Table 1. $Q(O_2)$ VALUES OF FRESH NORMAL AND RACHITIC RAT PALATAL MUCOSA

	No substrate added	Phosphorylated vitamin D added	Succinate added	Succinate + phosphorylated vitamin D added	Malate added	Malate + phosphorylated vitamin D added
Normal rat mucosa	1·96 ± 0·03 (35)	2·13 ± 0·016 (18)	3·34 (3)	3·53 (3)	1·94 (3)	2·29 (3)
Rachitic rat mucosa	1·92 ± 0·02 (17)	1·94 ± 0·018 (7)	2·65 (3)	3·92 (3)	2·53 (3)	4·49 (3)

1 ml. of $M/50$ solutions of various intermediates of the Krebs citric acid cycle were added to the reaction flasks in a total volume of 2·55 ml. 0·05 ml aqueous 0·5 per cent phosphorylated vitamin D solution was introduced where indicated. Figures in brackets indicate number of observations. Where only three observations were made the mean value is given and the t test was applied to the results which were found to be significant at the 5 per cent level for succinate and the 3 per cent level for malate in the case of rachitic tissue.

was used. This action of vitamin D was found only in fresh tissue, since after storage for one day at 4° C. no such effect on oxygen uptake could be detected although the metabolites still increased the oxygen consumption above the basal value.

Zetterström[9] has described an activation of aerobic oxidation in kidney mitochondria by phosphorylated vitamin D using glutamic acid as oxidizable substrate. De Luca *et al.*[10], found that vitamin D suppressed citrate oxidation in kidney mitochondria of normal and rachitic rats, but no effect of vitamin D on citrate oxidation was observed in liver mitochondria of these animals.

The above observations seem to be at variance as to the precise mode of action of vitamin D in metabolic processes. Our results would suggest that this vitamin may play an important part in the direct regulation of the oxidative phase of carbohydrate metabolism. However, the possibility must be considered that vitamin D is exerting its influence indirectly by affecting the permeability of the metabolites of the citric acid cycle through the cell wall or mitochondrial membrane.

Further work is in progress to elucidate this mechanism.

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[1] Glickman, I., Turesky, S., and Hill, R., *J. Dent. Res.*, **28**, 83 (1949).
[2] Glickman, I., Turesky, S., and Manhold, J. H., *J. Dent. Res.*, **29**, 429 (1950).
[3] Zar, L., and Irving, J. T., *S. Afr. J. Med. Sci.*, **23**, 102 (1958).
[4] Zar, L., and Irving, J. T. (unpublished).
[5] Bacharach, A. L. (private communication).
[6] Coward, K. H., "The biological standardisation of the vitamins", second ed. (London, Bailliere, Tindall and Cox, 1947).
[7] Zetterström, R., *Nature*, **167**, 409 (1951).
[8] Umbreit, W. W., Burns, R. H., and Stauffer, J. F., "Manometric Techniques", pp. 13, 149 (Burgess Publishing Co., Minneapolis, 1957).
[9] Zetterström, R., *Acta Chem. Scand.*, **5**, 343 (1951).
[10] De Luca, H. F., and Steenbock, H., *Science*, **126**, 258 (1957).

Blood Acetylcholinesterase in *Phalaris* Staggers

RUMINANTS grazing pastures containing a large proportion of growing *Phalaris tuberosa* on occasions develop a nervous disorder, phalaris staggers[1,2], the symptoms of which are an initial hyperexcitability followed by muscular tremors, unco-ordinated gait and stumbling or falling when driven. Demyelination in the spinal cord ensues and complete recovery has not been observed. On the grounds that similar symptoms and pathological changes occur in cats[3], dogs[4] and hens[5] treated with cholinesterase inhibitors, Dr. J. H. Gaddum (personal communication to Dr. H. R. Marston) suggested that some knowledge of the cholinesterase activity in the blood of animals suffering from phalaris staggers might illuminate the origin of the disorder.

Blood was collected into heparinized tubes from the jugular veins of sheep that had recently developed visible symptoms of the malady and which were still grazing on *P. tuberosa*. Control samples of blood were collected from animals grazing the same pasture but which were protected from staggers by prior administration of cobalt pellets[6]. Further controls were obtained from penned animals fed on a wheaten hay chaff-gluten diet.

Acetylcholinesterase was measured in whole blood by Ammon's[7] method after dilution 1 in 5 with distilled water to dilute the enzyme suitably and to hæmolyse the red cells.

The results obtained (Table 1) show that no significant difference exists between the blood acetylcholinesterase activities in normal and staggering sheep. These findings support results obtained by Lee and Good (unpublished) who demonstrated that the blood cholinesterase level of sheep could be reduced to a very low value by feeding the powerful anticholinesterase ethylthioglycol diethyl thiophosphate (Systox) without producing symptoms of nervous debility.

Table 1. ACETYLCHOLINESTERASE ACTIVITY OF BLOOD OF NORMAL AND STAGGERING SHEEP

	Normal	Staggering
Number sampled	12	12
Range of values obtained*	35–56	33–62
Mean value	48·2500	45·1667
Standard deviation	3·3844	
't' value $\left\{\frac{\text{difference of the means}}{\text{standard deviation}}\right\}$	0·91 (not significant)	

* Activity expressed as μmoles acetylcholine hydrolysed/hr./ml. blood.

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[1] McDonald, I. W., *Aust. Vet. J.*, **18**, 12 (1942).
[2] Lee, H. J., and Kuchel, R. E., *Aust. J. Agric. Res.*, **4**, 88 (1953).
[3] Feldberg, W., and Sherwood, S. L., *J. Physiol.*, **125**, 488 (1954).
[4] Koelle, G. B., and Gilman, A., *Pharmacol. Rev.*, **1**, 166 (1949).
[5] Barnes, J. M., and Denz, F. A., *J. Path., Bact.*, **65**, 597 (1953).
[6] Dewey, D. W., Lee, H. J., and Marston, H. R., *Nature*, **181**, 1367 (1958).
[7] Ammon, R., *Arch. f. d. ges. Physiol.*, **233**, 486 (1934).