First, antiserums labeled with fluorescein failed to detect immunoglobulins on the surfaces of MOLT-4 cells pretreated with NHS. These cells were tested because they lack membrane-bound immunoglobulin and fragment c receptors (*19*). Second, incubation of RAJI cells with NHS heated at 56°C for 45 minutes failed to induce lysis of these cells after addition of fresh guinea pig serum as a source of complement. The guinea pig serum supported complement-dependent lysis of erythrocyte-antibody complex but was minimally lytic (2.5 ± 0.9 percent ^{51}Cr release) for RAJI cells. Third, as indicated above, absorption of fresh NHS with RAJI cells at 4°C had little effect on its lytic activity against these cells. Fourth, serums from two agammaglobulinemic patients lysed RAJI cells as efficiently as NHS. In addition, lysis of RAJI cells is probably not produced by antibodies to Epstein-Barr virus (EBV), since these cells, although carrying a repressed EBV genome, are free of detectable EBV-related antigens (*20*).

Activation of the properdin pathway and lysis of lymphoblasts bearing C3b immune adherence receptors may represent a natural mechanism of in vivo surveillance to limit the growth of B-type lymphoma and lymphoblastic leukemia cells. The antitumor effect of certain polysaccharides is related directly to their capacity to activate the properdin pathway (*21*), while infusion of fresh, but not heated, NHS has been used to treat leukemia in AKR mice (*22*). It is possible that an absolute or functional deficiency of a properdin complement pathway component may exist in patients with leukemia or lymphoma, which would limit the efficiency of lysis of tumor cells bearing C3b immune adherence receptors mediated by this pathway. It would be worth knowing whether B-type lymphoma and leukemia cells obtained directly from patients have components of the properdin system fixed to their surface membranes and whether the properdin pathway is activated in their serums.

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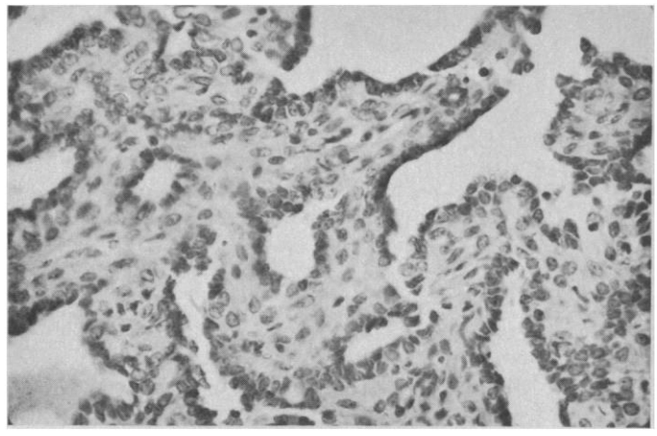
Angiogenesis: A Marker for Neoplastic Transformation of Mammary Papillary Hyperplasia

Abstract. *Mouse mammary papillomas elicit new formation of vessels when transplanted onto the rabbit iris. This angiogenic capacity is a property of carcinomas but not of the resting mammary gland. In mouse papillary hyperplasias, however, this property appears much earlier than any morphological or clinical sign of carcinoma. A test for angiogenic capacity may reveal a step in the progression toward clinical malignancy and thus could be used to screen for neoplastic potential of hyperplastic epithelium in biopsy tissues.*

The hypothesis that overt clinical neoplasia develops in cell populations through a sequence of changes that are often disguised as hyperplasia has been extensively debated and is accepted by many cancerologists (*1–3*). The mammary gland is an organ particularly prone to hyperplastic lesions. Some of them have been labeled preneoplastic to suggest their high risk of becoming clinically

Table 1. Angiogenic response of benign mouse mammary papillomas.

Experiment	Transplant generation	Latent period (weeks)	Fraction of iris implants with neovascular response		
			Tumor	Boiled tumor	Mouse liver
		C57BL mice (urethane-induced)			
1	2	14	19/19	0/3	
2	2	14	23/23	0/7	
3	1	15	32/32	0/5	0/5
4	1	15	13/13		0/9
5	3	14	19/19	0/12	
6	3	14	30/30	0/7	
7	3	14	29/29	0/9	
8	1	47	34/34	0/11	
9	1	51	25/26	0/6	
		BALB/c mice (DMBA-induced)			
10	3	13	26/26	0/16	
11	5	10	23/24		0/10
12	5	10	18/18		0/16
13	1	16	16/16		0/17
Total			307/309	0/76	0/57
Percentage			99	0	0



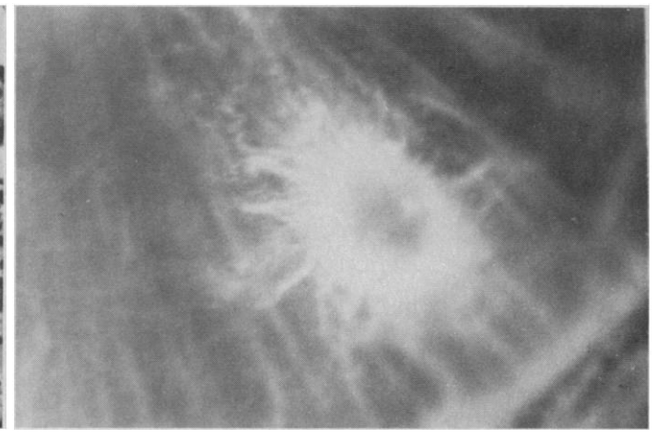


Fig. 1 (left). Papillary structure in large duct hyperplasia of mouse mammary gland characterized by a well-differentiated epithelium. Despite the absence of morphological signs of carcinoma, all fragments induced neovascularization (× 200). Fig. 2 (right). A positive neovascular response. A fragment of mouse mammary papilloma 5 days after transplantation to the rabbit iris has induced a field of tortuous, new blood vessels directed toward the tumor (× 10). For more details see (*5*).

malignant tumors, although there is no possibility of predicting this neoplastic potential. Ductal hyperplasia, often accompanied by proliferation of the stromal cells and collagenosis, is one of these lesions (*3, 4*).

In previous work we showed that the ability to induce new formation of vessels is characteristic of mammary carcinomas, the exception in resting glands, and present in about 30 percent of mammary hyperplasias (*5, 6*). To test our hypothesis that ability to induce angiogenesis could be used to identify tissues in which progression toward clinical malignancy had already begun, we studied a mouse model of mammary papillary hyperplasia of ductal origin (*7*).

Papillary hyperplasias were induced by urethane or 7,12-dimethylbenz[*a*]-anthracene (DMBA), respectively, in C57BL and BALB/c mice (*7*). The primary lesions were then transplanted into the gland-free fat pad of syngeneic mice. After a latent period of 10 to 51 weeks (Table 1), tumors appeared. Clinically as well as histologically (Fig. 1), the tumors behaved as benign papillomas, which the animal could carry for many months, in contrast to mammary carcinomas, which grow rapidly and are fatal to the mouse (*8*). Papillomas serially transplanted gave rise to mammary carcinomas; however, the frequency and the latent period have not been definitively established since it is not yet clear whether transplantation plays a role in selecting malignant populations.

To test for the angiogenic capacity of papillomas, 311 fragments from 25 ductal papillomas showing no morphologic signs of carcinoma were transplanted onto the rabbit iris by previously described techniques (*9*). Within 3 to 5 days, 309 of the 311 fragments produced a clear neovascular response characterized by a ring of vessels directed toward the implant and extending as far as 2 mm from its edge (Fig. 2). Further documentation of the new-formed nature of the vascular response could be obtained by injecting fluorescein (0.1 ml of a 10 percent solution) intravenously. Newformed vessels are more permeable to the dye than surrounding vessels, and within seconds a green halo appears around the transplant when it is observed with a slit-lamp stereomicroscope and cobalt blue light (*5*). By contrast, 76 fragments of heat-killed papilloma tissue and 57 fragments of normal mouse liver did not elicit a vascular response. Previously we showed that normal resting mouse mammary gland lacked the angiogenic capacity but mammary carcinomas virtually always produced angiogenesis (*5*).

Berenblum and Shubik (*10*) developed the concept that normal epithelium is converted to hyperplastic or altered epithelium by an "initiator" factor (*11*). Hyperplastic or altered epithelium could either (i) revert to normal, (ii) remain hyperplastic, or (iii) proceed toward overt carcinoma, depending on the presence of promoting factors (*1, 10, 11*). The development of carcinomas from papillomas under the influence of chemical or hormonal "promoters" has been demonstrated experimentally (*1, 10, 11*). In the set of experiments reported here, the carcinogens urethane and DMBA can be viewed as the initiators of papillomas, and angiogenesis may indicate the process of promotion of papillomas toward overt carcinoma, which may appear months later. In experimental models it has been shown (*12*) that the acquisition of an angiogenic capacity by cells is associated with rapid invasive growth, whereas the blockade of angiogenesis results in a small, clinically innocuous tumor. The present study indicates that angiogenesis can be used to test directly for neoplastic progression of tissue from biopsy material. We want to stress the concept that angiogenic capacity may be useful in detecting the potential of hyperplastic tissues without morphologic signs of malignancy to become overtly malignant. The clinical usefulness of the angiogenesis test has yet to be evaluated.

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Endothermy During Terrestrial Activity in Large Beetles

Abstract. *The large tropical American beetles* Strategus aloeus *(Scarabaeidae) and* Stenodontes molarium *(Cerambycidae) can endogenously maintain metathoracic temperatures 5° to 7°C or more above ambient temperature for many hours. During such periods, their activity is exclusively terrestrial and their oxygen consumption equals that of active mammals of the same size. Before and during flight they elevate metathoracic temperatures by an additional 8° to 10°C.*

Insects belonging to at least five different orders can attain thoracic temperatures 10° to 20°C above ambient temperature by retaining some of the heat produced by the contraction of the flight muscles (*1*). The primary functional result of these elevated temperatures is an increase in the power output and the frequency of wingbeat. However, heat produced by the wing muscles can also be important in processes not involved with flight, for example, brooding in bees and stridulation in katydids (*2*). The endogenous heat production of beetles has received less attention than that of bees and moths, but preflight warm-up was demonstrated 35 years ago in a scarabaeid, *Geotrupes stercorarius* (*3*), and the role of high pterothoracic temperatures in large beetles has been critically examined (*4*). We report here prolonged, endothermically supported, elevated body temperatures during terrestrial activity as well as during preflight warm-up in *Strategus aloeus* (Scarabaeidae; Dynastinae) and *Stenodontes molarium* (Cerambycidae; Prioninae).

We measured the energy metabolism of both resting and strenuously active animals in a closed system with a paramagnetic oxygen analyzer (*5*). To measure oxygen consumption during activity we rotated the glass respirometer chambers manually so that the beetles were either continuously walking or continuously attempting to right themselves. We made instantaneous measurements of thoracic temperatures by inserting a thermocouple probe 0.2 mm in diameter into the metathorax anteriorly through the fold of thin chitin at the base of the third walking leg. We obtained continuous records of body temperatures from 40-gauge copper-constantan thermocouples inserted laterally or dorsally into the abdomen, metathorax, and prothorax. The signals from the implanted thermocouples were either read from a thermocouple thermometer or recorded on the servo channel of a polygraph. The activity of the implanted beetles included walking about freely in a tabletop enclosure, attempting to walk on the table surface while tethered by threads attached to the mesothoracic legs, or walking on a running wheel rotated by hand at a speed which kept the beetle climbing uphill or walking horizontally. The frequencies of respiratory pumping were counted by eye and timed with a stopwatch. All measurements were made in an air-conditioned room (air temperature 22° to 25°C) illuminated by fluorescent lights.

The body temperatures of *Strategus* and *Stenodontes* were indistinguishable from ambient temperature, but during activity each showed two different patterns of endothermic temperature elevation. One pattern, a preflight warm-up, consisted of a rapid and sustained rise in body temperature that continued until flight temperature (38° to 41°C, depending on the species) was attained and the animal took wing. The other pattern did not involve flight and consisted of an initial rise followed by sustained maintenance of body temperatures at a level intermediate between resting and flight temperatures.

The preflight warm-up of *Strategus* and *Stenodontes* (Fig. 1B) is generally similar to that for *Geotrupes stercorarius* (Scarabaeidae) (*3*) and *Acilius sulcatus* (Dysticidae) (*6*) and resembles preflight warm-up in moths (*7*). In view of the heavy wing loading of both *Strategus* and *Stenodontes*, it is to be expected that they, like heavily wing-loaded moths and bees, require high thoracic temperatures for flight (*8*). Under laboratory conditions immediately after flight, metathoracic temperatures ranged from 39.0° to 40.2°C in *Strategus* and from 38.2° to 40.6°C in *Stenodontes*. However, these beetles not only warmed up as a prelude to flight but also frequently maintained metathoracic temperatures 5° to 7°C or more above ambient for several consecutive hours during which they made no attempt to fly but remained continuously active and in almost constant motion. During these periods of sustained endothermy, metathoracic temperatures oscillated over a range of several degrees Celsius, sometimes with great regularity (Fig. 1A). In both species the oxygen consumption during activity was many times that at rest; in a 6-g *Strategus*, oxygen consumption increased to more than 100 times the resting level (Table 1). The dependence of metathoracic temperature on the rate of energy metabolism is shown by the strong correlation between increases in metathoracic temperature and specific metabolic scope (Fig. 1C). However, our data do not allow us to as-

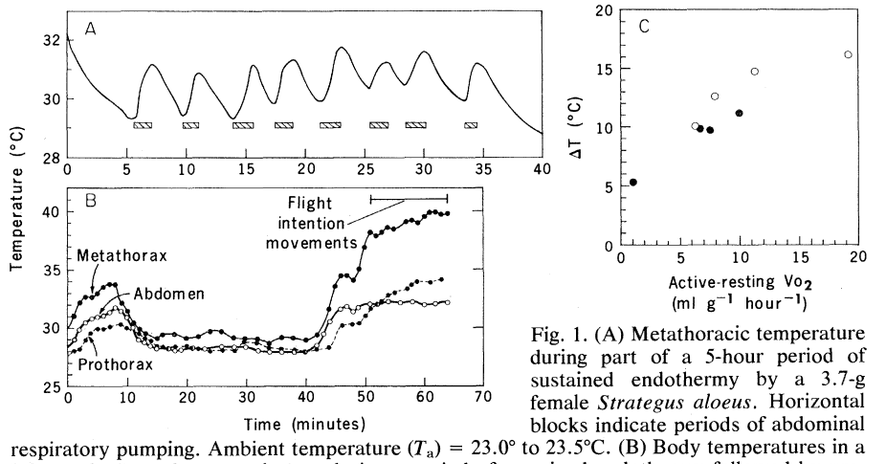

Fig. 1. (A) Metathoracic temperature during part of a 5-hour period of sustained endothermy by a 3.7-g female *Strategus aloeus*. Horizontal blocks indicate periods of abdominal respiratory pumping. Ambient temperature (T_a) = 23.0° to 23.5°C. (B) Body temperatures in a 2.8-g male *Stenodontes molarium* during a period of sustained endothermy followed by preflight warm-up and take-off: T_a = 22.6° to 23.0°C. (C) The relation of elevated body temperature to increased oxygen consumption ($\dot{V}_{O_2}$) for *Stenodontes molarium* (closed circles) and *Strategus aloeus* (open circles); ΔT = metathoracic temperature minus ambient temperature; T_a = 22.3° to 25.0°C.