epithelial cells (figure 1). In 3 animals, contacts were found between axons and parietal cells (figure 2).

The dimensions of the cross sections of the axons were measured on the electron micrographs of 3 animals. From these, 20 axons were measured. There was a range of axon diameter between 0.06 and 0.20 µm. These were the smallest and largest diameters found. The nerves at the points of contact were of roughly the same diameter as axons in the nerve trunk and no evidence of expansion or narrowing was observed. The illustrated axon at a contact point was 0.11 µm in diameter (figure 2). At a point of contact, there was a very narrow gap between the axonal membrane and the epithelial cell membrane (figure 2). Neither membrane showed any specialized feature. No synaptic vesicles were observed. The width of the gap varied between 6 and 14 Å with a mean width of 11 Å. In the illustrated example there are, however, short areas in which no gap can be found and the cell membranes may be fused.

Discussion. In the methylene blue preparations, nerves were identified clearly in the muscle coat in all 10 animals, in the muscularis mucosae in 7 and in the mucosa in 3 animals. A number of these fibres could be followed to the parietal cells[4]. However, it was not possible to determine the exact termination points using these methylene blue preparations.

These electron microscopical studies have shown that the parietal cells and nerve fibres have points of contact, but in these areas there were no specialized functional structures, no synaptic vesicles and no mitochondria. However, points of contact between axons and gastric epithelial cells have not been as clearly shown before it, in fact, similar observations have been made before. In the innervation of vascular smooth muscle, physiologists occasionally discussed 'en passant' synapses, or points of contact between fine axons and the muscle cell membrane. The functional significance of these has not been elucidated, and as with our findings, it cannot be stated that they were, in fact, synapses of functional importance. The intention in illustrating these was to record their presence so that the matter can be pursued in future experiments. These findings will now allow us to study the changes which occur after vagotomy regarding vagal nerve degeneration and possible regeneration, for if this occurs clinically, it may be an important cause of recurrent peptic ulceration after vagotomy.

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The effect of dietary amino acids on the growth of tumors[1]

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Summary. In C3H mice, a direct dose response relationship between tumor growth and dietary amino acid is seen for fibrosarcoma and mammary carcinoma, extending over a range the lower limit of which is defined by the minimum amino acid requirements, and the upper limit by the amino acid level found in most stock diets.

Inhibition of the incidence and growth of malignant tumors has been reported in animals fed diets deficient in protein or in essential amino acids[3-16]. In these studies, most test diets produced impairment of body growth, although a critical level of moderate phenylalanine restriction was found which slightly inhibited tumor growth without affecting host weight[11]. In some studies, the casein content of the 'control' diet varied from 12 g/100 g diet[11] to 18%[6], 20%[15] and 28%[12]. In other studies, the 'control' diet was the standard mouse or rat chow usually containing protein in excess of 20%[3-5,7,8,10,13-16]. Thus the above-mentioned reports deal only with the effect of dietary amino acid restriction on tumor growth, comparing an amino acid deficient diet with a 'control' diet containing an arbitrary amount of amino acid, usually in excess of minimum requirements. This report describes the influence of dietary

Effect of 4 weeks dietary treatment in 6-week-old C3H/CrlBR male mice

Dietary treatment	Body weight (g)		Food intake (g food/ mouse/24 h)	Relative spleen weight[c]	Total serum protein (g/100 ml)	Serum albumin (g/100 ml)	Blood leucocytes (cells/mm^3)	Neutrophils (cells/mm^3)	Lympho-cytes (cells/mm^3)
	Initial weight[a]	Final weight (%)[b]							
Purina	22.2±0.4	122.9±2.6	3.53±0.16	52.0±2.1	5.35±0.14	3.74±0.1	5908±833	1980±186	3750±182
Diet 1	21.2±0.3	120.8±2.0	3.24±0.15	45.6±5.0	5.39±0.22	3.92±0.12	4895±809	1482±170	3240±175
Diet 2	21.3±0.5	112.1±2.7[d]	3.20±0.19	39.5±1.5[e]	4.77±0.21	3.63±0.18	4134±673[d]	1177±106[e]	2851±118[e]
Diet 3	22.0±0.2	123.0±3.0	3.30±0.17	49.3±4	5.43±0.23	3.80±0.14	5230±780	1700±112	3400±178

[a]) Mean of 10 mice ± SEM. [b]) Percentage of initial weight. Superscripts indicate statistically significant difference from the mean for the mice fed Purina lab chow: [d]) $p < 0.02$; [e]) $p < 0.01$ (Student's t-test).

[c]) $\frac{\text{Spleen weight}}{\text{Body weight}} \times 10^{-4}$.

amino acid contents not only below, but also above minimum requirements, on the development of implanted fibrosarcoma and mammary carcinoma.

8-week-old inbred male C3H/NCr1BR mice (C3H) purchased from Canadian Breeders, Montreal, were fed ad libitum 1 of 3 defined formula diets or Purina mouse chow. The standard amino acid diet (diet 1) contains approximately the same amino acid distribution as casein; the nitrogen content is 1.8% or 11.94 g casein equivalent/100 g diet. Detailed composition of diet 1, is given in a previous paper[17]. In diet 2, L-methionine is reduced by 25%, cystine is absent, and L-phenylalanine and L-tyrosine are reduced by 66%. This diet is rendered isonitrogenous to diet 1 by corresponding increments of nonessential amino acids. Lipids, carbohydrates and vitamins are identical to those of diet 1. In diet 3, the total amount of amino acid mixture is double that of diet 1, with identical amino acid distribution (22 casein equivalents/100 g of diet). Diets were prepared in similar proportions with substitution of dextrose-cornstarch for the free amino acid mixture to achieve a final amino acid concentration of 11.94% in diets 1 and 2, and 22% in diet 3. The particular brand of Purina mouse chow used in our studies contains approximately 18% protein.

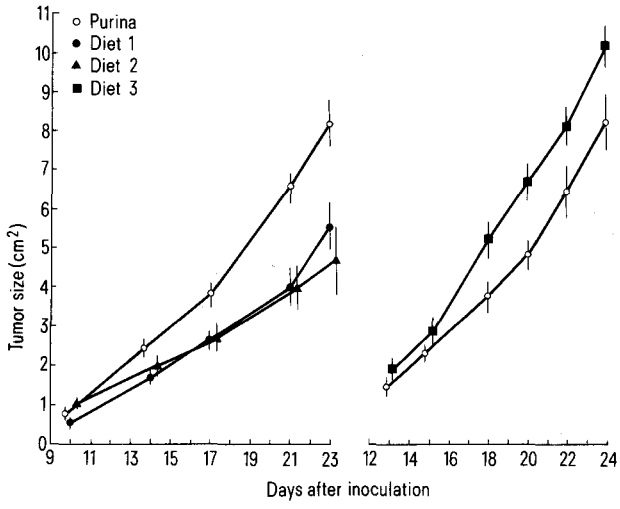

Fig. 1. Statistical analysis (Student's t-test): Left hand graph: Diet 2 v diet 1 NS. Diet 1 v Purina $p < 0.05$ or less (day 17–23). Right hand graph: Diet 3 v Purina $p < 0.05$ or less (day 18–24). Influence of diets upon the growth of implanted fibrosarcoma 1038 (National Institute of Health) in C3H mice. 10 mice per group. Mean ± SEM.

Tumor 1038 fibrosarcoma was originally induced by a pellet of 1% 3-methylcholanthrene in the s.c. tissue of a C3H/HeNIcr mouse at the National Institute of Health, Bethesda, Maryland. It was transferred to our laboratory as a monolayer growing in vitro and passaged serially in C3H/NCr1BR mice. Mammary adenocarcinoma H2712 was obtained from the Jackson Laboratories, Bar Harbour, Maine. It is a spontaneous tumor, originating in a C3H/HeJ female mouse which has been transplanted serially in syngeneic mice. Spontaneous regressions are rarely observed and metastasis does not occur with either tumor. Tumor cells (1×10^6 in 0.15 ml) were injected s.c. in the shaved flank of the mouse on the day that the dietary regimen was commenced. Tumor measurements were taken along the greatest diameter, and at 90° to this diameter with a dial gauge calipers. Tumor size is expressed as the approximate surface area obtained by multiplying the 2 diameter measurements. 10 mice per dietary group, all of which survived the observation period, were used.

Results and comments. Our nutritional studies show that diet 1, with 11.94 g casein equivalent/100 g, sustained normal growth of the mice, and that diets 3 and Purina, with a higher protein content, do not enhance body growth beyond that with diet 1 (table). These findings are consistent with recent evidence suggesting that a 12% casein diet is sufficient to maintain normal post-weaning growth in various strains of mice including C3H[11,17–19].

The time of appearance of palpable fibrosarcomas was similar for all dietary groups but the sequence of appearance of palpable mammary carcinomas was diet dependent: tumors appeared earliest in the groups fed diet 3 and Purina (higher protein) and later in the groups fed diets 1 and 2 (lower protein). Figures 1 and 2 show the effect on the growth of fibrosarcomas and mammary carcinomas of diet 1 and 2 in comparison with Purina chow and diet 3. Diet 2, with essential amino acid restriction, produces some impairment of body growth (table), and a greater inhibitory effect on mammary tumor but no differential effect on the growth of fibrosarcoma, in comparison to diet 1. More importantly, diet 3, with an amino acid content in excess of requirements, was found to enhance tumor growth to a value above that seen in mice fed the standard amino acid diet (diet 1). The growth rate of both tumors in the Purina fed groups is also higher than that of diet 1 and lower than that of diet 3 mice. Making allowances for differences in protein quality and digestibility, the protein content of Purina lies between that of diets 1 and 3.

Our findings indicate that a certain dose-response relationship appears to exist between dietary amino acid and tumor

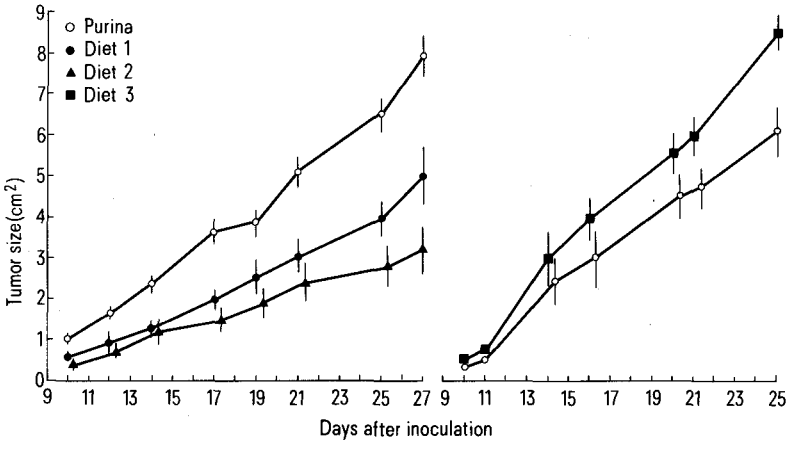

Fig. 2. Statistical analysis (Student's t-test): Left hand graph: Diet 2 v diet 1 $p < 0.05$ (day 27). Diet 1 v Purina $p < 0.05$ or less (day 14–27). Right hand graph: Diet 3 v Purina $p < 0.01$ (day 25). Influence of diet upon the growth of implanted mammary adenocarcinoma H2712 (Jackson Laboratory) in C3H mice. 10 mice per group. Mean ± SEM.

growth. These data also indicate that dietary amino acid restriction need not produce malnutrition in order to inhibit tumor growth. This finding was made possible by the use of 4 diets containing graded amounts of amino acids extending both above and below the minimum requirements represented by diet 1. The correlation between dietary amino acids and tumor growth suggested by our data appears to emphasize the tumor enhancing effect of amino acids in excess of minimum requirements, rather that the inhibitory effect of amino acid restriction. Whether or not this phenomenon is similar in nature to that underlying the well-documented inhibition of tumors with amino acid restriction below requirements[3-16], is a matter of speculation.

One might also consider the likelihood that the lower incidence of chemically-induced colonic carcinoma associated with a high-fibre diet[14] could, in fact, be explained on the basis that dietary fibres protect the host by preventing the intake of protein above requirements. The enhancing effect of dietary protein above minimum requirements has been shown in a similar type of tumor[16]. Finally, these findings may have some significance for man, since the typical North American diet is well in excess of minimum protein requirements[20].

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The selective accumulation of vitellogenin in the locust oocyte

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Summary. The selectivity of vitellogenin absorption by the locust oocyte was examined by comparing the uptake of vitellogenin and a haemolymph protein of similar molecular weight (MHP). Though both proteins occurred in the haemolymph at approximately the same concentration there occurred a 500-fold difference in accumulation of vitellogenin over MHP during a 24-h period. Surprisingly MHP did not accumulate in the oocyte during vitellogenesis.

Since the initial demonstration of preferential uptake of haemolymph vitellogenin by the developing *Cecropia* oocyte[1], several authors have examined the process of its sequestration during vitellogenesis in *Locusta*. The rate of uptake of trypan blue was found to be proportional to the oocyte surface area[2]. This is confirmed by the observation that the amount of vitellin in the oocyte shows a geometric correlation with the oocyte length (figure 2, a). Ferenz[3] measured the haemolymph protein concentration and the vitellogenin concentration (using an RIA of iodinated vitellogenin) in relation to vitellogenin uptake. ^{125}Iodinated bovine serum albumin and ^{125}I-immunoglobulin G were incorporated more slowly than the vitellogenin. In a similar study Gellissen and Emmerich[4] measured the titre of vitellogenin and diglyceride carrier lipoprotein (DGCL) in the haemolymph of vitellogenic *Locusta* females. They detected fluctuations in the titre of vitellogenin which were correlated with oocyte growth but little change in the concentration of DGCL, which is also taken up by the oocyte but in lesser amounts. These authors did not follow the uptake of these 2 proteins into the oocyte.

In the present study the haemolymph titre of vitellogenin and a 2nd haemolymph protein of comparable size (the major haemolymph protein (MHP)[5]) were measured in *Locusta migratoria* (gregarious phase) by rocket immunoelectrophoresis during the vitellogenic cycle. The amount of both these proteins in the developing oocyte was determined in a similar fashion. Both of these proteins are synthesized by the fat body[6,7] and absorbed from the haemolymph into the oocyte via pinocytosis[8]. Antisera to locust vitellin and MHP were raised in rabbits and each shown to produce a single band when allowed to react with haemolymph or oocyte extracts in Ouchterlony double diffusion plates. No immunological cross reaction between isolated vitellin and MHP could be detected. Ouchterlony double diffusion plates were run to show that vitellogenin from the haemolymph was immunologically identical to vitellin from the oocytes. Oocyte lengths were measured with the aid of an ocular micrometer in a dissecting microscope (Intralux 500-H, Leitz) after a haemolymph sample from the same insect had been collected. Oocytes were homogenized in insect Ringers solution[9], centrifuged at 10,000 × g for 10 min and the supernatant retained. The oocyte extracts and the haemolymph samples were analyzed by rocket immunoelectrophoresis against a) antivitellin serum and b) anti-MHP serum. An appropriate