(d) Rhamnose and glucosamine were fermented but no methane was produced.

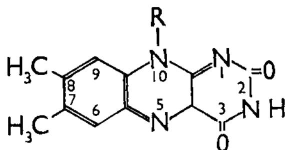
The fermentation of sucrose and its constituent hexoses was accompanied by a transient accumulation of lactate. On the other hand, fermentation of rhamnose and glucosamine resulted in a permanent accumulation of lactate. No increases in the amounts of lactate were observed when other sugars were fermented.

Although the overall rates of fermentation varied widely, the amount of methane produced was related to the amount of carbohydrate fermented, irrespective of the type of carbohydrate used as substrate, except for rhamnose and glucosamine. The mean amount of methane produced was equivalent to 6 cal/100 cal substrate fermented. The amount of steam-volatile acids formed was also directly related to the amount of carbohydrate fermented. The results are consistent with a hypothesis that methanogenesis involves a pathway that utilizes precursors which may result from the dissimilation of carbohydrates and other substrates by non-methanogenic micro-organisms.

REFERENCE

Czerkawski, J. W. & Breckenridge, G. (1969). *Br. J. Nutr.* **23**, 51.

A study of cultures of rumen anaerobic bacteria in the presence of excess riboflavine (vitamin B_2). By P. N. Hobson and R. Summers, *Microbiology Department, Rowett Research Institute, Aberdeen*, and E. C. Owen, Jane C. Spencer and D. W. West, *Biochemistry Department, Hannah Dairy Research Institute, Ayr*



For riboflavine (B_2) (7, 8-dimethyl-10[D-ribityl]-isoalloxazine) R is $-CH_2\,(CHOH)_3\,CH_2OH$
For hydroxyethylflavine (HEF) R is $-CH_2$, CH_2OH
For formylmethylflavine (FMF) R is $-CH_2$ CHO

Riboflavine (B_2) in the ruminant especially if fed in amounts in excess of requirement gives rise to metabolites in milk and urine the chief of which is HEF (Owen, 1962). Accompanying HEF is FMF the corresponding aldehyde (Owen & West, 1968). B_2 metabolites including FMF and HEF arise when rumen contents are incubated in vitro with B_2 but are not produced by the animal when B_2 is subcutaneously injected. This and other evidence now being prepared for publication indicate that rumen micro-organisms are responsible for HEF and FMF production. Pure cultures of rumen anaerobic bacteria were grown for 24 h in the presence of excess B_2. The bacteria were then separated by centrifugation from the supernatant medium and ground in a mortar with glass wool in the presence of 10% (w/v) trichloroacetic acid. The extract was neutralized and, using phenol, an aqueous concentrate was prepared (Owen, 1962). The supernatants and the extracts were chromatographed on thin filter paper in the upper phase of a mixture of n-butanol, acetic acid and water (4:1:5, v/v). On chromatograms isoalloxazines fluoresce

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yellow in ultraviolet light. Marker spots of B_2 and HEF were applied at the origin alongside the test spots. R_f values of B_2, HEF and FMF in this system are 0·40, 0·53 and 0·63 respectively but there were no yellow spots at or near 0·53 or 0·63 though the B_2 was prominent at 0·40. Yellow-fluorescent spots of R_f <0·40 were from the medium itself. All the bacteria except *Streptococcus bovis* showed a yellow-fluorescent spot at 0·87. The species tested were *Butyrivibrio* sp, *Lactobacillus bifidus*, *Escherichia coli*, *Veillonella gazogenes*, *Peptostreptococcus* sp, *Lact. fermenti*, *Strep. bovis* and *Selenomonas ruminantium*. However later isolations of rumen bacteria from B_2-enriched media have confirmed that some unidentified rumen bacteria can produce HEF or FMF or other metabolites of B_2. Isolated pure cultures of these are being studied for classification.

REFERENCES

Owen, E. C. (1962). *Proc. int. Congr. Fd Sci. Technol.* 1. *London.* Vol. 3, p. 669.
Owen, E. C. & West, D. W. (1968). *J. chem. Soc.* (C) p. 34.

A hypothetical mechanism for the regulation of food intake in relation to energy balance. By G. R. HERVEY, *Department of Physiology, School of Medicine, University of Leeds*

Animals take in and expend amounts of energy which over long periods are found to be equal within the limits of practical measurement. Since any difference between energy gain and loss necessarily alters the store of energy in the body, and this is in chemical form, the maintenance of a near-constant adult body-weight depends upon maintaining energy balance. If the difference between energy intake and output is calculated from the changes which typically do occur in body-weight over significant parts of the life-span, the error of balancing, as a fraction of the total turnover, is seen to be extremely small. Near-equality of energy intake and output could be achieved by physiological regulation of either or both. Adjustment of food intake in relation to energy balance can be readily demonstrated, for example by changing the calorie concentration of the diet; adjustment of energy expenditure occurs if intake is restricted, but is otherwise controversial.

Whatever the effector, the information used in energy balancing is a problem. Since there is no correlation between energy intake and output over short periods such as a day, and the accuracy of adjustment is higher the longer the period considered, one possibility is that the level of energy stores in the body is monitored. It is the purpose of this communication to suggest how this could be done. The proposal stems from two lines of experiment: (1) experiments with parabiotic rats suggesting a blood-borne signal to the hypothalamus; (2) the apparently centrally integrated effects of hormonal and synthetic steroids on body-weight, fat content, food intake and activity.

The suggestion is that a humoral agent could measure the amount of fat in the body by the dilution principle. If (like some steroids) it had a high fat: water partition

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