dition, 0.1% Mo, 0.15% W + 0.1% Mo and 0.15% W + 0.4% Mo respectively, whereas 0.15% W gave only 28 µg of N. With 50 ng of riboflavin/tube the tubes containing 0.15% W showed only 5 µg of N and the nitrogen in the other tubes was halved.

We believe that, considered in relation to our own earlier work and to that of others to whom we made reference (Owen & Proudfoot, 1968), the present results indicate that *L. casei* requires molybdenum. The implications of this finding on the microbiological assay of riboflavin will be discussed elsewhere.

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Growth of the Riboflavin-Splitting Rumen Organism *Eubacterium fissicatena* in the Presence of Tungstate and Molybdate

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Owen and his colleagues showed that ruminants produce metabolites of riboflavin and that these originate from bacteria in the gut (Owen & West, 1970). Owen, West & Coates (1970) found the chief of these metabolites, which Owen (1962) had identified as 10-(2'-hydroxyethyl)isoalloxazine (I), in conventional but not in germ-free rabbits, and West & Owen (1969) found the metabolites in the urine of man. Known species of rumen bacteria could not produce riboflavin metabolites (Hobson, Summers, Owen, Spencer & West, 1969), but t.l.c. of extracts from riboflavin-enriched cultures revealed new species of anaerobes that could produce compound (I). *Eubacterium fissicatena* n.s. (M. M. Taylor, D. W. West & E. C. Owen, unpublished work) is one of these.

We now report a study of this bacterium in the presence of molybdate and tungstate in two different media, A and B. Each medium contained Oxoid yeast extract, Oxoid proteose peptone, NaCl and riboflavin, but medium A also contained Oxoid liver digest, whereas medium B also contained rumen fluid, a salt mixture providing Mg^{2+} , Zn^{2+} , Mn^{2+} , Fe^{2+} , Cu^{2+} , Co^{2+} and MoO_4^{2-} , EDTA and phosphate buffer.

Turbidimetry of 20 ml cultures in Universal bottles and of uninoculated incubated blanks was used to assess growth at 37°C. The extinctions at 600 nm of seven triplicated cultures with neither molybdate nor tungstate averaged 0.478 (± 0.043 s.d.) for medium A and 0.791 (± 0.090 s.d.) for

medium B. Two series of cultures were grown in the presence of 0.2%, 0.4%, 0.6% and 0.8% Mo or W (calculated as the element), a third series had 0.2% Mo added at each concentration of tungsten and a fourth series had 0.2% W added at each concentration of molybdenum. Thus the Mo/W concentration ratio varied over the third and fourth series from 4:1 to 4:16.

Differences between the effects on growth of *E. fissicatena* of tungstate and molybdate in either medium were small, but molybdate or tungstate or their mixtures stimulated growth in medium A and diminished it in medium B, for the average extinctions of all the cultures, excluding those to which neither molybdate nor tungstate had been added, were 0.762 (± 0.061 s.d.) for medium A and 0.619 (± 0.046 s.d.) for medium B. Thus unlike *Lactobacillus casei* (see Owen & Pringle, 1970) *E. fissicatena* shows no antagonism between molybdenum and tungsten. It is of interest that McKillen & Spencer (1970) reported that *Salmonella typhimurium* is inhibited by molybdate.

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A Protected Intermediate for the Preparation of Semisynthetic Insulins

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Porcine insulin has only three amino groups, the α -amino groups of glycine A-1 and phenylalanine B-1 and the ϵ -group of lysine B-29 (Brown, Sanger & Kitai, 1955). Our experiments form part of an attempt to develop methods that make it unnecessary to synthesize a whole protein when a structure is required that departs only slightly from a naturally occurring one (Offord, 1969). In its application to insulin it resembles that of Brandenburg & Ooms (1968), who used the phenyl isothiocyanate procedure to attack, and remove, the N-terminal residues of both chains. They subsequently coupled an amino acid back to replace them. During this procedure lysine B-29 was (irreversibly) protected by acetylation.