Polyacrylamide is not antigenic in itself, but when conjugated to bovine serum albumin, antibody capable of specifically binding radioactively labeled PAA is elicitable. Its affinity appears to be quite small; however, material of higher specific activity would increase the sensitivity, which in turn would increase the titer of the antibody proportionately. A final dilution of 1:5000 or more would be entirely practical for the routine analysis of polyacrylamide in effluent waters. The results indicate that RIA may prove useful in analysis of other water soluble synthetic polymers that enter the environment, such as electroconductive resins or polyethyleneimine, and could be extended to analysis of samples of downstream effluents.

## Proteolysis in dystrophic hamster diaphragm and abdominal muscle

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*Summary.* Proteolysis, as measured by tyrosine release, was estimated in abdominal and diaphragm muscle of hamsters. There did not appear to be a difference between dystrophic and control hamsters.

Wasting of muscle is one of the characteristics of muscular dystrophies. In spite of research interest in these diseases for several years, the etiologies are still unknown. Early reports by Weinstock, Epstein and Milhorat[2], and others led to the suggestion that abnormal protein breakdown might be an important factor in the progress of some of the muscular dystrophies. Thus the objective of this study was to determine whether increases in proteolysis could be detected prior to detection of other changes described in the literature. In this communication we report the degradation of protein in muscle of young hamsters.

Table 1. Tyrosine release by abdominal and diaphragm muscles of dystrophic hamster

| μg Tyrosine release/mg noncollagen protein in 2 h | | | | |
|---|---|---|---|---|
| Age | Diaphragm | | Abdominal | |
| (days) | Control | Dystrophic | Control | Dystrophic |
| 3 | 0.77 ± 0.24 (6) | 0.84 ± 0.38 (10) | 0.88 ± 0.17 (6) | 0.98 ± 0.20 (10) |
| 7 | 1.04 ± 0.46 (6) | 0.62 ± 0.25 (6) | 0.89 ± 0.09 (6) | 0.89 ± 0.15 (6) |
| 11 | 0.76 ± 0.14 (6) | 0.46 ± 0.32 (6) | 0.89 ± 0.14 (6) | 0.71 ± 0.27 (6) |
| 15 | 0.51 ± 0.13 (6) | 0.63 ± 0.12 (6) | 0.53 ± 0.02 (6) | 0.72 ± 0.20 (6) |

Incubations were conducted in Krebs-Ringer bicarbonate solution which contained 0.5 mM cycloheximide. Values given are means ± SD; numbers in brackets indicate the number of animals.

Table 2. Tyrosine release by dystrophic hamster muscle in the absence of cycloheximide

| μg Tyosine release/mg noncollagen protein in 2 h | | | | |
|---|---|---|---|---|
| Age | Diaphragm | | Abdominal | |
| (days) | Control | Dystrophic | Control | Dystrophic |
| 3 | 1.36 ± 0.83 (6) | 0.74 ± 0.36 (10) | 0.84 ± 0.54 (6) | 0.97 ± 0.75 (10) |
| 7 | 0.58 ± 0.29 (7) | 0.38 ± 0.17 (7) | 0.44 ± 0.09 (6) | 0.51 ± 0.10 (5) |
| 11 | 0.39 ± 0.09 (7) | 0.59 ± 0.43 (6) | 0.40 ± 0.12 (7) | 0.48 ± 0.33 (6) |
| 15 | 0.43 ± 0.03 (5) | 0.85 ± 0.58 (8) | 0.45 ± 0.02 (5) | 0.91 ± 0.76 (8) |
| 20–40 | 0.43 ± 0.14 (7) | 0.60 ± 0.17 (6) | 0.56 ± 0.25 (8) | 0.62 ± 0.18 (7) |

Conditions were the same as for table 1 except that cycloheximide was excluded.

*Materials and methods.* The hamsters used for these experiments were UM-X7.1 and the controls were Syrian Golden Hamsters; both sexes were used. Because of the young age of the animals required, they were all bred and raised in the animal quarters of Queen's University. Hamsters were sacrificed by decapitation and their diaphragms and abdominal muscles were removed as quickly as possible. The rate of tyrosine release from the muscles was then estimated by the method of Fulks, Li and Goldberg[3] to provide an index of proteolysis. Briefly the tissues were preincubated (37 °C) in 3 ml of Krebs-Ringer bicarbonate solution (KRB) for 30 min and then incubated in a further 3 ml of KRB for 2 h. After removing the tissues the tyrosine contents of the incubation media were determined by the method of Waalkes and Udenfriend[4]. This procedure involves reacting tyrosine with 2-nitrosonaphthol and measuring fluorescence intensity. Protein as noncollagen protein was measured by the method of Lilienthal et al.[5], using bovine serum albumin as the reference.

*Results and discussion.* Homburger et al.[6] have reported that the earliest detectable morphological lesion in dystrophic hamsters occurs at about 20 days of age. Since we wished to learn whether alterations in protein degradation contributed to such observations it was necessary to examine animals younger than 20 days. Table 1 shows the rates of tyrosine release from diaphragms of animals as young as 3 days. These were the youngest animals which we could handle because of their small size. At any of the ages studied there was no difference between the dystrophic and control hamsters. These data are consistent with those of Goldspink and Goldspink[7] at the youngest age they used (30 days).

In the present studies experiments were conducted in the presence of cycloheximide to inhibit re-incorporation of tyrosine and also in its absence. Several reports in the last 2 years have indicated that the action of cycloheximide may not be simple blockade of protein synthesis as had been formerly assumed. For example, Woodside[8] found

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that 18 μM cycloheximide reduced protein synthesis by 93% in perfused rat liver but this concentration of drug also reduced protein degradation by 60%; Wildenthal and Griffen[9] observed decreased cathepsin-D-activity due to cycloheximide in cultured fetal mouse hearts. It can be seen from table 2 that in the absence of cycloheximide there was still no difference between control and dystrophic samples.

In addition to diaphragm, we have also studied abdominal muscle because, like diaphragm, it is a flat, thin sheet and lends itself to diffusion of small molecules into and out of the tissue. It also had the advantage of being readily recognizable and removable in these small animals. Tables 1 and 2 show that in this muscle also, the rates of tyrosine release were the same in dystrophic and control hamsters. The present data are tentatively interpreted to mean that increased proteolysis may not be essential to the development of the muscle lesion but rather could be a result. This interpretation is based on our observations that muscles from dystrophic hamsters did not show increased tyrosine release at ages younger than those at which the morphological lesion appeared[6]. Furthermore Goldspink and Goldspink[7] demonstrated increased tyrosine release at 100 and 230 days when the disease is well advanced. In making an interpretation it is well to remember that this method only allows for estimates of overall protein degradation and does not allow one to determine the fate of a particular protein which could be instrumental in the development of a lesion. We also recognize that it is not possible to relate directly tyrosine release to assays of cathepsin activity because cathepsin assays are conducted using synthetic or artificial substrates.

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## Studies on substrate specificity of X-prolyl dipeptidyl-aminopeptidase using new chromogenic substrates, X-Y-p-nitroanilides

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*9 August 1977*

*Summary.* Substrate specificity of X-prolyl dipeptidyl-aminopeptidase (dipeptidyl aminopeptidase IV) was examined by using newly synthesized 8 chromogenic substrates, X-Y-p-nitroanilides. Homogeneous enzyme from human submaxillary gland hydrolyzed glycylproline p-nitroanilide almost specifically, except alanylalanine p-nitroanilide which had 11% activity.

X-Prolyl dipeptidyl-aminopeptidase[1] is an enzyme which cleaves N-terminal X-proline from peptides. The enzyme was purified from either porcine kidney[2-4] or human submaxillary gland[5]. We synthesized several new chromogenic substrates, p-nitroanilides of the dipeptides, glycylproline, alanylproline, lysylproline, arginylproline, glutamylproline, and aspartylproline for X-prolyl dipeptidyl-aminopeptidase purified from human submaxil-

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Table 1. Analytical data for X-Y-p-nitroanilide · tosylates

| Compound | Melting point (decomp) °C | Optical rotation $[\alpha]_D$ | Temperature (°C) | Molecular formula | Found / calculated (%) C | H | N |
|---|---|---|---|---|---|---|---|
| Gly-Pro-pNA · TosOH | 223 ~ 225 | −81.0 (C1 MeOH) | 30 | $C_{20}H_{24}O_7N_4S$ | 51.82<br>51.72 | 5.19<br>5.21 | 12.24<br>12.06 |
| Gly-Leu-pNA · TosOH | 124 ~ 140 | −12.3 (C1 DMF) | 25 | $C_{21}H_{28}O_7N_4S \cdot H_2O$ | 50.73<br>50.59 | 5.90<br>6.06 | 11.01<br>11.24 |
| Gly-Sar-pNA · TosOH | 183 ~ 185 | | | $C_{18}H_{22}O_7N_4S$ | 48.92<br>49.29 | 4.98<br>5.07 | 13.00<br>12.78 |
| Gly-Gly-pNA · TosOH | 220 ~ 222 | | | $C_{17}H_{20}O_7N_4S \cdot H_2O$ | 45.92<br>46.15 | 4.95<br>5.01 | 13.01<br>12.66 |
| Gly-Hyp-pNA · TosOH | 142 ~ 146 | −36.3 (C1 DMF) | 25 | $C_{20}H_{24}O_8N_4S \cdot H_2O$ | 48.35<br>48.19 | 5.24<br>5.26 | 10.98<br>11.24 |
| Gly-Ala-pNA · TosOH | 239 ~ 241 | −31.1 (C1 DMF) | 25 | $C_{18}H_{22}O_7N_4S \cdot 1/4H_2O$ | 48.88<br>48.81 | 4.93<br>5.12 | 12.82<br>12.65 |
| Ala-Gly-pNA · TosOH | 244 ~ 247 | −16.3 (C1 DMF) | 25 | $C_{18}H_{22}O_7N_4S \cdot 1/4H_2O$ | 48.88<br>48.81 | 4.98<br>5.12 | 12.48<br>12.65 |
| Ala-Ala-pNA · TosOH | 122 ~ 141 | −11.1 (C1 DMF) | 25 | $C_{19}H_{24}O_7N_4S \cdot 1/2H_2O$ | 49.07<br>49.45 | 5.21<br>5.46 | 11.95<br>12.14 |