were getting a continual small supply of 'oestrogenic substances' from the diet. Bennetts *et al.*⁴ demonstrated that sheep grazing a pasture of subterranean clover could ingest sufficient substance of this type to bring about symptoms of oestrogen hyperactivity. The work of Phillips *et al.*⁵ directs attention to the effect of climatic conditions on the distribution of both the rate of calvings and of lamb-weights in West Wales. It seems that climatic conditions affect farm animals both directly, and indirectly, through the pasture. Apart from the general basic nutritive differences, it is possible to predict that factors such as the above should be taken into account in assessing these problems.

I. ANTICE EVANS
W. CHARLES EVANS

Department of Animal Health,
University College of Wales,
Aberystwyth.
Dec. 14.

¹ Morris, S., and Wright, N. A., *J. Dairy Res.*, **4**, 177 (1933); **5**, 1 (1934). Morris, S., Wright, N. A., and Fowler, A. B., *ibid.*, **7**, 97 (1936).

² Friedman, M. H., and Friedman, G. S., *Amer. J. Physiol.*, **125**, 486 (1939). Friedman, M. H., and Mitchell, J. W., *Endocrin.*, **29**, 172 (1941). Mitchell, J. W., Borasky, R., and Bradbury, J. T., *ibid.*, **31**, 283 (1942). Bodenheimer F. S., and Sulman, F., *Ecology*, **27**, 255 (1946).

³ Bartlett, S., Folley, S. J., Rowland, S. J., Curnow, D. H., and Simpson, S. A., *Nature*, **162**, 845 (1948).

⁴ Bennetts, H. W., Underwood, E. J., and Shier F. L., *Aust. Vet. J.*, **2**, 2 (1946).

⁵ Phillips, R., and Davies, J. L., *Nature*, **160**, 429 (1947). Phillips, R., "Fat Lamb Production in West Wales" (unpublished).

The Tricarboxylic Acid Cycle in Nematode Parasites

In nematode parasites the anaerobic breakdown of glycogen to pyruvate and lactate with a consequent formation of adenosine triphosphate follows a course similar to that in vertebrate muscle¹. Since it appears that aerobic mechanisms may play an important part in the metabolism of certain nematode parasites², studies on the carbohydrate metabolism of the parasites have been extended to include some aspects of the oxidative utilization of pyruvate. The organisms used were *Ascaridia galli* from the chicken small intestine and *Nematodirus spathiger* and *N. filicollis* from the sheep small intestine. Some experiments were also carried out with *Neoplectana glaseri*, a parasite of the Japanese beetle, which, however, was grown *in vitro* for the present experiments. Owing to its relatively large size and its position in the host, *A. galli* may not have an active aerobic metabolism *in vivo*. However, it has been shown that *Nematodirus* spp. probably respire actively *in vivo*, and oxygen is essential for the survival *in vitro* of *Neoplectana glaseri*.

Experiments were carried out using brei prepared from whole parasites suspended in Krebs-Ringer phosphate buffer at pH 7.3. The results were not appreciably affected by omitting calcium from the medium. Oxygen consumption at 38° C., determined by the 'direct' method of Warburg, varied somewhat with different lots of parasites, but Q_{O_2} values (μ l. of oxygen taken up per hour per mgm. dry weight, taken over the first period of 30 min.) were usually in the region of 3.8 (*Nematodirus* spp.), 4.0 (*Neoplectana glaseri*), or 4.2 (*A. galli*). Cytochrome c or methylene blue increased oxygen uptake, and, as a routine measure, experiments were carried out with the brei in *M*/3,000 methylene blue.

Co-factors such as co-carboxylase, coenzymes I and II, pantothenate, riboflavin, nicotinic acid and pyridoxine did not markedly affect the oxygen uptake. However, in increasing order of activity the substrates pyruvate, malate, α -ketoglutarate, fumarate and succinate, at concentrations of *M*/100, all caused increased oxygen uptake. Oxalacetate (*M*/1,000) caused a stimulation of the same order as pyruvate. Citrate (*M*/100) had little effect. These results suggest that some form of Krebs tricarboxylic acid cycle was functioning in the parasite tissues. However, compared to pigeon breast muscle, the parasite tissue was relatively insensitive to malonate. Further, results taken over the first 30 min. of an experiment always showed that malonate inhibition was increased in the presence of added succinate. When the period of observation was confined to the first 10 min. of the experiment, the effect of succinate on malonate inhibition was less marked; but as a rule, as shown in the accompanying table, inhibition was increased. Pyruvate, fumarate and malate, at concentrations of *M*/100, all markedly reduced the inhibition caused by *M*/50 malonate. One interpretation of these results would be that, though a form of the Krebs tricarboxylic acid cycle is functional in the parasite tissues, succinate oxidation, relative to the total oxidative activity, is less important than in pigeon breast muscle.

Effect of malonate and succinate on the oxygen consumption of brei from pigeon breast muscle and nematode parasites. The figures give the percentage inhibition found during the first period of 10 min.

Conditions	Pigeon breast muscle	<i>Nematodirus</i> spp.	<i>Ascaridia galli</i>	<i>Neoplectana glaseri</i>
Malonate, <i>M</i> /100	50-70	25	11	8
Malonate, <i>M</i> /100 succinate, <i>M</i> /100	—	40	43	16
Malonate, <i>M</i> /1000	30	0-10	0-10	0
Malonate, <i>M</i> /1000 succinate, <i>M</i> /1000	5			

The difference in the reaction of the parasite tissues and pigeon breast muscle to malonate was also examined by determining the accumulation of succinate, by the method of Krebs³, in brei from the different organisms which had been incubated for 1 hr. at 38° C. In the absence of malonate, no succinate was found in the parasite preparations and very little in the breast muscle. However, in *M*/100 malonate, succinate accumulated in the muscle brei but still was not found in the parasite material. Even when *M*/100 malonate and *M*/100 fumarate were added to the parasite brei, succinate accumulation did not occur or was very small indeed.

A detailed account of this work, which was part of the research programme of the Division of Animal Health and Production, Council for Scientific and Industrial Research, will be published elsewhere. A note on the nature of substrates, other than those of the Krebs tricarboxylic acid cycle and the mechanism by which they are metabolized by the parasites, will be published shortly.

VINCENT MASSEY
(Ian McMaster Fellow)
W. P. ROGERS

McMaster Laboratory,
Division of Animal Health and Production,
C.S.I.R., Sydney.
Nov. 26.

¹ Rogers, W. P., and Lazarus, M., *Parasitology* (in the press).

² Rogers, W. P., *Nature* [163, 879 (1949)].

³ Krebs, H. A., *Biochem. J.*, **31**, 2095 (1937).