# TECHNICAL PAPERS

## Cinephotomicroscopy of Normal Blood Circulation in the Cheek Pouch of the Hamster[1]

GEORGE P. FULTON and RUBY G. JACKSON
*Arthur D. Little, Inc., Cambridge, Massachusetts*

BRENTON R. LUTZ
*Boston University*

Precise microscopic studies of the peripheral circulation in the living animal require the use of thin, nonpigmented tissues which can be illuminated by transmitted light to reveal the structure of the walls of the smallest blood vessels. The number of suitable preparations is small, particularly in the mammal. Direct microscopic investigations in mammals have been made largely on the newly-formed blood vessels in transparent chambers inserted in the ear of the rabbit (*2*) and on the blood vessels in the mesentery of the gut (*1*). More recently, microscopic observations on the circulation in the wing of the bat have been reported (*4*). The investigators in this laboratory have used a new preparation for cinephotomicroscopic studies on small blood vessels, namely, the cheek pouch of the golden hamster (*Cricetus auratus*).

The hamster cheek pouch is a paired structure located inside the mouth cavity and used for temporary storage of food. *In situ* the wall of the pouch possesses numerous longitudinal folds which make it adaptable to considerable distention. The normal pouch is approximately 1½ inches in length and extends posteriorly to a position near the shoulder.

Its histological structure has been examined in sections stained with hematoxylin and eosin. The pouch is lined with stratified squamous epithelium consisting of from two to five layers of cells which become progressively flatter toward the surface, on which a slight amount of cornification is present. A relatively dense layer of fibrous connective tissue is found beneath the epithelium. Longitudinally arranged skeletal muscle fibers are numerous at the edge of this layer near the open end of the pouch but absent at the blind end. A layer of loose areolar connective tissue joins the wall of the pouch to the subcutaneous tissue of the cheek. Blood vessels are numerous in both connective tissue layers and skeletal muscle.

When the pouch is everted by gentle traction with forceps, the loose connective tissue between the subcutaneous tissue of the cheek and that of the pouch wall separates. The thin, nonmuscular portion of the pouch nearest the blind end is selected for use.

The animal is anesthetized with 6.5 per cent pentobarbital sodium (initial dose, .15 cc./animal, with fortification by .05 cc. increments at hourly intervals) and placed in a specially adapted Petri dish (6 inches in diameter). The dish is equipped with a glass platform cemented to the bottom. The pouch is everted and the portion near the tip extended over the glass platform and pinned on paraffin, producing two flat layers. A crescent-shaped cut is made in the upper layer, forming a flap of pouch wall with the limbs of the crescent directed toward the free end of pouch. The flap is pulled back, exposing a portion of the inner or connective tissue aspect of the lower layer of the wall. A considerable amount of the loose connective tissue is removed by careful dissection under the binocular microscope. The pouch may be turned over and the inverted single-layered flap repinned with the epithelial aspect uppermost. The dish is fastened to the mechanical stage of a microscope equipped with a light-splitting prism for simultaneous viewing and motion-picture recording.

The effect of a substance in solution on a small blood vessel is studied by application with a micropipette to either the epithelial surface or the connective tissue side of the pouch.

Spontaneous intermittency of flow is very frequent in the small blood vessels of the hamster's cheek pouch. This is of two kinds: (1) that produced by the action of sphincters at the arterial end of capillaries; and (2) that produced by changes in the blood-pressure differential between the two ends of the capillary.

Intermittency of flow resulting from the action of smooth-muscle sphincters at capillary origins has been observed in the frog's retrolingual membrane (*3*). Similar sphincteric activity occurs in the small blood vessels of the hamster cheek pouch. A decrease in diameter of the capillary wall and deformation of red cells were observed at the point of branching of a capillary from the supplying precapillary arteriole, which did not change in caliber. This observation has been recorded in a motion-picture sequence taken at a magnification of 1,200 times. In the frog the arterioles, as well as capillary sphincters, may contract completely, stopping the flow of blood. In the hamster the arterioles rarely contract completely, and only small areas of tissue are deprived temporarily of a blood supply by the action of sphincters at capillary origins.

Intermittency of flow has been observed in capillaries when sphincteric action was not occurring in these vessels. In favorable preparations demonstrating this type of intermittency, the wall of the capillary remained completely open throughout the entire length. The flow stopped periodically in the capillary but continued without observable change in either the arteriole or the venule. Actual reversals in direction of flow in capillaries have been observed frequently. Intermittency of this type may be explained on the basis of changes in the blood-pressure differential between the two ends of the capillary involved.

Both types of intermittency, that involving sphincteric activity of the capillary origin and that without, have been observed in adjacent small blood vessels in the same vascular area. It is possible that pressure changes producing the in-

[1] This research work was supported by Bristol-Myers Company, New York.

termittency may be caused by small changes in caliber of the arterioles or by sphincteric activity in an anastomosing portion of the vascular bed.

## References


1. CHAMBERS, R., and ZWEIFACH, B. W. *Amer. J. Anat.*, 1944, **75**, 173–205.
2. CLARK, E. R., and CLARK, E. L. *Amer. J. Anat.*, 1943, **73**, 215–250.
3. FULTON, G. P., and LUTZ, B. R. *Science*, 1940, **92**, 223–224.
4. NICOLL, P. A., and WEBB, R. L. *Fed. Proc.*, 1946, **5**, 76.


# Vascular Changes in the Wings of Bats


RAYMOND B. COWLES

*Department of Zoology, University of California, Los Angeles*


In a series of articles published since 1939 it has been proposed that acquisition of progressively higher operating temperatures may have been one of the fundamental factors in the evolutionary processes of terrestrial animals (*2*, *4*, *5*). It has also been suggested that transgression of the threshold of either somatically or germinally tolerable temperatures, particularly the latter, constitutes one of the major hazards to survival (*3*, *4*, *5*, *7*) and even may be the key to extinction in some the the major groups of terrestrial animals (*2*; for contrasting views, see *1*). In these papers it has also been stated by the present writer that the existence of heat sensitivity in male germinal processes, in combination with the thermal progression seeming to characterize major groups of terrestrial animals, may have expedited the process of evolution on land.

Thinking along this line inevitably points to the importance of investigating all instances in which there is an appearance of testicular thermal tolerance equal to that of the somatic cells.

Among terrestrial animals, the exceptionally high normal body temperatures of the birds, especially when considered in conjunction with the internal location of their testes, would seem to indicate that they would fit this category since their spermatogenic activity must take place at abnormally high temperatures. However, until additional observations have been made, this will remain only a partially acceptable assumption. The universality of heat susceptibility in other groups and Riley's findings on interrupted spermatogenesis (*9*) suggest the possibility that temperature may influence spermatogenesis in the English sparrow, and this view is strengthened through the finding by Cowles and Nordstrom (*8*) of an avian analogue to the mammalian phenomenon of testicular descent and possible scrotal thermoregulation. In this avian phenomenon it was demonstrated that the testes of Brewer's blackbird migrate a short distance, so that, while they are spermatogenetically active, they lie between two folds of the abdominal air sac.

Following the same lines of thought that led to investigations on the air sac-testes relationship in birds, it became apparent that a somewhat similar situation might be encountered in some Nearctic bats.

Several species of temperate-zone bats are known to breed in late summer or early fall, and throughout the summer months many of them are daytime occupants of hot attics; yet, despite this exposure to theoretically unfavorable temperatures, they are among the minority of nonscrotal vertebrate animals which reach the peak of spermatogenic activity toward the end, rather than before, the season of maximum summer temperatures. This situation is in marked contrast to conditions found in other terrestrial organisms, since the great majority of birds, most nonscrotal mammals, reptiles, and amphibians give at least the appearance of requiring a prolonged period of cool or cold winter weather prior to resumption of spermatogenesis. This condition (winter rest?) strongly suggests that there is need of moderate thermal conditions for testicular rehabilitation from heat effects. It seems possible that our long, hot summers may explain the widespread regression of the testes characterizing so many temperate-zone animals, a condition that frequently sets in not long after the onset of hot summer weather. Certainly there is a strong resemblance between this situation and that produced in *Xantusia vigilis* by artificial heat sterilization (*7*), and a similar condition is illustrated by the 13-lined ground squirrel (*10*).

Because of the effectiveness of their thermoregulatory device, scrotal mammals seem not to be so rigorously limited to early spring or summer breeding. Although exceptions to this general rule of postwinter breeding are known, they are not numerous. However, because of the known effects of light on gonadal activities it will be necessary to test the respective missions of these two factors.

Although Nearctic bats are hemipoikilothermic organisms, they are characterized by testicular descent and testes migration into the tail membrane, the uropatagium, where cooler conditions should prevail. In spite of this presumable protection, it is possible that this effect might be canceled by two heating factors: (1) the nature of their daytime retreats, which might prevent the normal daytime fall in temperature; and (2) their heat-generating nocturnal activities, chiefly the pursuit of food, which is to a large extent captured while in flight. The importance of this latter factor is accentuated by the bat's notable capacity for heat generation, a requisite for these animals to enable them to preheat their bodies in preparation for flight. Taken together, these two conditions suggest that the bats may either furnish an example of an animal that has succeeded in achieving identical somatic and spermatogenic heat tolerance or that they have some unusually effective means for heat dissipation and thermal regulation.

For a preliminary excursion into the thermal relationships of these animals it was clear that the effectiveness of the flight membranes should be evaluated as heat-dissipating and thermoregulatory structures. These comparatively large areas are usually devoid of hair and are highly vascularized and thus suitable for heat exchange mechanisms. A preliminary rough survey of ratios between the surface areas of the furred body and the naked flight membranes shows that these proportions range from 4:1 to as much as 8:1 in Nearctic bats but 12:1 in the tropical fruit bats.

In the common and rather typical bat, *Myotis yumanensis sociabilis*,[1] a superficial observation on the degree of vascular change in the network of blood vessels in the flight membranes clearly revealed marked changes in the amount of blood passing through these tissues. These changes were readily correlated with body temperatures.

In experiments conducted up to the present time it has been found that engorgement of the vascular plexus in the membranes is induced by elevating the body temperature to be-

[1] For the identity of this bat and for information on the position of the testes in the uropatagium I am indebted to Mr. Kenneth Stager, of the Los Angeles Museum.