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

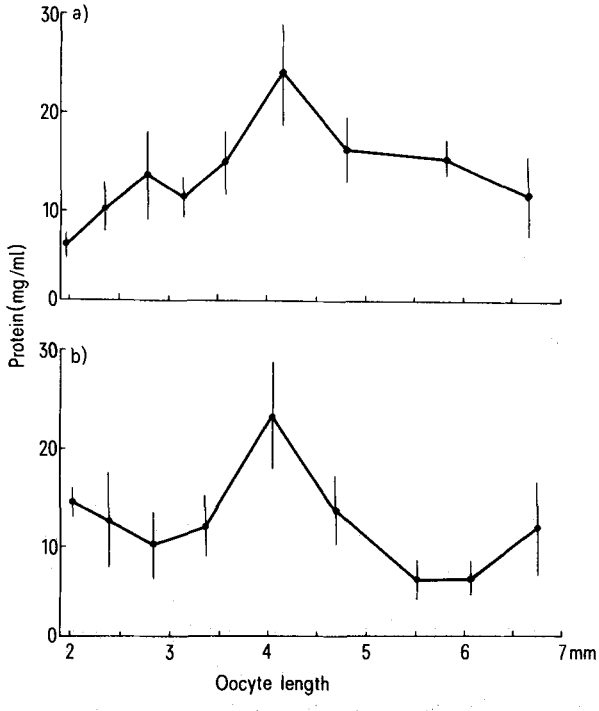

Fig. 1. Concentration of locust vitellogenin (a) and MHP (b) in the haemolymph of female locusts during vitellogenesis. Concentration of the specific proteins were estimated by rocket immunoelectrophoresis. Each point is the mean of 6 analyses from separate insects. Vertical bars indicate twice the SE.

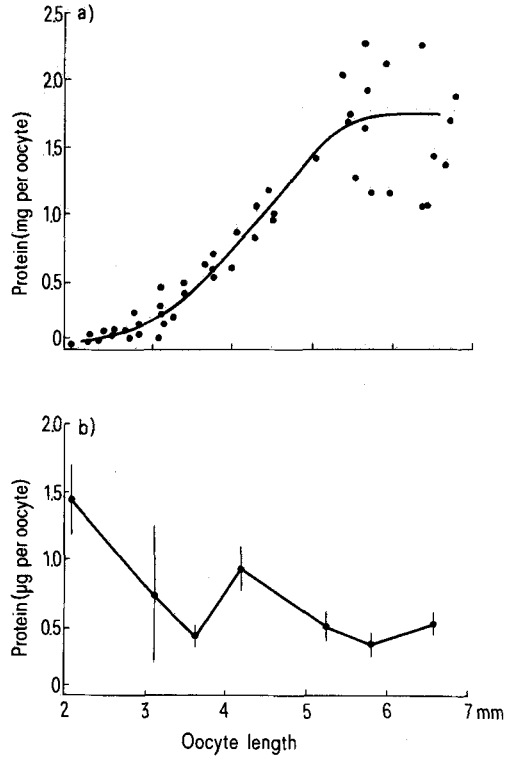

Fig. 2. Amount of vitellin (a) and MHP (b) in the oocyte of *Locusta* during vitellogenesis. Methods as for figure 1. *a* Each point represents 1 determination. Line drawn by inspection. *b* Each point is the mean of 6 analyses from separate insects. Vertical bars indicate twice the SE.

standard of either vitellin or MHP was always run concomitantly. The concentrations of MHP and vitellogenin in the haemolymph of the locust during the vitellogenic cycle are shown in figure 1. It can be seen that both proteins have a peak concentration when the oocyte length reaches 4 mm and that the overall concentration of both these proteins in the haemolymph is rather similar (about 23 mg/ml maximum). Figure 2 shows the amounts of vitellin and MHP in the oocyte. It can be seen that whereas vitellin accumulates as the oocyte increases in size the amount of MHP present in the oocyte does not. Vitellogenin accumulates in the haemolymph as the oocyte reaches 4 mm in length. As the oocyte grows towards its maximum size the rate of vitellogenin uptake increases and the titre of vitellogenin in the haemolymph declines (figures 1, a, and 2, a). The amount of MHP in the oocyte remains low throughout vitellogenesis, thus verifying the apparent selectivity of vitellogenin uptake by the oocyte. Thus, as the oocyte increased in size from 3.6 mm to 4.15 mm the amount of vitellin present increased by about 260 µg whereas the amount of MHP in the oocyte only increased by about 0.5 µg. The general pattern of MHP content of the oocyte tends to follow the concentration of this protein in the haemolymph. In order to ensure that the MHP measured in oocyte extracts was not the result of contamination from the haemolymph, yolk was withdrawn directly from the developing oocyte with a syringe and subjected to rocket immunoelectrophoresis. Concentrations of MHP comparable to those determined after homogenizing whole oocytes (from the same insect) were detected. Rocket immunoelectrophoresis of extracts of the oocyte membranes failed to demonstrate the presence of MHP. Thus it is clear that MHP enters the oocyte. Surprisingly there is no evidence of an accumulation of MHP in the oocyte during the vitellogenic cycle, suggesting that MHP sequestered during absorption is preferentially degraded or eliminated by the oocyte. The selective degradation of proteins has been proposed to account for the selective passage of immunoglobulins across the placenta[10] but the mechanism for this degradation has not been elucidated. In a recent study Opresko[11] found that vitellogenin iodinated by the chloramine-T method was preferentially degraded by amphibian oocytes following endocytosis. In addition I^{125} bovine serum albumin was degraded at approximately the same rate as it entered the oocyte. Hence it appears that the low levels of MHP in the oocyte can be explained satisfactorily by selective degradation following absorption, though it has generally been assumed in the past that the oocyte plasma membrane or the follicular epithelium provided the 'filter' limiting entry of non-specific proteins into the oocyte[12].

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