LETTERS TO THE EDITOR

FAILURE OF *CLOSTRIDIUM CHAUVOEI* VACCINES TO PROTECT AGAINST BLACKLEG

In the August 1977 issue of the *Australian Veterinary Journal*, Messrs Reed and Reynolds wrote concerning the failure of *Clostridium chauvoei* vaccines to protect against blackleg. They discussed possible explanations for the failure, and indicated that they had incorporated another strain into the vaccine to overcome the problem. A number of questions may be asked based on the contents of their letter. Some months after its publication a conference on clostridial vaccines was held in Melbourne. The report of that conference has not yet been made available; this is unfortunate as many readers who are interested in this subject and who were not invited to the conference, had hoped that this report would provide some of the answers to the questions raised in the letter by Messrs Reed and Reynolds.

In general these questions all relate to antigenic identification of *Cl. chauvoei*, the selection of protective strains and the evaluation of vaccines. Any rational discussion of a vaccine breakdown is difficult unless we know the nature of the protective antigens and the immune response to those antigens. Immunity to *Cl. chauvoei* is antibacterial rather than antitoxic, but the protective antigen(s) are not known. Therefore any standard vaccine strain can only be evaluated by animal inoculation and subsequent challenge, which conforms with the requirements of the British Veterinary Codex. Any strains which appear to overcome the immunity induced by the standard strain can be compared only by the same procedure. That is, while no knowledge of protective antigens exists, *Cl. chauvoei* strains cannot be compared as potential bacterins by their antigenic composition.

This ignorance is highlighted by the unfortunate situation described by Messrs Reed and Reynolds. If other commercially available vaccines were also associated with losses, it seems that either a strain(s) of *Cl. chauvoei* was encountered against which the immunity produced by the standard vaccine strain in

any of the commercial products was ineffective or that the strain(s) against which the vaccines are evaluated has varied. What evidence is there that field strains have changed? What strain(s) are used to evaluate vaccines and what steps are taken to preclude change in their antigenicity (as has happened with recognised challenge strains for other vaccines)? The Yeerongpilly isolate appears to be effective as a bacterin against available 'official' and field strains of *Cl. chauvoei*. This raises the question as to why 2 strains need be present in the currently available products? On what basis did the National Biological Standards Laboratory in Canberra sanction the inclusion of the Yeerongpilly isolate in the new vaccine?

The veterinary profession in this country prides itself on the contribution it has made to an understanding of the pathogenesis and control of clostridial disease. However, it is clear that there are still many areas open to investigation, and this is well illustrated by our present ignorance of the antigenic and immunogenic composition of such a well-known organism as *Cl. chauvoei*. The absence of freely available information on the subject is to be deplored. Our *ad hoc* approach to these problems is unlikely to be improved unless there is open discussion, exchange of results, and financial support for research into clostridial antigenic identification and immunity.

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3 April 1978

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THE EFFECTS OF BIOTIN ADMINISTRATION ON GLUCOSE SYNTHESIS RATES IN STARVED PREGNANT SHEEP

Fatty liver and kidney syndrome (FLKS) of chickens and pregnancy toxæmia of sheep are both characterised by low plasma glucose concentrations, low glucose synthesis rates and fatty infiltration of the liver and kidneys (see Balnave *et al* [1977a, b] for review of FLKS in chickens, and Reid [1968] for review of pregnancy toxæmia).

The specific activity of hepatic pyruvate carboxylase, an enzyme involved in gluconeogenesis and which requires biotin as a cofactor, is markedly reduced in chickens affected by FLKS. Incubation of liver slices from affected birds with biotin returns pyruvate carboxylase activity to normal (Bannister 1976). Biotin administration to chickens on diets where FLKS occurs prevents the onset of the condition and increases glucose synthesis rates, glucose pool sizes and plasma glucose concentrations (Balnave *et al* 1977b).

Since pyruvate carboxylase is a key rate-limiting enzyme in gluconeogenesis in sheep and since glucose availability appears to be implicated in pregnancy toxæmia (McClymont and Set-

chell 1955), the effects of biotin administration on the rate of glucose synthesis in starved, pregnant ewes was investigated.

Twenty Merino ewes and 20 Border Leicester/Merino crossbred ewes in moderate body condition were housed in the last 50 days of pregnancy and given free access to lucerne chaff. The animals were then divided into groups of 10 of the same breed and the sheep in one group from each breed were given a daily intramuscular injection of 500 µg of biotin for 14 consecutive days up to and including a starvation period of 48 h. The sheep in the remaining group from each breed were injected daily with saline. Several animals from each group were removed from the experiment because they had low feed intakes.

Glucose entry rates were measured 24 h and 48 h after removal of feed, using [2-³H] glucose as a single injection according to the methods of Judson and Leng (1972). Dates of lambing and numbers of lambs per ewe were recorded. Results are shown in the accompanying table.

TABLE 1

The Effects of Starvation and Injections of Biotin on Plasma Glucose Concentrations and Glucose Synthesis Rates in Merino and Border Leicester-Merino Crossbred Ewes Close to Lambing

Group	Ewes Bearing Single Lambs	Twin Lambs	Average No. of days from Lambing	Plasma Glucose Concen- tration (mg/100ml)	Glucose Synthesis rate (g/kg ^¾ /d)	Level of Significance †
<i>Merinos</i>						
24 h starvation						
Biotin	9	0	23	36.8 ± 1.3	7.4 ± 0.5	*
Placebo	9	0	31	35.5 ± 2.2	6.1 ± 0.4	
48 h starvation						
Biotin	9	0	23	35.6 ± 1.3	6.6 ± 0.4	***
Placebo	9	0	31	31.9 ± 2.3	4.8 ± 0.1	
<i>Crossbreds</i>						
24 h starvation						
Biotin	6	1	20	30.0 ± 1.3	5.4 ± 0.1	N.S.
Placebo	5	3	14	27.2 ± 1.6	4.8 ± 0.4	
48 h starvation						
Biotin	6	1	20	27.0 ± 1.4	4.8 ± 0.1	*
Placebo	5	3	14	24.5 ± 1.4	4.2 ± 0.2	

† Significance of differences in glucose synthesis rates.

* P < 0.05
*** P < 0.001

Glucose synthesis rates were significantly higher in both groups of biotin-treated ewes after 48 h starvation, the differences being 38% in the Merinos (P < 0.001) and 14% in the crossbred sheep (P < 0.05).

Pregnancy was more advanced in the Merino ewes injected with biotin but the reverse was the case with the crossbred ewes. The demand for glucose by the foetus increases as pregnancy advances (Kronfeld 1958), and although a pregnant sheep on a fixed feed intake increases its glucose entry rate as it approaches term, in the same sheep starved for four days there were no differences in glucose entry rates at any period of pregnancy (Steel and Leng 1968). The differences observed in the animals in this study are therefore unaffected by the time from lambing especially since at a maximum the mean difference was only about 8 days. Therefore it seems reasonable to suggest that the biotin increased glucose synthesis rates in both breeds of animals when starved for 48 h.

The results suggest that biotin may become a factor limiting the rate of gluconeogenesis in fasted pregnant sheep which have been on a high plane of nutrition. This may indicate a rapid loss of biotin in the tissues of pregnant sheep, particularly after the onset of starvation. Further studies are planned on the effects of biotin on glucose metabolism and the incidence of pregnancy toxæmia.

We would like to thank Roche Chemical Company for providing the injectable biotin and the Australian Wool Research

Corporation for financial support. Mr. J. Hiscox gave valuable technical assistance.

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17 February 1978

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ENTERIC CORONAVIRUS-LIKE PARTICLES IN SHEEP

An outbreak of diarrhoea in Merino x Corriedale weaner sheep on a property in Gippsland, Victoria, was investigated during November 1977. Approximately 10% of a flock of 1500 were affected. Although the faeces were fluid in nature and

greenish in colour, the sheep showed no ill-effects and recovered uneventfully within 3 to 4 days. At the time of the outbreak the pasture was drying off and conditions that may have predisposed to nutritional diarrhoea were considered