these three subjects familial hyperlipemics, since they clear ingested fat in a normal period of time, it is fairly certain that they would have been included in such a group on the basis of fasting specimens only. This not only points out the importance of the fat-tolerance test in the diagnosis of familial hyperlipemia but also accentuates the importance of recognizing the possible heterogeneity of biochemical causes for the primary elevation of neutral fat in the blood (*11*).

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11. A detailed description of our results is in preparation. We wish to express deep gratitude to Dr. Gunnar Wallenius of the clinical chemistry department of Uppsala University for invaluable advice and for the cholesterol determinations.

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Predaceous Feeding in Two Common Gooseneck Barnacles

Abstract. *Lepas anatifera* and *Mitella polymerus,* while relatively unselective omnivores, behave at times like predatory macrophagous carnivores. Observations suggest a greater range of food size for gooseneck barnacles than is generally suspected and clearly indicate that large organisms, when available, are effectively captured and handled.

Thoracican barnacles are generally considered to feed on *small* organisms and particles of detritus caught by combing the water with highly setose thoracic appendages. Very few observers have described barnacles feeding on larger forms: Darwin (*1*) notes prehensile behavior of the cirri in capturing crustaceans and other prey; Batham (*2*) notes the curling motions of the cirri in *Mitella* (= *Pollicipes*) *spinosus,* which deposit crustaceans "often of no mean size" in the mouth; Brown (*3*) states that in the laboratory *Lepas* will feed on animals even larger than itself; but Gruvel (*4*) is apparently the only worker who has described the coordinated behavior of the cirri and mouth parts of *Lepas* in seizing and ingesting food of various sizes, and his observations have gone largely unheeded. It is the purpose of this report to present evidence that at least two common gooseneck barnacles not only capture and feed upon large prey but, on occasion, show the feeding habits and food-capturing mechanisms, and have the diets, of macrophagous predators, exhibiting a behavior more reminiscent of the lion than of the anteater.

Initial observations made on *Lepas anatifera* in 1951 at the Hopkins Marine Station of Stanford University by one of us (H.S.) have recently been confirmed and extended by further work on this species (by both of us) and on *Mitella polymerus* (by G.H.). In the laboratory, feeding was observed in both species. A large cluster of *Lepas* attached to driftwood was allowed to feed freely for 2 hours on the brine shrimp *Artemia salina* (5 to 11 mm in length) and on the small tide-pool copepod *Tigriopus californicus* (approximately 1 mm in length). A cluster of *Mitella* freshly removed from intertidal rocks was similarly fed these two animals. The gut contents of the barnacles were then examined. *Lepas*, ranging in size from 11.2/7.0 mm (shell length = ratio of total length of capitulum to gut length) to 7.7/4.8 mm, all ingested both *Artemia* and *Tigriopus*; smaller animals, ranging in size down to 3.6/2.3 mm, took copepods only. The largest individual *Lepas* took 56 *Tigriopus* and two *Artemia*. The second largest (10.8/6.8 mm) took four copepods and four brine shrimp. Individuals of *Mitella* greater in size than 5.8/7.2 mm ingested both *Artemia* and *Tigriopus*; smaller *Mitella,* down to 1.6/2.1 mm, took only copepods. Ingested organisms originally longer than the barnacle digestive tract (Fig. 1) were bitten in pieces, folded, or compacted to fit the gut.

How is the prey of these animals captured and handled? Activity of the cirri and mouth parts of these barnacles is most clearly demonstrated in *Lepas,* though the behavior of *Mitella* is similar. Individuals of *Lepas,* immersed in a suspension of *Artemia* and *Tigriopus,* consistently seized the animals with ravenous grabs, lassoing and caging the prey and holding it in their cirri. The six pairs of thoracic cirri may operate together or move quite independently of one another, a behavior not observed in balanoids but previously seen in *Lepas* (*4*), in *Scapellum* and *Verruca* (*5*), and in *Mitella* (*2*). If a brine shrimp contacts the cirri of *Lepas,* there is a total clutching motion of all the cirri; these then contract, forcing the food toward the mouth parts.

When the animals are feeding on smaller organisms, the functions of the individual cirri can be seen. Here the anterior three thoracic appendages. which are equipped basally with spinous pushing brushes, direct food to the mouth parts, where it is gripped and compacted. At the same time copepods may be trapped by individual rami of the last three cirri, which curl around them and hold them in reserve. We noted that one animal held seven copepods in separate coiled posterior rami while ingesting a brine shrimp. *Mitella* has cirri and mouth parts which are anatomically and functionally similar to those of *Lepas.* The cirri are thicker and shorter and, in general, less active than those of *Lepas,* but when cirri are presented with food, their response is quick. Food may be trapped separately by the last three pairs of rami and passed to the very heavily bristled pushing pads at the bases of the second two pairs of cirri and on the rami of the first cirri, which in turn cram it against the mouth parts for compacting and swallowing.

The differences in feeding behavior between *Lepas* and *Mitella* seem to be in accord with their respective ways of life. *Mitella* characteristically occurs in clusters on a fixed rocky substrate exposed to a great deal of wave action. Individuals are generally oriented in fixed positions to receive the down-wash

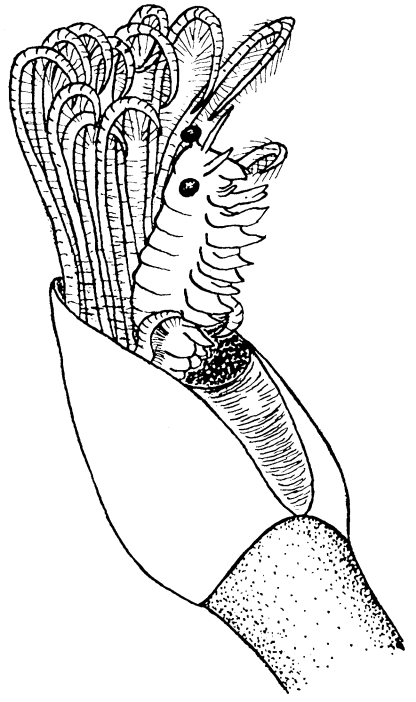

Fig. 1. *Lepas* ingesting *Artemia.*

of waves; cirri are held maximally extended as water, which is often rich in food, sweeps over them. *Lepas,* on the other hand, occurs on freely floating timbers moving *with* the current. The peduncle is highly mobile and swings in every direction; the cirri are more active, though they seldom exhibit the rhythmic beating so characteristic of the balanoids. These motions could be those of foraging, in a situation where the probability of obtaining food by random contact with organisms is greatly reduced (even though at sea certain forms tend to congregate below floating objects).

What is the natural diet of these organisms? Examination of the gut contents from monthly collections of *Mitella* taken over a period of a year showed that copepods, algae, and unidentified particulate matter are nearly always present; cirriped molts, amphipods, cypris larvae, small clams, and hydroids occur frequently; polychaetes and barnacle nauplii are taken occasionally. Batham's list for *Mitella spinosus* is comparable. Polychaetes and plant fragments over one-half the gut length were found coiled inside.

The guts of a number of field specimens of *Lepas* were also inspected, disclosing polychaetes, amphipods, (gammarids, hyperiids, and caprellids), caridean shrimps, gastropods, clams, pycnogonids, algae, and detritus. Some of these items were doubtless captured while the timber bearing the barnacles was washed about in the intertidal zone. The largest crustaceans measured nearly one-half the length of the barnacle gut holding them.

Despite the effective predaceous behavior often exhibited by both *Lepas* and *Mitella,* these forms, as feeders, are opportunistic rather than selective; *Lepas,* attached to timbers stranded on the beach, will fill their guts with sand, while small chunks of granite and calcareous shell have been noted in the gut of *Mitella* (*6*).

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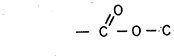
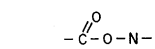
Structural Correlation between Esterase and Protease Activities of Trypsin

Abstract. It is tentatively concluded from ultraviolet and x-ray studies that the two tryptic activities are mediated by overlapping "enzymatic sites." Crucial to this conclusion were studies of the factors which can modify the measured inactivation rates. The data are interpreted in the light of postulated mechanisms of inactivation.

Some enzymes are presumed to have more than one catalytic activity since they act upon substrates that are quite different. Whether these activities are mediated by a single site or a number of separated sites on the enzyme surface is a problem of current biochemical interest and importance. Previous workers concluded that the esterase and protease activities of trypsin (that is, the ability to hydrolyze, respectively, the



structure of esters and the



structure of polypeptides and proteins) must be located at a single site. The evidence is twofold (*1*): (i) Inhibitors of fairly large molecular weight produce equivalent decreases in the two activities, and (ii) trypsin hydrolyzes either type of substrate at a decreased rate in the presence of the other type. However, this evidence does not preclude the existence of closely adjacent or overlapping sites.

The radiation studies reported here were designed to examine this question without use of large-sized inhibitors. Presumably, x-ray inactivation is initiated by radiation-produced radicals of low molecular weight (*2*). Inactivation by ultraviolet irradiation (2537 A) is probably localized within a small region of trypsin, since only one molecule is inactivated per approximately 60 quanta absorbed (each of 4.9 ev). In both studies, the two activities should be inactivated at identical rates if only a single site is involved but probably, although not necessarily, at different rates if adjacent or overlapping sites exist.

The same two activities are inactivated unequally when chymotrypsin is x-irradiated in dilute solution (*3*) or oxidized with sodium periodate (*4*). This has been attributed to concentric sites having different areas or charge configurations (*3*).

While such conclusions are possibly correct, they now appear to require additional justification, since we have found that measured inactivation rates are affected by the treatment afforded irradiated molecules prior to and during assay. Specifically, our experiments are consistent with these postulates: (i) At least three classes of trypsin molecules are present in solution after irradiation—active, damaged, and inactive. (ii) Damaged molecules have an average of one to three more intact H-bonds than do inactive ones and therefore can be converted to the inactive class either by urea ($> 5.5M$) or by thermal treatment prior to the addition of a substrate. (iii) Damaged molecules have normal activity either when only substrate is added or after urea is added if they have been previously exposed to substrate at a *p*H consistent with activity. (iv) This prior exposure to substrate "reactivates" damaged molecules to active ones.

I obtained results similar to those of Aronson, Mee, and Smith (*3*) when 0.4 to 5.0μM solutions of trypsin (twice recrystallized, from Worthington Biochemical Co.) in 0.001M Na_2HPO_4 were irradiated at 0°C with 250 kv (peak) x-rays. Protease activity was determined by hydrolysis of hemoglobin (*5*) (Hb); esterase activity, by hydrolysis of benzoyl-arginine ethyl ester (*6*) (BAEE). The decrease (inactivation) of both activities with increasing dose fitted (± 10 percent) the kinetics of a first-order reaction. The two inactivation rates depend upon the initial trypsin concentration and are quite different. That measured by Hb assay (which employs 5.5M urea) was about 1.5 times that measured by BAEE assay (no urea) when both were extrapolated to infinite solute concentration (*2*). If a one-to-one correspondence between reaction probability and fraction of surface area is assumed, the number of amino acids in the proteolytic site which are reactive to radiation-produced radicals is estimated to be between two and four (*2*).

Only slightly different results were obtained when ultraviolet irradiation instead of x-ray was carried out at 0°C (see plots for H and B, 0° in Fig. 1): the inactivation rates were essentially independent of trypsin concentration between 0.4 and 5.0μM and in a ratio of about 3:1 or 4:1 rather than 1.5:1. The four postulates depend critically upon the following preliminary results with ultraviolet irradiation (see Fig. 1).

1) The rate of inactivation measured by Hb assay appears to be independent of the temperature during irradiation, from 0° to 60°C [see (H, 0–60°)].

2) When samples irradiated at 0° are heated at 60° for 30 minutes (B, 0°:P60°, 30 m) or 100 minutes (B, 0°:P60°, 100 m) immediately after irradiation, and thus prior to assay, the "BAEE-measured rate" approaches the standard "Hb-measured rate."

3) The rate of inactivation measured