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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CAROL I. WASLIEN
DORIS HOWES CALLOWAY
SHELDON MARGEN

Department of Nutritional Sciences,
University of California,
Berkeley, California.

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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.

A more marked effect was obtained when isolations were made in a continuous human embryo lung cell line—L132[9], obtained from the American Type Culture Collection. These cells were maintained in 2 per cent foetal calf serum in Eagle's medium in roller tubes at 33° C. Nasal washings collected from volunteers who had been inoculated with the B814 organ culture strain induced a maximum cytopathic effect in L132 cell cultures about 5 days after inoculation, but the effect often regressed later. The cytopathic effect can be passed serially: sera obtained from volunteers after recovery from colds induced by the B814 virus were at least four times more effective in inhibiting this cytopathic effect than sera obtained from the same volunteers before inoculation. Of eight volunteers inoculated three developed colds, and the cytopathic agent was isolated from each of them and also from two symptomless volunteers. The cytopathic effect could not be passed if tissue culture fluids were treated with ether or acid. 5-Bromodeoxyuridine (BUDR) at 25 μg/ml. did not inhibit the cytopathic effect in L132 cells, although it reduced the titre of a DNA virus (vaccinia)—by 10^5 TCD_{50}, compared with a control titration. The titre of poliovirus type 1 in these cells was not affected by this concentration of BUDR. Organ culture fluids which had been shown to contain infectious B814 virus also produced the cytopathic effect in L132 cells, indicating that the virus had been propagated in tissue culture.

There is preliminary evidence that three other ether labile viruses, previously cultivated only in organ culture—LP, EVS and MR isolates[10]—have now been propagated in this way. Fig. 1 shows normal L132 cells in a stained roller tube culture and Fig. 2 shows a tube of these cells inoculated with a second tissue culture passage of LP virus. Both tubes were rolled for 5 days at 33° C. The LP and EVS viruses have morphology typical of a "coronavirus" (J. D. Almeida, personal communication). Isolates of B814 and EVS viruses will inhibit plaque production of 229E virus in monolayers of L132 cells in plastic Petri dishes, presumably as a result of some sort of viral interference. This inhibition is reversed by mixing the isolate with convalescent human serum at room temperature before the cells are inoculated. After passage in L132 cells, LP virus has become adapted to diploid lung fibroblast W1-38 cells.

Influenza virus C (strain Jhb/1/66)[11] can also be isolated in the L132 cell line from infectious nasal washings and can be passed serially. Furthermore, the cells support the growth of rhinoviruses (E. J. Stott, personal communication) and are sensitive to respiratory syncytial virus.

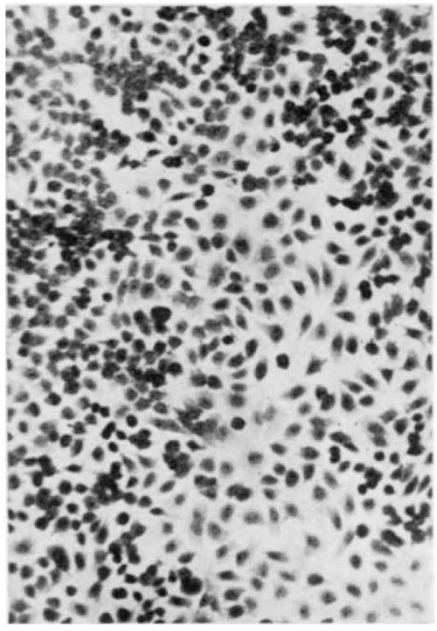

Fig. 1. Normal L132 cells in a stained roller tube culture.

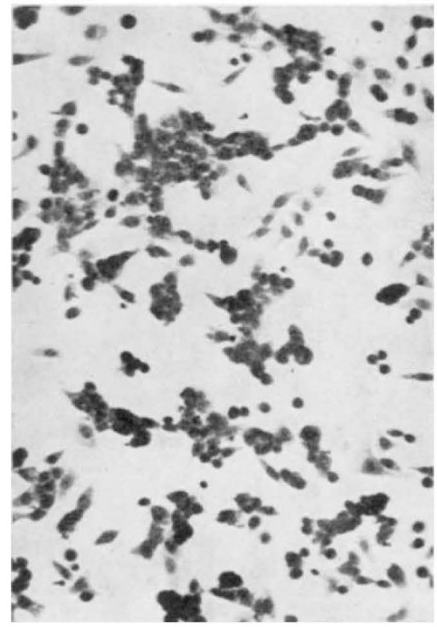

Fig. 2. L132 cells inoculated with a second tissue passage of LP virus.

A. F. Bradburne

Clinical Research Centre,
Common Cold Research Unit,
Salisbury, Wiltshire.

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Occurrence and Determination of Inositol in the Oviducts of Turkey and Hen

At the junction of uterus and vagina of the turkey a few scattered tubular glands have been observed, Spermatozoa are stored there[2] and retain their fertilizing ability for about 45 days[2]. Turkey semen collected for insemination, however, has to be used within an hour of ejaculation, because fertility decreases considerably after that time.

Weighed segments of parts of the turkey oviduct (isthmus, uterus, vagina and junction of uterus and vagina) were treated in a Bühler homogenizer with water for 1 min. The solutions obtained were deproteinized with alcohol. After centrifugation, they were evaporated and the residue was dissolved in a known quantity of water. Adequate amounts were submitted to electrophoresis at *p*H 1·9 followed by chromatography in a solvent system of butanol, acetic acid and water (4 : 1 : 5). The resulting paper chromatograms were sprayed with