Table 1. APPROXIMATE R_F VALUES OF HYDROXAMIC ACIDS CHROMATOGRAPHED

Solvent system	Acetyl radical of hydroxamate: Brain extract	γ-OH-butyryl	γ-OH-valeryl	Acetyl	Carbamyl
Butanol, water saturated	0·45	0·45	0·60	0·52	0·19
Phenol, water saturated	0·65	0·65	—	0·68	—
Isobutyric acid, water saturated	0·50	0·50	—	0·55	0·52
Butanol/isopentanol/formic acid/water, 2:2:1:3	0·32	0·32	—	0·40	0·21
Butanol/formic acid/water, 3:1:3	0·40	0·40	0·55	0·50	0·32

gives R_F values of the unknown and standards in various solvents.

The amount of γ-hydroxybutyrate in brain tissue was estimated by comparing the recovery of standard material carried through the same procedures. Rat brain is approximately 1–2 millimolar in γ-hydroxybutyrate and a human brain (no central nervous system disease) taken 3 h *post mortem* was 0·3 millimolar.

The successful use of the sodium salt of γ-hydroxybutyrate in anæsthesia has been reported by Laborit *et al.*[6]. We have confirmed this effect in mice and rats (500 mg/kg IV) and humans (100 mg/kg IV). In all species sleep begins within 15 min and lasts for about 90–120 min. Preliminary tissue investigations in rats show that the sleep period corresponds to an increment of γ-hydroxybutyrate in brain of about 2 millimoles/kg and lasts as long as this level is exceeded. This corresponds to an initial blood-level of about 3 millimolar. Preliminary data on human beings show sleep to occur while the blood-level exceeds 1 millimolar.

The significance of these findings that a normal brain intermediate in relatively small doses can produce sleep is being investigated.

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Lipase Formation by Micro-organisms grown on Hydrocarbons

WE have been investigating the utilization of hydrocarbons by micro-organisms, and have already described formation of amino-acids from kerosene by a fermentation process[1]. On the other hand, it is reported[2] that some authors have been examining the production by micro-organisms of feed supplements, fatty acids and other oxidation products from hydrocarbons. Recently, we investigated whether some enzymes are formed by micro-organisms grown on hydrocarbons as a sole carbon source, and have found that extracellular lipase is formed by 5 strains out of 26 micro-organisms tested.

Cultures were grown in the basal medium containing 2·5 per cent kerosene, 0·2 per cent urea (or 0·3 per cent NH_4NO_3) and mineral salts ($MgSO_4 \cdot 7H_2O$ 0·1 per cent; K_2HPO_4 0·1 per cent; Na_2HPO_4 $12H_2O$ 0·05 per cent; KH_2PO_4 0·05 per cent; $FeSO_4$ 0·002 per cent, $MnSO_4$ 0·001 per cent), at 26·5° C on the reciprocal shaker. Though the amounts of lipase formed by those strains are small (Table 1), it seems that fungi which utilize hydrocarbons all show lipase. Lipase activity in the cultured broth was determined by Nord's method modified according to K. Yamada and Y. Ota[3]. One unit is the amount of enzyme which liberates 1 μequiv. acid/min.

Table 1. LIPASE-FORMING STRAINS AND UNITS OF LIPASE FORMED

Strains	Period of cultivation (days)	Nitrogen source	Lipase activity (unit/ml.)
*S*169*M*1 (mould-like)	7	urea	0·4
*S*190*M*3 (mould-like)	7	urea	0·35
*S*192*Y*1 (yeast-like)	2	NH_4NO_3	0·6
*S*200*M*1 (mould)	3	NH_4NO_3	0·3
*S*209*M*1 (mould)	7	urea	0·85

Table 2. EFFECTS OF 'TWEEN-20' AND OLIVE OIL ON LIPASE FORMATION BY *S*192*Y*1 STRAIN

Medium		Lipase activity (unit/ml.)	Period of cultivation: 2 days	3 days
Basal			0·55	0·4
Basal	'Tween-20'	0·005 per cent	0·95	0·4
Basal	'Tween-20'	0·05 per cent	0·4	0·25
Basal	Olive oil	0·05 per cent	0·3	0·05

Effects of 'Tween-20' and olive oil on formation of lipase by *S*192*Y*1 strain were tested. As shown in Table 2, formation of lipase seems to be slightly stimulated by adding 0·005 per cent 'Tween-20' to the basal medium, while it is prevented when 0·05 per cent of 'Tween-20' or olive oil is added.

Identification of those lipase-forming strains are yet to be made.

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Inhibition of Pyruvate Metabolism in Mitochondrial Suspensions by Cobalt

IN a recent publication, Strickland and Goucher[1] claim that, in brief polarographic experiments, cobalt does not inhibit the oxidation by mitochondrial preparations from rat liver of keto-glutarate, β-hydroxybutyrate or pyruvate. On the basis of these findings, the authors suggest that our observations[2] on the particular sensitivity of keto-acid oxidation to inhibition by cobalt may have been artefacts caused by prolonged incubation of the mitochondria in the absence of a phosphate acceptor.

We have confirmed that in the medium used by Strickland and Goucher[1], and in a modification of this (medium II, Table 1), even relatively high concentrations of cobalt produce no significant inhibition of pyruvate oxidation by isolated mitochondria during the first 20 min of incubation in conventional manometric experiments. Strickland and Goucher's[1] incubation medium, however, differs from that used by us not only in the presence of ADP, but also in the contents of inorganic ions, particularly orthophosphate. In our previous papers[2,3] it was emphasized that in studies of this type it is essential to control the phosphate content of the medium, since the latter limits the concentration of free, ionic cobalt that can be maintained in solution. It seems that the failure of Strickland and Goucher[1] to observe the inhibitory action of cobalt on pyruvate oxidation was due to the high (20 mM) concentration of orthophosphate in their medium. Thus, as shown by the data of Table 1, the inhibition of pyruvate metabolism is immediately apparent when the phosphate content is reduced from 20 mM (media I and II) to 8 mM (medium III). In the experiments at the lower phophate concentration, the buffering capacity of the medium was maintained by the addition of 10 mM *tris*, *p*H 7·4. This prevented the fall-off in oxidation rate that occurred in the presence of 8 mM phosphate as the only buffer, but did not affect the inhibitory action of the cation. Under these conditions, the inhibition produced