the same configuration was observed repeatedly in the same pig. Coronary artery spasm never occurred when diphenhydramine, an agent that blocks histamine H_1 receptors, was given intravenously in doses of 1 mg/kg ($N = 3$) or diltiazem, a calcium antagonist, was given in doses of 100 μg/kg ($N = 3$). In contrast, when indomethacin was injected intravenously in doses of 2 mg/kg, coronary artery spasm was intensified rather than prevented ($N = 3$).

Histological examination revealed that the atherosclerotic changes were predominantly in the denuded portions of the left circumflex coronary artery, and consisted of intimal thickening with abundant accumulation of foam cells and migration of smooth muscle cells to the intima (Fig. 2). There was no thrombus formation or hemorrhage in the fibrous plaque. There was a close coincidence between the site of coronary artery spasm and the portion of coronary atherosclerosis. That is, coronary artery spasm occurred only in the atherosclerotic portion; there was one focus of coronary atherosclerosis in the one-focal type, multiple foci in the multiple-focal type, and diffuse atherosclerosis in the diffuse spastic type.

Since the first report of a relation between coronary artery spasm and variant angina appeared in 1959 (*8*), numerous attempts have been made to determine the role of coronary artery spasm in the pathogenesis of ischemic heart disease, mainly in the clinical field. Several experimental studies were also done to determine the pathogenesis of coronary artery spasm. Nevertheless, coronary artery spasm similar to that observed in humans has not been reported in animals (*9*). In previous work, we demonstrated the augmented response of the atherosclerotic canine coronary artery to ergonovine but failed to produce myocardial ischemia (*2*). In the present study, we succeeded in developing a working animal model of coronary artery spasm in miniature pigs, in which coronary atherosclerosis can be produced in a short period and the atherosclerotic changes bear many similarities to those observed in humans (*3*). In our model, histamine proved to be the most vasoactive agent, whereas in humans, ergonovine, which was largely ineffective in pigs, is most frequently used to provoke coronary artery spasm (*4*). Histamine is also a potent vasoactive agent in humans (*6, 10*).

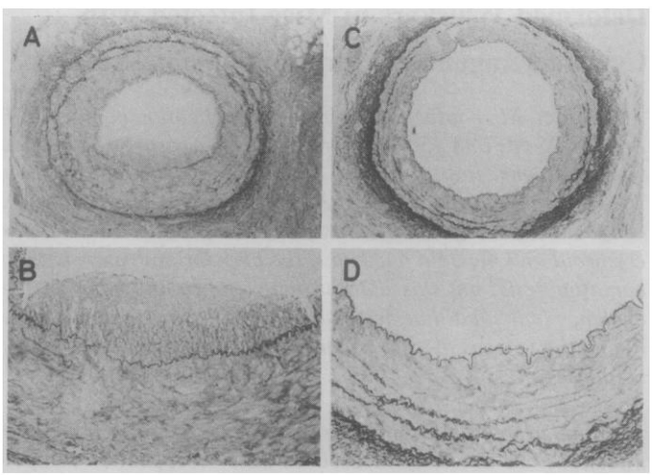

Fig. 2. Histology of the left circumflex coronary artery. (A and B) Site of induced coronary artery spasm. (C and D) Left anterior descending coronary artery (controls) (A and C, ×32; B and D, ×80). Atherosclerotic changes, characterized mainly by intimal thickening, are distinct, yet these changes were not apparent angiographically.

Coronary artery spasm was provoked repeatedly only in the area of the experimentally induced atherosclerotic lesion. Henry and Yokoyama stated that, in the rabbit, the atherosclerotic aorta constricts more potently in response to ergonovine (*11*). The important finding is that the coronary artery spasm occurred only in the denuded portion, histologically defined as being atherosclerotic, even when the degree of atherosclerosis was too slight to detect angiographically. Thus, coronary atherosclerosis is probably the primary factor in the pathogenesis of coronary artery spasm.

HIROAKI SHIMOKAWA
HITONOBU TOMOIKE
SHOZO NABEYAMA
HIDEO YAMAMOTO
HARUO ARAKI
MOTOOMI NAKAMURA*
Research Institute of Angiocardiology and Cardiovascular Clinic, Faculty of Medicine, Kyushu University, Fukuoka, Japan
YUTAKA ISHII
KENZO TANAKA
First Department of Pathology, Faculty of Medicine, Kyushu University

References and Notes

1. L. Wiener *et al.*, *Am. J. Cardiol.* **38**, 945 (1976); A. Maseri *et al.*, *ibid.* **42**, 1019 (1978).
2. M. Nakamura, Y. Kawachi, H. Tomoike, *Circulation* **64**, IV-121 (1981).
3. W. M. Lee and K. T. Lee, *Exp. Mol. Pathol.* **23**, 491 (1975); P. Clopath, *Artery* **3**, 429 (1977).
4. J. S. Schroeder *et al.*, *Am. J. Cardiol.* **40**, 487 (1977).
5. H. Yasue *et al.*, *Am. Heart J.* **91**, 148 (1976).
6. R. Ginsburg, M. R. Bristow, E. B. Stinson, D. C. Harrison, *Life Sci.* **26**, 2245 (1980); R. Ginsburg, M. R. Bristow, N. Kantrowitz, D. S.

Baim, D. C. Harrison, *Am. Heart J.* **102**, 819 (1981).
7. E. Müller-Schweinitzer, *J. Cardiovasc. Pharmacol.* **2**, 645 (1980).
8. M. Prinzmetal, P. Kennamer, R. Merliss, T. Wada, N. Bor, *Am. J. Med.* **27**, 375 (1959).
9. Segal *et al.* [S. A. Segal, D. L. Pearle, R. A. Gillis, *Eur. J. Pharmacol.* **76**, 447 (1981)] reported that they had produced coronary artery spasm in vagotomized cats by intravenous administration of picrotoxin. However, they observed no excessive vasoconstriction of the large epicardial coronary arteries and it is unclear why electrocardiographic ST-segment elevation of 0.30 mV occurred along with only a 25 percent increase in coronary vascular resistance yet the mean arterial pressure increased to 36 percent with picrotoxin administration.
10. The role of histamine in the cardiovascular system is complicated [K. J. Broadley, *Br. J. Pharmacol.* **54**, 511 (1975); R. Levi, G. Allan, J. H. Zavecz, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 1942 (1976)]. However, it has been accepted that the cardiac histamine system is analogous to the adrenergic system; that is, histamine H_1 receptors are analogous to α-adrenergic receptors and histamine H_2 receptors are analogous to β-adrenergic receptors [M. R. Bristow, R. Ginsburg, D. C. Harrison, *Am. J. Cardiol.* **49**, 249 (1982)].
11. P. D. Henry and M. Yokoyama, *J. Clin. Invest.* **66**, 306 (1980).
12. A brief report of this model was made at the International Teach-in, Regulation of Cardiac Function and Metabolism, 11 to 12 April 1983, Osaka, Japan. Supported in part by Scientific Research grants 56570338, 57440053, 57570353, and 58870058 and Developmental Scientific Research grant 587067 from the Ministry of the Education, Science and Culture Japan and Takeda Science Foundation, a grant from the Chiyoda Mutual Life Foundation, and a grant from the Taisha Ijo Foundation. We thank M. Mori for technical assistance and M. Ohara for critical reading of the manuscript.
* Correspondence should be addressed to M.N.

2 May 1983

Deformed Whiskers in Mice Infected with Certain Exogenous Murine Leukemia Viruses

Abstract. *Mice infected at birth with replication competent Friend, Moloney, Cas-Br-M, C2S-M, and 1504-A murine leukemia viruses developed abnormalities of the vibrissae consisting of erratic curvature, shortening, and loss. A number of other virus strains, as well as endogenous AKR-type ecotropic virus and AKR-type, mink cell focus-inducing (MCF) viruses, did not produce these abnormalities. In mice with erythroid and myeloid leukemia, the perivibrissal sinus is the site of extramedullary hematopoiesis, but this did not appear to be the basis of the deformities. Genetic evidence indicated that newly arisen MCF-type recombinant viruses are involved in the pathogenesis of the abnormalities, at least with some of the virus systems studied.*

Although interest in mouse retrovirus infections has naturally focused on cells of the hematopoietic system, various retroviruses can infect a wide variety of cell types in vivo. Other than hematologic malignancies and a form of demyelinating brain disease (*1*), disease manifestations of infection with nondefective, replication competent murine retroviruses have not been observed.

In the course of examining mice that had been infected at birth with a replication competent Friend helper virus, free of the spleen focus-forming component, we noticed that their vibrissae were markedly abnormal. The whiskers were sparse, short, thin, and erratically curved (Fig. 1). The anomalies ranged from a few clearly altered hairs to virtually complete loss of vibrissae. The rest of the pelage has shown no anomalies.

The Friend helper virus on which most of these observations were made is the NB-tropic virus that was molecularly cloned by Oliff *et al.* (*2*). In newborn NFS/N mice this virus induces erythroleukemia within 5 to 10 weeks. The abnormalities of the vibrissae often precede the splenic enlargement, in some cases by as much as 3 or 4 weeks. The N-tropic Friend murine leukemia virus (MuLV), F-S, also induces the whisker abnormalities, but with a longer latent period and less marked effects.

We have examined mice infected with a number of exogenous and endogenous murine retroviruses for the whisker anomalies (Table 1). Four viruses consistently induced the changes: Friend and Moloney viruses, which are laboratory-passaged, highly pathogenic variants, and two horizontally transmitted viruses recovered from wild mice, Cas-Br-M and 1504-A. Another isolate from wild mice, C2S-M, also induced the changes in a small proportion of mice; this low response rate was probably due to the effect of a gene segregating in the study mice, as described below, rather than to inefficiency of the virus. In contrast, other highly pathogenic viruses, such as Gross Passage A, SL3, and a variety of thymomagenic recombinant MuLV's of the mink cell focus-forming (MCF) type

Table 1. Frequency of abnormal vibrissae in mice inoculated as newborns with various helper-independent MuLV's and in mice carrying life-long high titers of endogenous AKR-type MuLV. The inoculated mice were of genotypes that are permissive for replication of the inoculated virus; most of the animals were NFS/N or AKR, or hybrids derived from them. All viruses were grown in tissue culture and had been biologically cloned by two cycles of limiting dilution purification. Mice from cages in which there was any suspicion of barbering were excluded.

Virus	Major type of disease induced	Number of mice with abnormal vibrissae/number examined (%)				
		All mice by age				Leukemic mice only
		< 2 months	2 months	3 to 6 months	> 6 months	
Inoculated mice						
Friend MuLV-NFS	Erythroleukemia	28/33 (85)	16/17 (94)			33/34 (97)
Friend MuLV-C57BL-derived strains with resistance to erythroleukemia	T lymphoma		4/5	22/22 (100)		1/1
Moloney MuLV	T lymphoma		6/29 (21)	31/58 (53)	7/11 (64)	13/37 (35)
FM-I2*	T lymphoma		4/5	27/37 (73)		12/16 (75)
Gross MuLV	T lymphoma		2/24 (8)	1/8	0/5	0/20 (0)
SL3 (3)	T lymphoma		1/30 (3)	0/8		0/21 (0)
Class I MCF viruses	T lymphoma		0/21 (0)	2/72 (3)	2/34 (6)	2/61 (3)
Cas-Br-M (4)	Neurologic			13/17 (76)	0/1	
C2S-M (5)	Myeloid leukemia			0/2	4/12 (33)	2/4
1504-A (4)	Lymphoma			4/4	14/16 (88)	1/1
Uninoculated high-ecotropic carriers						
AKR and AKR F1's	T lymphoma			1/25 (4)	1/30 (3)	1/19 (5)
NFS congenic for various ecotropic V loci	T and B lymphomas			0/20 (0)	3/80 (4)	0/18 (0)

*FM-I2 is an in vitro recombinant between molecularly cloned Friend and Moloney viruses constructed by P. Chatis and N. Hopkins.