References and Notes

1. J. B. Ranck, Jr., and W. F. Windle, *Exp. Neurol.* **1**, 130 (1959); W. F. Windle, H. N. Jacobson, M. I. Robert de Ramirez de Arellano, C. M. Combs, *Res. Publ. Ass. Res. Nervous Mental Dis.* **39**, 169; M. D. Faro and W. F. Windle, *Exp. Neurol.* **24**, 38 (1959); W. F. Windle, *Science* **140**, 1186 (1963).
2. C. J. Bailey and W. F. Windle, *Exp. Neurol.* **1**, 467 (1959).
3. Marked cerebral atrophy and neuronal loss from other causes can occur without hemorrhages [J. S. Munroe and W. F. Windle, in *Recent Developments in Comparative Medicine* (Academic Press, London, 1967), pp. 401–406].
4. Some of these findings were reported in discussions of papers at scientific meetings [E. Hibbard and W. F. Windle, *Physiologist* **4**, 47 (1961); A. Dekaban and W. F. Windle, *J. Neuropathol. Exp. Neurol.* **21**, 305 (1962); W. F. Windle, in *Brain Damage in the Fetus and Newborn from Hypoxia or Asphyxia* (Ross Laboratories, Columbus, Ohio, 1967), pp. 26–27].
5. M. I. Robert de Ramirez de Arellano and W. F. Windle, *Trans. Amer. Neurol. Ass.* **86**, 157 (1961).
6. W. F. Windle, *Develop. Med. Child Neurol.* **8**, 129 (1966).
7. J. Cammermeyer, *Amer. Med. Ass. Arch. Neurol. Psychiat.* **70**, 54 (1953); *Acta Psychiat. Neurol. Scand.* **28**, 9 (1953).

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Neutrophils: Their Role in the Formation of a Tick Feeding Lesion

Abstract. *Dogs infested with adult* Rhipicephalus sanguineus *were given nitrogen mustard to reduce leukocyte numbers. In the treated animals the tick lesions were insignificant, lacking the collagen destruction found in untreated hosts, but the ticks engorged normally. Feeding results from tick secretions causing vascular trauma and is independent of tissue damage associated with inflammatory responses.*

Some authors have suggested that the feeding lesions of ticks result from extra-oral digestion through the action of cytolysins in their salivary secretions (*1*). However, a study of the development of the feeding lesions caused by *Boophilus microplus* suggested that "specific vascular damage results from the saliva of the tick while tissue damage is caused by the host response" (*2*). This hypothesis was based on the finding that collagen destruction beneath the mouthparts of the tick was preceded by an intense infiltration of polymorphonuclear neutrophil leukocytes (PMN), a finding also noted with other Ixodidae. This infiltration is similar to that of the Arthus and Schwartzman reactions (*3*) where it is accepted that the intense infiltration of the involved tissues by PMN's leads to necrosis. Under conditions of polymorphonuclear leukopenia, necrosis does not occur (*3*).

To study the possible role of PMN in tick feeding, a leukopenia was induced by the administration of nitrogen mustard in four dogs infested with *Rhipicephalus sanguineus*.

Intravenous injections of mustine hydrochloride (Boots Pure Drug Co.) in distilled water (1 mg/ml) were given to two dogs at a rate of 1.75 mg/kg, while two other dogs received 2.0 mg/

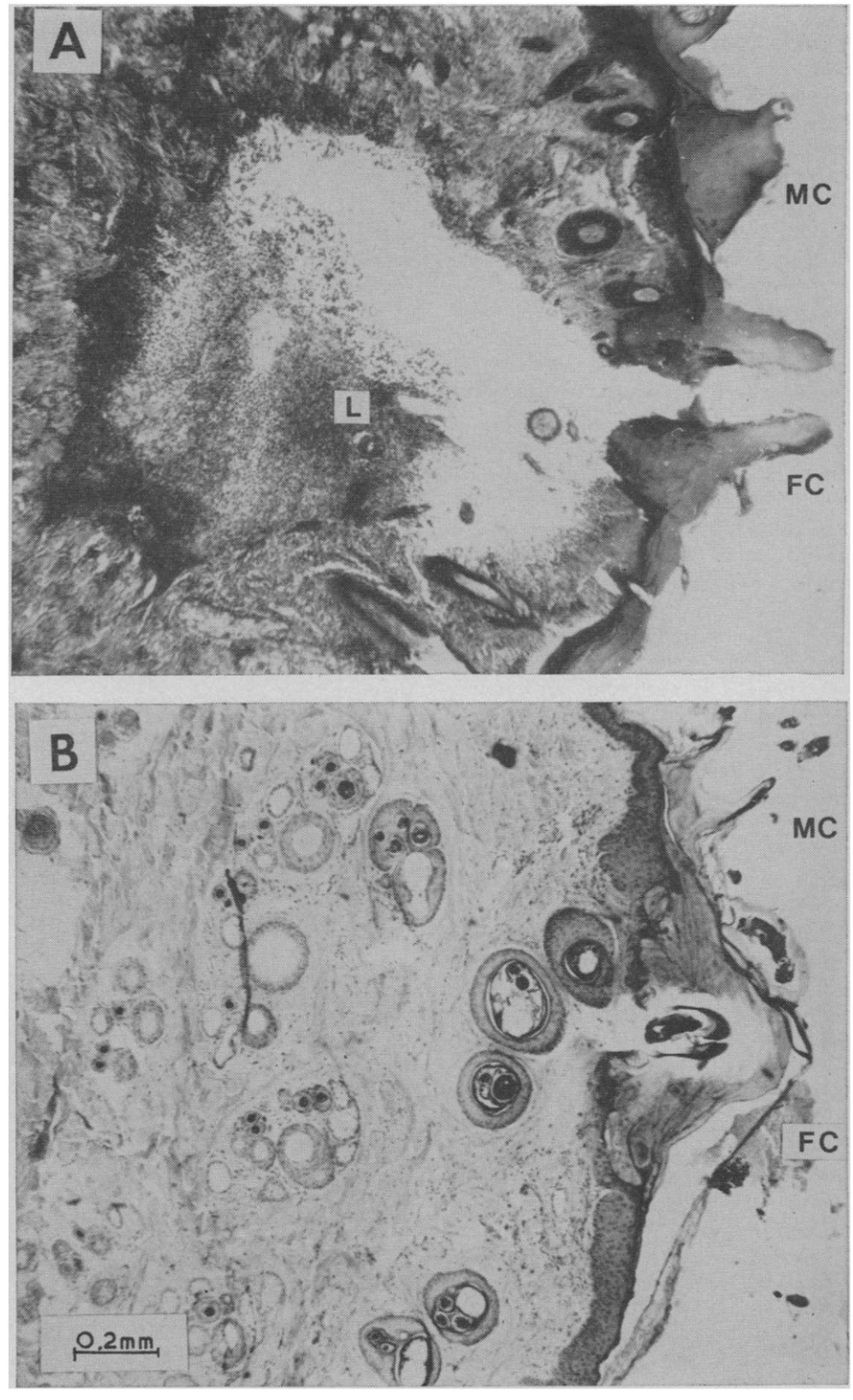

Fig. 1. Sections through the skin of dogs at sites of attachment of engorged females with associated males of *Rhipicephalus sanguineus*. (A) On a normal untreated dog. (B) On a dog treated with nitrogen mustard. *MC*, Cement of male tick; *FC*, cement of female; *L*, lesion.

kg and 2.5 mg/kg, respectively. Twenty-four hours before administration of the drug, the first two dogs were infested with unfed male and female *R. sanguineus* collected locally 7 days earlier. The other two dogs were similarly infested 30 minutes after treatment. We used both sexes of *R. sanguineus* because the full development of the adult feeding lesion is dependent on prior fertilization (*4*). For controls, two untreated dogs were also infested.

Skin biopsies including the attached ticks were taken daily from the two animals receiving the higher dosages of the drug and at the time of full engorgement of the ticks from the other dogs; the biopsies were processed as described previously (*5*).

In all dogs treated with nitrogen mustard, a decrease in the number of circulating leukocytes had occurred by day 3, and by day 5 or earlier the number was reduced to 2.2 to 7.6 percent of the amount present before treatment. Similarly, sections where the tick was attached, at all stages of its feeding, showed that PMN's were rare in the leukocyte-depleted animals, but there were heavy infiltrations of PMN's in the untreated animals. At the time of full engorgement of the tick, the untreated dogs had true cavities, averaging 1.3 mm in diameter, formed by collagen destruction below the mouthparts (Fig. 1A).

In the treated dogs (Fig. 1B) such cavities were either absent or insignificant. The dermal collagen appeared normal, but those capillaries and other blood vessels near the mouthparts were grossly dilated and hemorrhagic. Likewise, hair follicles and associated glands were unaffected. The ticks, as noted above, engorged naturally on the experimental animals and eventually laid eggs from which viable larvae hatched. From one animal, biopsies were also taken as the number of leukocytes returned to normal. Here the feeding lesions showed normal cavities infiltrated with PMN's together with depositions of secondary cement. These secondary cement depositions are invariably produced just before the final engorgement on normal animals. They were absent in the lesions of the treated dogs during the period of leukocyte depression. This supports our belief (*5*) that the function of secondary cement is to overcome mechanical instability resulting from the enlarging cavity.

Lesion formation is apparently a result of damage induced by the host's own inflammatory response and is not caused by the histolytic action of the salivary secretions of the tick. This is in keeping with the observations (*1*) that blood cells originating from these intense necrotic foci are ingested apparently unaltered. It is proposed that the ticks on treated dogs fed on hemorrhage induced by substances in tick saliva which exerted an effect on blood vessels.

These findings aid in the understanding of disease transmission. Thus, it has been suggested (*6*) that the infective form of *Theileria parva* is transferred by female *Rhipicephalus appendiculatus* from Till's type III acini (*7*) of the salivary gland at the time when supposed histolytic enzymes are being produced before the rapid final engorgement. However, some studies suggest that the salivary glands of *R. appendiculatus* and *R. sanguineus* are similar and that Till's type III acini contain the precursor of the cement material. The time of transmission is, therefore, better correlated with the deposition of the secondary cement.

We have seen these intense infiltrations of PMN's with a wide range of species of ticks; this suggests that much of the tissue destruction associated with ixodid feeding could be of host origin.

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References and Notes

1. D. R. Arthur, *Advan. Parasitol.* **3**, 249 (1965).
2. R. J. Tatchell and D. E. Moorhouse, *Parasitology* **58**, 441 (1968).
3. L. Thomas in *The Inflammatory Process*, B. W. Zweifach, L. Grant, R. T. McCluskey, Eds. (Academic Press, New York, 1965), p. 449.
4. J. S. C. Pollock and K. C. K. Williams, *Proc. Aust. Vet. Ass.*, in press.
5. D. E. Moorhouse and R. J. Tatchell, *Parasitology* **56**, 623 (1966).
6. L. P. Joyner and R. E. Purnell, *ibid.* **58**, 715 (1968).
7. W. M. Till, *Mem. Entomol. Soc. S. Afr.* **6**, 1 (1961).
8. We thank the Australian Meat Research Committee for their financial support, and Mrs. C. H. Carlisle for professional assistance in the administration of the drug.

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Alanine: Key Role in Gluconeogenesis

Abstract. *Of 20 amino acids measured, alanine is the principal amino acid released by forearm muscle of man, in accord with its being the principal amino acid extracted by liver for gluconeogenesis. This occurs in both the postabsorptive state and after 4 to 6 weeks of starvation, when total amino acid release is markedly diminished.*

Starvation necessitates endogenous glucose synthesis for metabolism mainly by brain. With prolonged starvation, man's brain adapts to consumption of acetoacetate and β-hydroxybutyrate as fuel; this adaptation diminishes the need for gluconeogenesis (*1*). Concomitantly, there is a decrease in urinary nitrogen and hepatic glucose output. This decrease in the rate of gluconeogenesis appears to result from a reduction in alanine supply to the liver, alanine being the principal amino acid extracted from man's splanchnic circulation in both postabsorptive and prolonged starved states (*2*). The primacy of alanine as a gluconeogenic precursor prompted examination of its source.

Two groups were studied, one composed of six subjects of normal weight and two obese subjects (20 to 45 years) and the other composed of seven obese subjects (23 to 40 years) whose body weight was 140 percent of ideal or greater (*3*). Differences of amino acids and glucose between arteries and veins across the forearm muscle and the blood flow in the forearm were determined (*4*). The first group was studied after

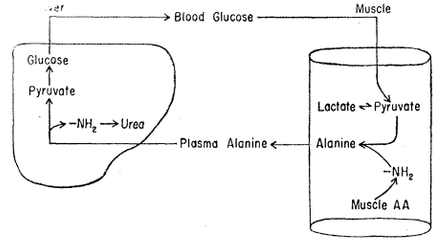

Fig. 1. Postulated cycle whereby alanine serves to convey amino groups and carbon substrate from muscle to liver for conversion to urea and glucose, respectively. Alanine is resynthesized in muscle by transamination of pyruvate derived from glucose, or from pyruvate derived from metabolism of other amino acids. *AA*, Amino acids.