550 °C. Nucleic acid contents were determined according to the methods of Smillie and Krotkov[17]. In addition, the content of vitamin A, thiamine, riboflavin, niacin and vitamin C was also determined[16,18]. The amino acid composition of the algal protein was determined using an amino acid analyser (Beckman Model 120C).

*Results.* The salinity of the sewage effluent was found to be 15 ppt. The initial concentrations of inorganic N and P in the sewage effluent are shown in the figure. Growth of the cells in the sewage effluent was found to be comparable to that of cells grown in the modified complete medium. Apparently, cells of *C. salina* CU-1 preferred $NH_4^+$-N as the major nitrogen source, and $NO_3^-$-N was only used by the cells to a much smaller extent. Both levels of $NH_4^+$-N and $PO_4^{3-}$-P in the sewage effluent dropped to zero on the 7th day of cultivation. It is conceivable that cells of *C. salina* CU-1 can be used for purification of wastewater having a high salinity due to their high efficiency in removing the inorganic nutrients.

Table 1 shows the chemical composition of the algal cells. The crude protein content of the cells was found to be 51%. It has been reported that sewage-grown algae or seawater-grown algae generally have a higher ash and a lower protein content[19]. However, the protein content of *C. salina* CU-1 was found to be higher than that of most other sewage-grown algae[5], and comparable to that of others[6]. The content of vitamin A, vitamin C and riboflavin in the cells was higher than that of most other sewage-grown algae, while that of niacin was lower than average[5]. The levels of the vitamins tested were in general found to be higher than those reported for *Scenedesmus acutus* grown in fertilizer with added molasses[20] and for *Chlorella-Scenedesmus* cultures grown in sewage effluent[5]. On the other hand, the low level of total nucleic acids (2.09%) of the cells provides an advantage in using the algal cells as possible supplementary animal feed, since high levels of nucleic acids might cause harmful effects on animals[21]. It is also conceivable that the low crude fibre content (7.2%) of the cells offers yet another advantage, in increasing the digestibility of the algal biomass when used as animal feed. Analysis of the protein of the algal cells revealed a rather complete amino acid profile and all the essential amino acids were present in the protein (table 2); this compares well with the FAO patterns. The total amount of amino acids (64.4 g/16 g N) is comparable to levels reported for other *Chlorella* species grown in artificial media[22,23]. Experiments on pilot-scale outdoor cultivation of *C. salina* CU-1 in sewage effluent are now in progress.


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## A new host species for lactic dehydrogenase virus

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*Summary.* Lactic dehydrogenase virus has been found to replicate and maintain a chronic infection in the Asian mouse *Mus caroli* as it does in *Mus musculus.* However, the level of viraemia is lower and the increase in plasma lactate dehydrogenase activity very much less.

Lactic dehydrogenase virus[2,3] (LDV), a non-pathogenic virus, readily infects all strains of wild and laboratory mice *Mus musculus,* in which its infectivity has been tested. In these mice there is lifelong viraemia and raised plasma enzyme levels. The plasma lactate dehydrogenase (LDH) activity is 5-10 times the normal level by 72 h after infection and it is by this increase that the infection is most easily diagnosed. Infection has not been reported in any other species. Plagemann and his colleagues[4] injected rats and golden Syrian hamsters with LDV but there was no elevation of LDH activity in the plasma and virus infectivity could not be demonstrated in the plasma 1-2 weeks after virus injection. Notkins[5] reported that the injection of LDV into rats, hamsters, guinea-pigs or rabbits did not cause a rise in plasma LDH activity and attempts to demonstrate infectious virus in the plasma of these animals were unsuccessful. Deer mice, *Peromyscus maniculatus* have also been reported as insusceptible[6].

In an attempt to find another host species for the virus, dwarf hamsters *Phodopus sungorus* and the Asian mouse *Mus caroli* were used. Young adult animals were injected i.p. with a large dose of virus ($10^7$ $ID_{50}$) and blood samples

taken from the retro-orbital sinus[7] for LDH estimations and virus titrations. The hamsters showed no increase in plasma LDH activity and circulating viral infectivity declined steadily to become undetectable after 48 h. In contrast the *M. caroli* developed a raised plasma LDH activity after 4 days and a stable viraemia which remained at a level of $10^3$–$10^4$ $ID_{50}$ per ml of blood for at least 6 months. However, from the table it can be seen that their plasma LDH level did not rise as fast, nor to the height observed regularly in *M. musculus* and that it returned to near normal level by 13 days after virus injection. It seemed likely that the less dramatic change in plasma LDH activity in *M. caroli* as compared with *M. musculus* probably resulted from less active virus replication, and to test this hypothesis the level of viraemia was followed in 3 *M. caroli* injected i.p. with $10^3$ $ID_{50}$ of LDV. 45 min after injection the virus titre in the blood was $10^{2.5}$ $ID_{50}$ per ml. By 24 h this had increased to $10^6$ after which there was a slow fall to a stable level of $10^3$–$10^4$. In *M. musculus* peak levels of viraemia of $10^9$ $ID_{50}$ are regularly present 24 h after infection. It thus seems likely that the smaller increase in plasma LDH activity in *M. caroli* is the result of less active virus replication but that the pathological process is the same in both species. *M. caroli* has the same chromosome number as *M. musculus* but differs in a number of biochemical characters[8]. Asian mice in the genus *Mus* have been divided into 3 subgenera: *Pyromys, Coelomys* and *Mus*[9]. Species in the first 2 subgenera have not yet been tested for susceptibility to infection with LDV. The availability of a 2nd host species should be of value in the study of the virus and perhaps in the production of antisera.

Plasma lactate dehydrogenase activity in *Mus caroli* and *Mus musculus* at intervals after infection with lactic dehydrogenase virus

| Days after virus injection | Plasma lactate dehydrogenase activity (IU/ml plasma) in | |
|---|---|---|
| | *Mus caroli* | *Mus musculus* |
| 0 | 200 | 200 |
| 3 | 200 | 1200 |
| 4 | 500 | 1500 |
| 6 | 600 | 1400 |
| 10 | 400 | 1500 |
| 13 | 300 | 1200 |

Individual mice were bled only once and the values given are means of readings obtained on four animals using the forward reaction[10].

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## 5′-Nucleotidase activity in liver homogenates of rats treated with $CCl_4$, colchicine, cycloheximide, emetine, ethanol, ethionine and 5-fluorotryptophan

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*Summary.* 5′-Nucleotidase activity, an enzyme marker of the plasma membranes, increases in female rat liver homogenates following ethionine administration, while homogenates from males show no changes. Treatment with $CCl_4$, colchicine, cycloheximide, emetine, ethanol and 5-fluorotryptophan does not significantly modify the 5′-nucleotidase activity of liver homogenates of either female or male rats.

A number of steatogenic substances are considered to interact primarily with the endoplasmic reticulum, causing inhibition of protein synthesis, blockage of lipoprotein release and accumulation of triglycerides in the liver[1]. However it seems possible that the plasma membranes are also involved in liver injury[2]. Since 5′-nucleotidase activity (5-N) appears to be mainly located at the surface of the hepatocytes[3], we have undertaken a study of 5-N in liver homogenates of rats treated with various compounds. The following substances, reputed to be either steatogenic poisons or inhibitors of protein synthesis[1], were examined: $CCl_4$, cycloheximide, emetine and ethionine. The possible effects of colchicine, a drug believed to interfere with lipoprotein secretion without affecting protein synthesis[4], were also investigated. The study was extended to 5-fluorotryptophan, a substance incorporated in vitro into protein in mouse fibroblasts thus replacing the tryptophan residue, and inhibiting protein synthesis[5]. Furthermore, tryptophan has a stimulating effect on hepatic polyribosomes and on protein synthesis under a variety of experimental conditions, such as in different nutritional states or after treatment with hepatotoxic agents[6]. The possible effect of ethanol, whose mechanism of action is poorly understood[1], has also been observed.

*Materials and methods.* Wistar rats of both sexes, bred in our colony, weighing 150–200 g were used. They were fed on a complete commercial diet (Piccioni, Brescia, Italy) and tap water ad libitum. The animals were maintained in a climate-controlled room (23 °C), artificially illuminated with a light-dark cycle of 12:12 h daily. The dosage of drugs, time of killing and means of administration were chosen on the basis of bibliographical data so as to produce the most striking effects. Colchicine (0.5 mg/100 g b.wt)[4], cycloheximide (0.1 mg/100 g b.wt)[1], emetine (2 mg/100 g b.wt)[1], and 5-fluorotryptophan (30 mg/100 g b.wt)[7], dissolved in 1 ml of 0.9% NaCl (pH 7.4), were injected i.p. DL-ethionine was administered i.p. at a dosage of 100 mg/100 g b.wt (2.5% water solution) given in 2 equal doses at zero time and after 1 h[8]. 1 ml of a 1:1 solution of $CCl_4$ in maize oil was given by stomach tube under light ether anesthesia[8].