Table 3. ACTION OF SOME COMPOUNDS AND N_2 ON THE EVOLUTION OF H_2 (μl.) BY *Chloropseudomonas* IN THE DARK IN THE PRESENCE OF DIFFERENT SUBSTRATES

Conditions	*Chl. ethylica* 3C	*Chloropseudomonas* sp. 51
	Glucose	Formate
Ar	367	78
Ar + NH_4Cl 10^{-3} M	130	0
N_2	14	0
	Pyruvate	
Ar	122	78
Ar 10^{-5} M $NaHPO_2$	—	39
Ar 10^{-3} M $NaHPO_2$	124	0
	Citrate	
Ar	109	—
Ar malonate 10^{-3} M	100	—

—, Not done.

Hypophosphite, acting on formate dehydrogenase, inhibited the production of hydrogen by *Chloropseudomonas* sp. from formate, but did not interfere with this process in *Chl. ethylica* in the presence of pyruvate.

These results suggest that the production of hydrogen by *Chl. ethylica* is connected with the cleavage of pyruvate of the phosphoroclastic type, similar to that observed for *Chromatium* sp.[8]. The pyruvate also seems to be an intermediate in the production of hydrogen by this bacterium with glucose and other sugars as substrates[7].

In other cases the evolution of hydrogen by *Chl. ethylica* seems to be directly connected with the cleavage of α-ketoglutarate. With *Chloropseudomonas* sp. 51 formate seems to be the substrate for hydrogen production. Thus two closely related green bacteria seem to differ in the process of hydrogen production in the same way that *Chromatium* sp. and *Rh. rubrum* differ[8].

We could not observe any hydrogen production by *Chloropseudomonas* sp. in the presence of thiosulphate. But the same conditions for *Rhodopseudomonas* sp. have been shown to produce hydrogen[9].

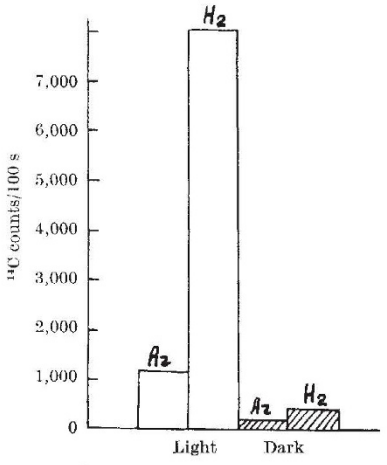

Fig. 2. Assimilation of $NaH^{14}CO_3$ by cell suspensions of *Chl. ethylica* 3C in the light and in the dark under gas phase of Ar or H_2.

The production of hydrogen by *Chloropseudomonas* in the presence of different organic substrates does not depend on illumination. In the light the evolution of H_2 is decreased or stopped. On the other hand, in the presence of light, *Chl. ethylica* utilizes hydrogen for the assimilation of $^{14}CO_2$ (Fig. 2).

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Human Intolerance to Bacteria as Food

DEMANDS for new means to solve man's nutritional needs when the pressure of population intensifies or when he attempts to travel in space have suggested the use of single cell organisms as food. *Hydrogenomonas eutropha*, a hydrogen-fixing bacterium, could effectively control the atmosphere of a space cabin as well as providing nourishment[1]. The protein is of high biological value[2] and is well tolerated even in high concentrations in rat diets[3]. Human feeding trials seemed warranted and were attempted.

At the Battelle Memorial Institute, several lots of bacterial cells were collected from a semi-continuous culture, washed free of medium[4] by repeated centrifugation and resuspension, and frozen for shipment to this laboratory. Removal of medium was verified by examination of the supernatant for non-protein nitrogen before a slurry of the cells and distilled water was boiled. The boiled cells had a halogen-like taste which was removed by successive treatment in the cold (4° C) with cationic and anionic exchange resins ('Dowex 50-4X' and 'Dowex 21K'). The bacterial cells were then filtered through nylon cloth and lyophilized.

Preliminary tests included feeding this lot of bacteria to albino mice at twice the maximum anticipated intake for humans (60 per cent of mouse diet). As in earlier, more extensive rodent experiments with other lots of *H. eutropha* (refs. 2 and 3 and our unpublished results), the preparation did not produce any adverse effects. The mice continued to gain weight over a period of 3 weeks, at a slower rate than the chow-fed animals but not below the usual rate of mice fed purified diets of this approximate composition.

Bacteriological examination of the unprocessed cells received revealed very minor contaminants: two *Streptococcus* spp., one of which was haemolytic, and a Gram-positive bacillus. No species of *Staphylococcus* or *Clostridia* were present. In the fully processed cell preparation only the Gram-positive bacillus remained viable, indicating that it was a spore-forming type.

Six adult male volunteers were confined in a metabolic ward. Four were fed 15 to 25 g of *H. eutropha* and two control subjects were given isonitrogenous amounts of casein. The bacterial cells and casein were served with specially processed low-protein wheat products in sauces which masked the identity of the test substances. The untoward symptoms that ensued are summarized (Table 1). Another volunteer (*G*), a laboratory worker,

Table 1. HUMAN RESPONSE TO INGESTION OF AUTOTROPHICALLY GROWN *Hydrogenomonas eutropha*

Subject	Time of feeding (h) (g dry *H. eutropha* fed) 0830	1230	1730	2130	Symptoms (time of first occurrence) Vertigo	Nausea	Vomiting	Diarrhoea	Stool weight (g/24 h)
A	8·6	17·2	—	—	None	1000	1300	1500	No record
B	8·6	8·6	—	—	0930	1000	1500	1315	921
C	—	—	9·6	5·1	1900	1930	None	2230	423
D	—	—	17·2	—	1930	2030	2100	Evening	1,265
E	—	—	—	—	None	None	None	None	0
F	—	—	—	—	None	None	None	None	277

Table 2. HUMAN RESPONSE TO INGESTION OF HETEROTROPHICALLY GROWN *Hydrogenomonas eutropha* AND *Aerobacter aerogenes*

Subject	Day	*H. eutropha*, time of feeding (h) 0830 (g fed)	1230 (g fed)	1730 (g fed)	*A. aerogenes*, time of feeding (h) 0830 (g fed)	1230 (g fed)	1730 (g fed)	Symptoms (time of first occurrence) Asthenia	Nausea	Vomiting	Diarrhoea	Stool weight (g/24 h)
F	1	6	—	6	—	—	—	None	1000	None	None	77
	2	12	—	12	—	—	—	1000	1000	None	None	74
H	1	—	—	—	—	6	6	1230	1230	None	2100	251
	2	—	—	—	12	—	—	0900	0900	None	1800	206
I	1	6	—	6	—	—	—	None	None	None	None	88
	2	12	—	12	—	—	—	None	1630	2300	None	160
J	1	—	—	—	—	6	6	1230	1300	None	2200	213
	2	—	—	—	12	—	—	1030	1030	None	None	162
K	1	—	—	—	—	—	—	None	None	None	None	164
	2	—	—	—	—	—	—	None	None	None	None	183
L	1	—	—	—	—	—	—	None	None	None	None	112
	2	—	—	—	—	—	—	None	None	None	None	78

was given two 6 g doses of bacteria with different foods. His reactions were identical to those of the confined subjects.

A second lot of *H. eutropha*, grown and collected as the first but which had no off-flavour, was washed, boiled and lyophilized before blind administration to two of six volunteers in the metabolic unit. Subject *H* was fed 12 g at 0830 and 6 g at 1230 h. At 1100 h he complained of abdominal discomfort and thereafter he had thirteen bowel movements weighing a total of 955 g. He also complained of headache, weakness and, later, of pain in the extremities. These symptoms persisted for 12 h. The second man (*D*) had been fed the first lot of bacteria 2 weeks earlier (Table 1). On the second occasion he did not become nauseated but complained of feeling less fit than usual during 5 days of feeding graduated doses (12, 18, 21·7, 21·7 and 21·7 g/day). He also passed large volumes of soft to liquid stools. His temperature, pulse and respiration rate were normal throughout this period, and blood samples taken on the last day of feeding showed normal indices of hepatic and renal function.

Another lot of *H. eutropha* and one of *Aerobacter aerogenes* were purchased from a commercial supplier (Grain Processing Company, Cedar Rapids, Iowa. The *A. aerogenes* was marketed as *E. coli*). Both species were grown on media containing sucrose and casein hydrolysate. The cells were washed, boiled and lyophilized before feeding to subjects in the metabolic unit. *Aerobacter* was pale grey in colour and became unpleasantly slimy on wetting, whereas *Hydrogenomonas* was the usual light tan colour and powdery or granular in texture. To mask the identity of treatments, all men were fed a starch based formula with a small amount of herbs added, with or without bacteria.

A summary of the feeding schedule and symptoms (Table 2) shows that response to this lot of *Hydrogenomonas* was less severe than previously. The *Aerobacter* produced the same symptoms as in the first tests of *H. eutropha* except that subject *J* also developed a rash on his arms and trunk the second day of feeding which disappeared when he returned to his normal diet. In all cases, blood pressure was unaffected and there was no elevation of body temperature. One day later concentrations of blood cells, glucose, serum uric acid and key enzymes were the same as values before the test. No blood was detected in stools at any time.

None of a variety of animal species tested has shown any evidence of gastrointestinal disturbance after peroral administration of *H. eutropha* at dosages far in excess of that which affected men (Table 3). *E. coli* is also well tolerated by rats[5] and chicks[6].

Table 3. SUMMARY OF ORAL DOSAGES OF *Hydrogenomas eutropha*

Species	No. of animals	Mode of introduction	Amount given in one day (g/kg body wt.)
Man	8	Mixed with diet	0·14–0·38
Chimpanzee	2	Mixed with diet	0·5
Chimpanzee	4	Gastric tube	0·5 and 1·0
Dog	2	Mixed with diet	0·5–0·7
Miniature swine	1	Mixed with diet	0·7
Monkey	2	Mixed with diet	0·8 and 1·1
Mouse	6	Gastric tube	2·5

The bacterial cells had been washed free of medium before feeding, so the material responsible for gastrointestinal disturbances in man must be within or bound to the cell. The time at which symptoms occur suggests that digestion of the cell is necessary for release of the toxicant. Variation in response with different lots of *H. eutropha* may represent the development of tolerance to the organism with chronic or slow introduction, or it may simply reflect subject variation. It is also possible that with different growth conditions varying amounts of toxicant are produced.

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Sensitivity of L132 Cells to Some "New" Respiratory Viruses

THERE are several reports of the isolation from cases of human upper respiratory infections of viruses with a structure known previously only in the viruses causing avian infectious bronchitis[1] and mouse hepatitis[2]. The first isolate, named B814, was propagated in organ cultures of human embryo respiratory epithelium[3], and most of the other isolates have been made in organ culture[4]. Some viruses of humans, of the type 229E, multiply in tissue cultures of human embryo kidney fibroblasts where they cause a slight cytopathic effect, and in human embryo lung fibroblasts where they cause a definite progressive cytopathic effect[5]. Those viruses only cultivated in organ culture were detected either by electron microscopy, or by inoculation into human volunteers, or by stopping the ciliary activity of the organ culture epithelium. It has been proposed that the group of virsues with this morphology should be called "coronaviruses"[6].

I have found that the 229E virus can be isolated from infectious nasal washings in 'Bristol'[7] or S-3[8] strains of HeLa cells, but the cytopathic effect is not marked.