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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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

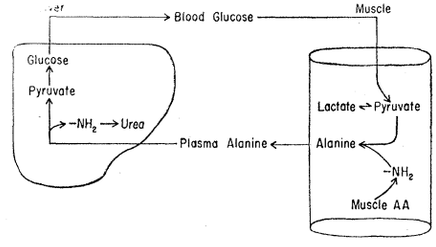

Fig. 1. Postulated cycle whereby alanine serves to convey amino groups and carbon substrate from muscle to liver for conversion to urea and glucose, respectively. Alanine is resynthesized in muscle by transamination of pyruvate derived from glucose, or from pyruvate derived from metabolism of other amino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

Table 1. Arterial concentrations and arterio–deep venous differences (A − DV) of plasma amino acids in the postabsorptive state and after prolonged (4 to 6 weeks) starvation. The arteriovenous difference of glucose (milligrams per 100 ml) was 3.8 ± 0.6 (mean ± S.E.) for the postabsorptive state and 1.6 ± 0.7 after starvation. The plasma flow (milliliters per minute per 100 ml of forearm) was 2.0 ± 0.3 for the postabsorptive state and 2.1 ± 0.5 after starvation. The results in the table are expressed as micromoles per liter (mean ± S.E.) Tryptophan was present in such low concentrations in the specimens from the starvation group that integration of its peak was not possible.

Amino acid	Postabsorptive ($N = 8$)		Starvation ($N = 7$)	
	Arterial	A − DV	Arterial	A − DV
Taurine	41.9 ± 3.8	− 3.0 ± 1.4*	51.8 ± 5.0	+ 3.0 ± 3.0
Threonine	129.1 ± 11.7	−28.0 ± 6.0†	172.7 ± 20.1	−10.6 ± 5.1*
Serine	111.6 ± 6.3	− 6.6 ± 5.5	114.8 ± 29.2	+ 1.1 ± 5.6
Proline	181.8 ± 16.6	−29.1 ± 9.7†	130.9 ± 12.3	− 5.6 ± 7.2
Citrulline	34.1 ± 2.1	+ 2.5 ± 1.2*	17.0 ± 2.5	− 0.4 ± 1.0
Glycine	178.8 ± 6.3	−35.8 ± 6.6‡	286.7 ± 28.1	−18.9 ± 9.7*
Alanine	240.3 ± 19.8	−111.4 ± 19.7‡	113.3 ± 10.4	−29.0 ± 9.0†
α-Aminobutyrate	19.4 ± 3.2	− 1.7 ± 0.7*	26.7 ± 3.2	+ 0.4 ± 1.5
Valine	245.1 ± 13.0	−15.5 ± 5.3*	141.6 ± 17.3	− 2.4 ± 4.4
Cystine	93.1 ± 8.1	+ 9.3 ± 2.9†	66.3 ± 5.3	+ 2.8 ± 2.1
Methionine	21.8 ± 0.8	− 5.8 ± 0.9‡	19.7 ± 2.2	− 3.1 ± 1.7
Isoleucine	59.5 ± 4.7	−10.9 ± 4.3*	55.3 ± 8.0	− 2.7 ± 1.4
Leucine	124.0 ± 6.9	−13.8 ± 3.3†	64.7 ± 13.9	− 5.9 ± 1.8†
Tyrosine	55.1 ± 4.3	− 8.9 ± 1.6‡	42.3 ± 1.5	− 3.0 ± 1.0*
Phenylalanine	50.1 ± 1.4	− 7.8 ± 1.6†	37.6 ± 2.8	− 2.9 ± 1.2*
Ornithine	57.5 ± 4.4	− 2.1 ± 2.2	32.9 ± 4.7	0 ± 1.0
Lysine	181.4 ± 8.7	−37.1 ± 9.0†	136.4 ± 9.5	−12.0 ± 1.5‡
Histidine	76.8 ± 3.3	−14.1 ± 2.9†	51.3 ± 4.8	− 4.1 ± 1.5*
Tryptophan	38.1 ± 2.1	− 4.2 ± 1.6*		
Arginine	78.5 ± 6.1	−23.3 ± 4.6†	43.1 ± 4.4	− 2.0 ± 1.6

* $P < .05$. † $P < .01$. ‡ $P < .001$. (Probability that A − DV is not different from zero.)

the subjects were fasted overnight, and the second after 4 to 6 weeks of total starvation (except for supplementary NaCl, KCl, and a daily multivitamin tablet). Blood samples were prepared for analyses of plasma amino acids and glucose as previously described (*2*, *5*).

In the postabsorptive state there was a significant release from resting forearm muscle of 16 amino acids, as indicated by the negative arteriovenous differences (Table 1). Of the remaining four amino acids, the net balance of serine and ornithine was not significantly different from zero, while a net uptake was observed for citrulline and cystine. Alanine release was far greater than that of other amino acids measured.

After prolonged starvation, muscle amino acid release decreased markedly (Table 1). Significant outputs were no longer demonstrable for taurine, proline, α-amino butyric acid, valine, methionine, isoleucine, and arginine. The magnitude of release of those amino acids for which a significant negative arteriovenous difference was still demonstrable fell by 60 to 75 percent. The absolute decline in output was greatest for alanine, which fell from −111 μmole/liter in the postabsorptive state to −29 μmole/liter during starvation. As in previous studies (*2*), the arterial concentration of most amino acids decreased after prolonged fasting, while that of glycine and threonine increased. There is also a significant decrease in glucose extraction ($P < .05$). Blood flow was unchanged.

Alanine comprises approximately 5 to 7 percent of muscle protein (*6*) and also is concentrated in free form within the muscle cell. Catabolism of a unique protein containing mainly alanine or dissipation of the free intracellular pool could explain the disproportionately large alanine output in the postabsorptive state. The continued predominance of alanine after 4 to 6 weeks of starvation, however, implies its formation in muscle *de novo*, presumably by transamination of pyruvate. Since during prolonged fasting the liver is enzymatically capable of high rates of gluconeogenesis, as evidenced by acutely accelerated glucose synthesis from intravenously administered alanine (*7*), the current data suggest that formation and release of alanine from muscle is the rate-limiting step for overall gluconeogenesis in starvation. A similar emphasis on the role of alanine concentration in controlling hepatic gluconeogenesis has been made (*8*) on the basis of liver perfusion studies in vitro.

Alanine also may serve an important role in the transport of ammonia from periphery to liver. In this context the following cycle may be operative: (i) uptake of glucose into muscle, (ii) glycolysis to pyruvate, (iii) formation of alanine and release into the circulation, (iv) uptake of alanine into the liver, and (v) formation of urea and glucose for release (Fig. 1). This cycle, although useful for ammonia transport, would not provide net substrate for gluconeogenesis *de novo* and is therefore analogous to the Cori cycle. For net gluconeogenesis, pyruvate must be formed from noncarbohydrate precursors in muscle. The responsible enzymatic processes involved in this alanine formation and release and their substrate and hormonal controls have yet to be defined.

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