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].

5'-Nucleotidase activity in liver homogenate of rats treated with CCl_4 and ethionine

	Control group		Experimental group	
	Female	Male	Female	Male
CCl_4	74.5± 8.59 (15)	78.6±10.39 (6)	73.5±13.30 (16)	70.1±13.68 (6)
Ethionine	92.9±12.45 (18)	104.5±11.52 (9)	160.5±11.52 (18)*	87.9±11.13 (11)

Values are given as μmoles substrate utilized/100 g b.wt and are the mean±SE of the number of determinations given in parentheses. * $p<0.01$ from the appropriate control.

Ethanol was administered as 1:1 water solution, by stomach tube, in 2 different doses. The first one (700 mg/100 g b.wt), described as inducing a well-defined fatty liver[1], produces in all the animals signs of strong intoxication, such as ataxia, somnolence and in some rats coma or even death before the 6th h following administration. Experiments were therefore also carried out using a lower dosage corresponding to 200 mg ethanol/100 g b.wt. The animals were killed by decapitation 6 h after the 1st injection of ethionine and 6 h after the administration of other drugs. About 50 mg (wet wt) of each liver was homogenized in 0.5 ml of 0.3 M sucrose (pH 7.4 with $NaHCO_3$) and 5-N determined at 37 °C, for 15 min, pH 7.4[9]. After the addition of trichloroacetic acid, the inorganic phosphate was estimated[10] in the supernatants. Each value was corrected for the inorganic phosphate present at zero time and for the possible hydrolysis of the substrate. All incubations were carried our between 16.00 and 17.00 h. The statistical significance was evaluated with Student's t-test[11]. No statistical significance has been attached to differences with a probability value $p > 0.05$. Since most of the experiments were performed at different times, a set of control rats was included with the experimental groups.

Results and discussion. The results for CCl_4 and ethionine are summarized in the table. Despite the well-controlled conditions of feeding and housing there is a certain degree of variability among the control values taken at different times. The variability does not appear to be linked to circadian rhythms since the incubations were carried out at about the same time of day. In view of the differences between the individual control groups, the experiments with ethionine were repeated several times, with slightly variable results. All these data have been collected in the table. As compared with corresponding controls, liver homogenates of female rats treated with ethionine show an increase of 5-N of about 73% while no significant differences in livers of male rats treated with the same drug were found. This result is in accordance with several findings showing that most morphological and biochemical changes in the liver caused by ethionine are much slighter or altogether absent in the livers of male as compared with female rats[12]. No significant changes in 5-N of liver homogenates either of female or male rats treated with CCl_4 or with the other compounds used in this research have been observed.

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Effect of daminozide on tomato fruit ripening

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Summary. Fruits of tomato (*Lycopersicon esculentum* Mill. cv. Fireball) were harvested 32 and 37 days after anthesis and dipped for 30 min in 10,000 ppm daminozide. Ripening of treated fruits, as manifested by an advancement in ethylene production and the respiratory climacteric, was accelerated by up to 7 days over control fruit.

Preharvest application of the growth retardant daminozide (succinic acid −2, 2-dimethylhydrazide, SADH, Alar, B-9) prevents premature fruit drop but delays the onset of both C_2H_4 production and the respiratory climacteric of apples after harvest[2,3]. However, this inhibition can be reversed by application of C_2H_4 gas[2,3]. In contrast to its effect on apples, preharvest daminozide treatment of peaches hastened ripening by accelerating the onset of the climacteric, by increasing internal flesh colour and external skin colour, and by decreasing flesh firmness[4]. Daminozide also significantly increased C_2H_4 production associated with peach fruit ripening[5]. An effect similar to ethephon (ethrel) treatment was induced by dipping mature-green tomato fruits in daminozide for 30 min[6]. This treatment enhanced cellulase activity, colour formation and softening of the fruits. Because daminozide is considered to be a ripening inhibitor and because the tomato appears to differ from other climacteric-type fruits in response to exogenous regulators such as C_2H_4[7,8], we were interested in studying the effect of daminozide on C_2H_4 production and the climacteric of tomato fruit.

Materials and methods. Seeds of 'Fireball' tomatoes (*Lyco-*