

Hernias as a Collagen Maturation Defect *

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THE INGESTION of the seeds of *Lathyrus odoratus* produces generalized connective tissue lesions in most mammals. The condition is referred to as lathyrism, or more specifically, as osteolathyrism, since bone lesions were among the earliest symptoms reported. Many reports have been made concerning the occurrence of hernias^{1, 2, 3, 5, 6} in young rats which had been fed a diet containing the lathyrus factor; it has never proved possible, however, to produce hernias in older rats by this method. This report deals with the correlation between these age differences and the concentration of extractable collagen in tissues of both young and old rats, which had been fed a lathyrus diet. The increased extractability of collagen, which is associated with lathyrism, is also demonstrated by histological techniques.

Experimental Methods

Two groups of Long-Evans rats, each consisting of 28 males and 28 females, were fed a diet containing 50 per cent *Lathyrus odoratus* seed meal and 50 per cent stock diet⁸ at different ages: 30 days of age and 88 days of age. Rats were sacrificed and autopsied after varying periods on the diet, and the incidence of peritoneal hernia determined. Litter mates of the lathyritic rats were in all cases used as controls.

Two areas of skin, approximately 2 × 2 cm., were excised from the anterior abdominal wall of each experimental and control animal and prepared for histological

examination by fixation in 10 per cent neutral formalin. Prior to fixation, one of the two specimens from each rat was immersed in a 1.0M NaCl solution at 4° C. for 24 hours,⁷ and will be referred to in this report as *extracted* tissue. Serial sections were stained as indicated on the figures. Peritoneal tissue and the hernial sacs were treated in an identical manner.

Collagen was extracted from peritoneal tissue and hernial sacs with 0.45M NaCl as previously described,⁹ and was assayed by determination of hydroxyproline by a micro method¹⁰ in hydrolysates of the unpurified extracts.

Results and Discussion

Hernia Incidence. A marked sex difference was noticed in the incidence and type of herniation in the group of rats placed on the diet at 30 days of age (Fig. 1-3). Thus 14 cases of hernia were found among the 28 males and only eight among 28 females. In the male, the inguinal type prevailed, occurring in 12 of the 14 cases, whereas in the females, all hernias were abdominal. The earliest observed hernia occurred on the 10th day of the diet in the males, while the earliest in the females occurred on the 14th day. It is felt, that these differences are not a reflection of growth rate differences, since we have previously shown that both sexes achieve maximum growth rates at the same age.⁹ In contrast to the herniation seen in young animals, none of the older group which was placed on the diet at 88 days of age developed hernias.

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Histology. Upon comparison of Figures 4 and 5 it can be seen that the collagen fibers in the unextracted lathyritic skin from the 30-day animals appear to be arrayed in a more random manner than those of the unextracted normal skin and in addition show increased reticulin staining material.

Following extraction with salt solution, a great deal of the dermal collagen is seen to be absent in the lathyritic skin (Fig. 7), while little change is seen in the similarly treated normal tissue (Fig. 6).

In contrast to skin, no histological differences were apparent between the peritoneum of normal and lathyritic rats when treated as above, and this may be due to the relatively small amount of collagen normally present in peritoneum as compared with the other tissue studied. No changes in any of the tissues could be demonstrated when rats were placed on the diet at 88 days of age.

It thus becomes apparent that the physical state of collagen is altered by lathyrism to such an extent that it can be largely removed by extraction with sodium chloride solutions, and this removal can easily be

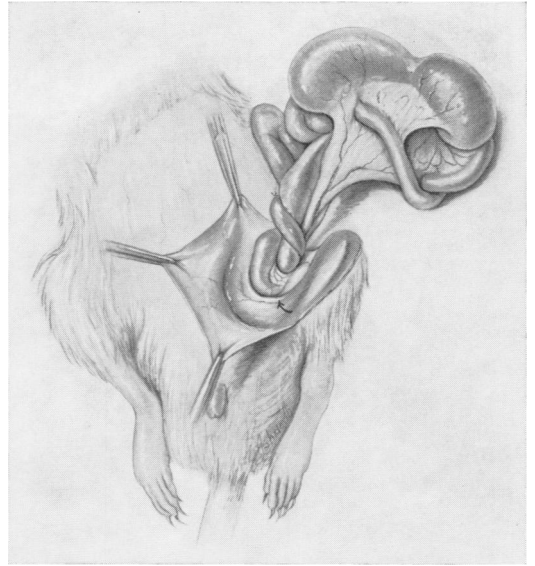


FIG. 2. Drawing of the defect in the peritoneum and the contents of the hernial sac.

seen by histological examination. The demonstration of extractable collagen histologically was first made by Jackson and Williams in 1956,⁴ who were able to demonstrate the disappearance of reticulin from normal tissue upon extraction with 0.2M sodium chloride solutions. The magnitude of the removal is, however, very much

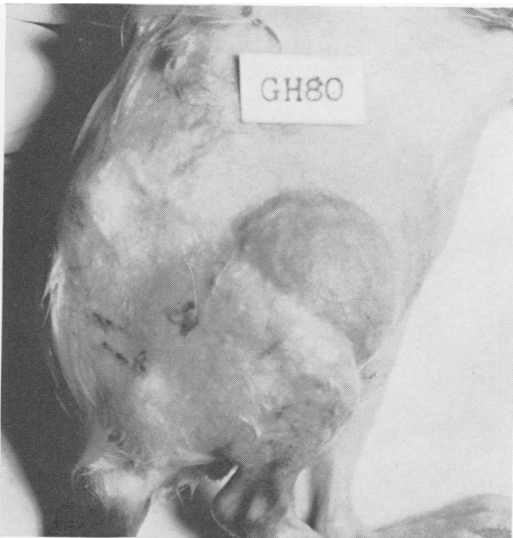


FIG. 1. Inguinal hernia in experimental animal, 44 days of age.

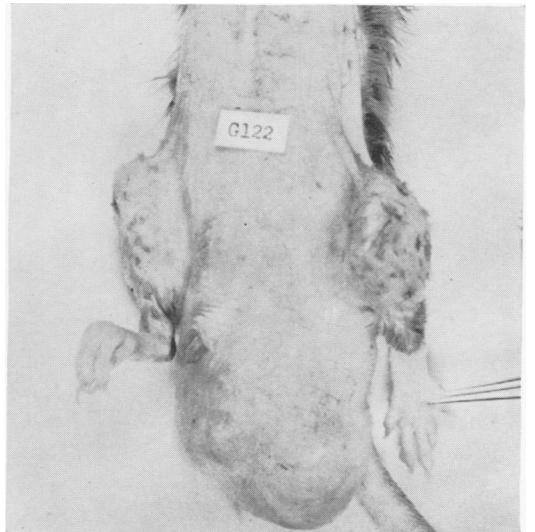


FIG. 3. Scrotal hernia of experimental rat, 50 days of age.



FIG. 4. Normal unextracted skin at 44 days. The collagen fibers are arrayed in a regular and orderly pattern. Wilder's reticulin stain ($\times 115$).

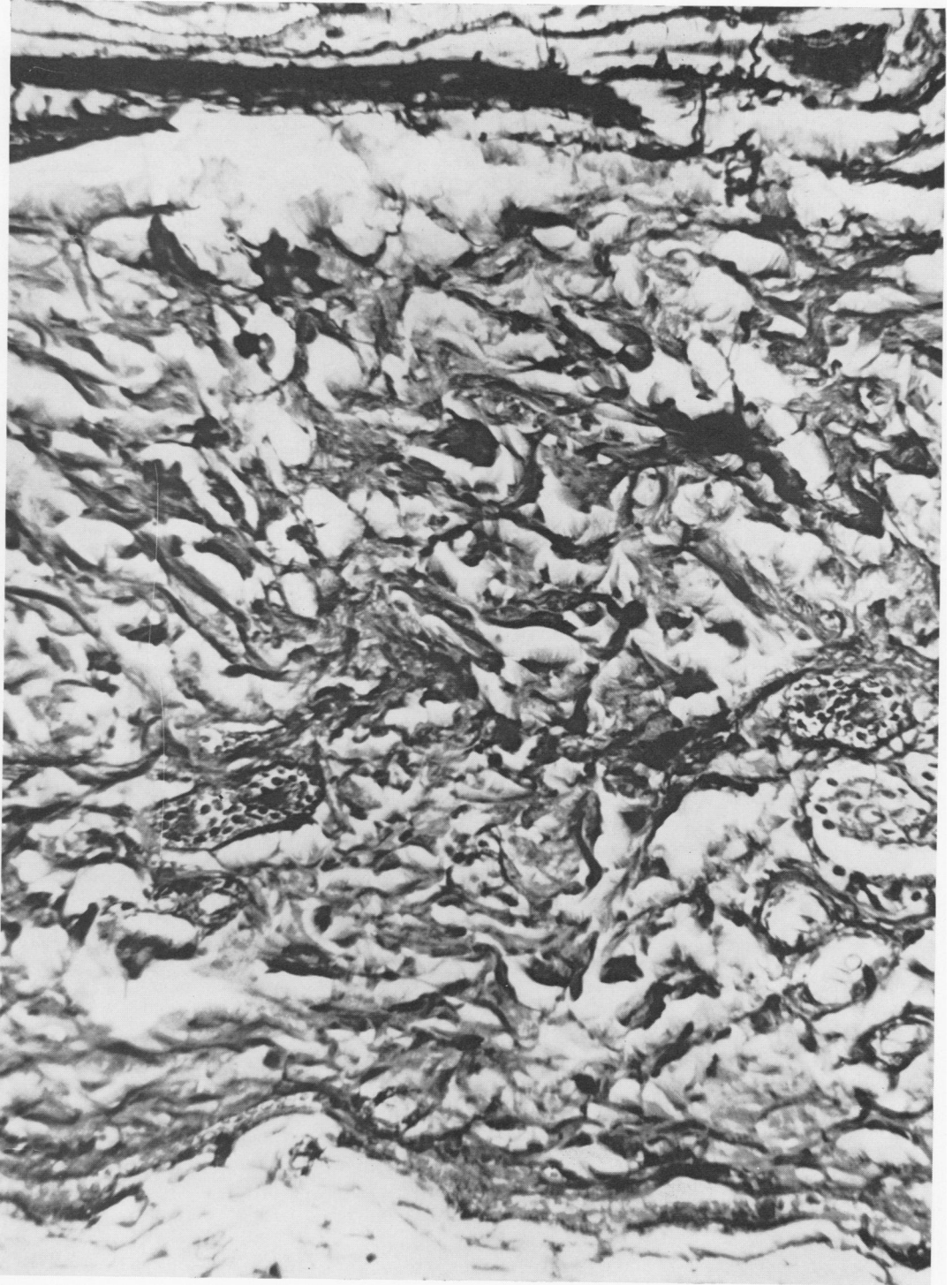


FIG. 5. Experimental unextracted rat skin at 44 days of age. The dermis is thinner than normal, the collagen fibers appear to be arrayed in a more random manner, and exhibit increased reticulin-staining material. Wilder's reticulin stain ($\times 115$).

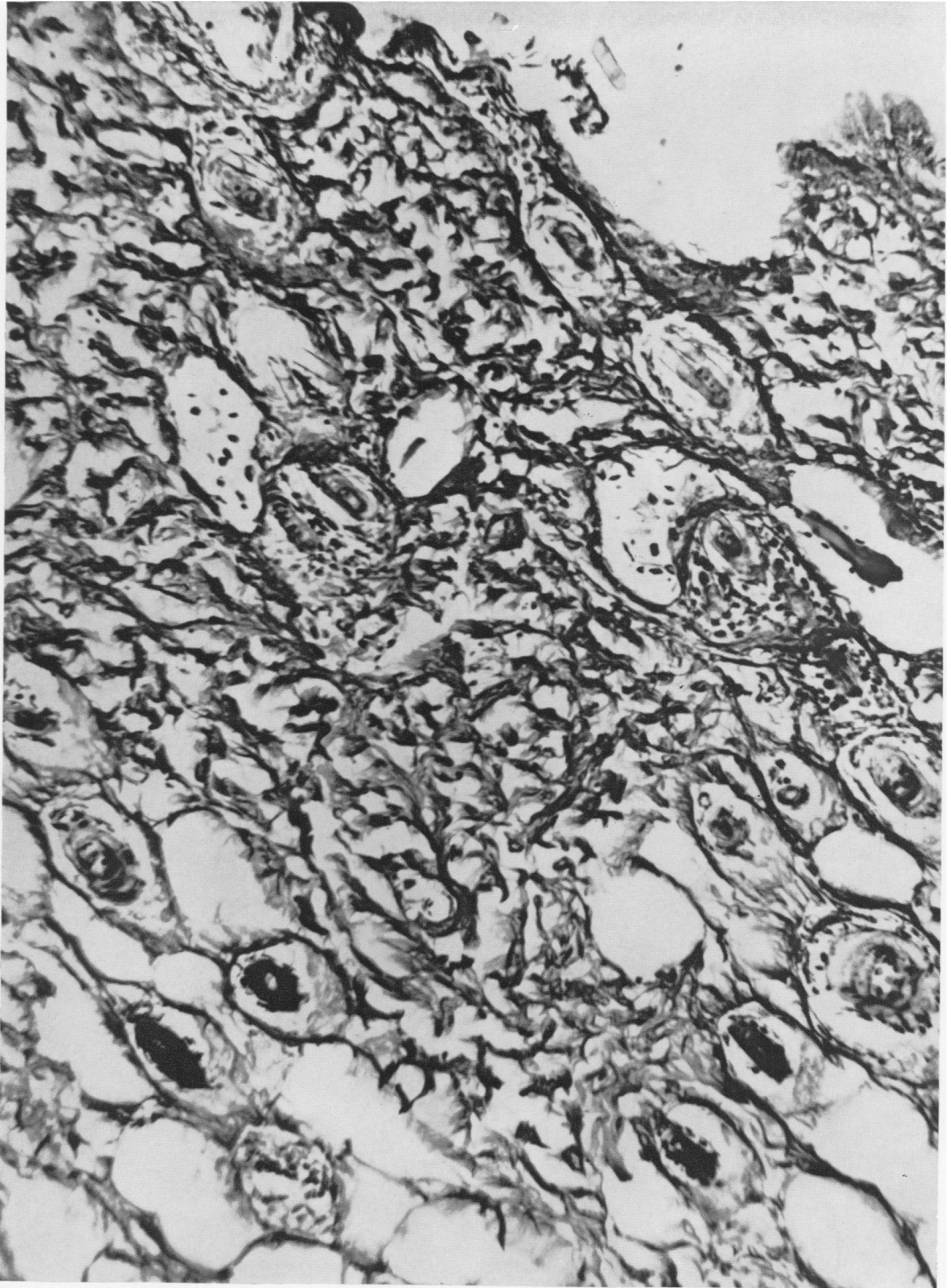


FIG. 6. Normal extracted skin at 44 days of age. Alterations in the collagen fibers are not observed. Wilder's reticulin stain ($\times 115$).

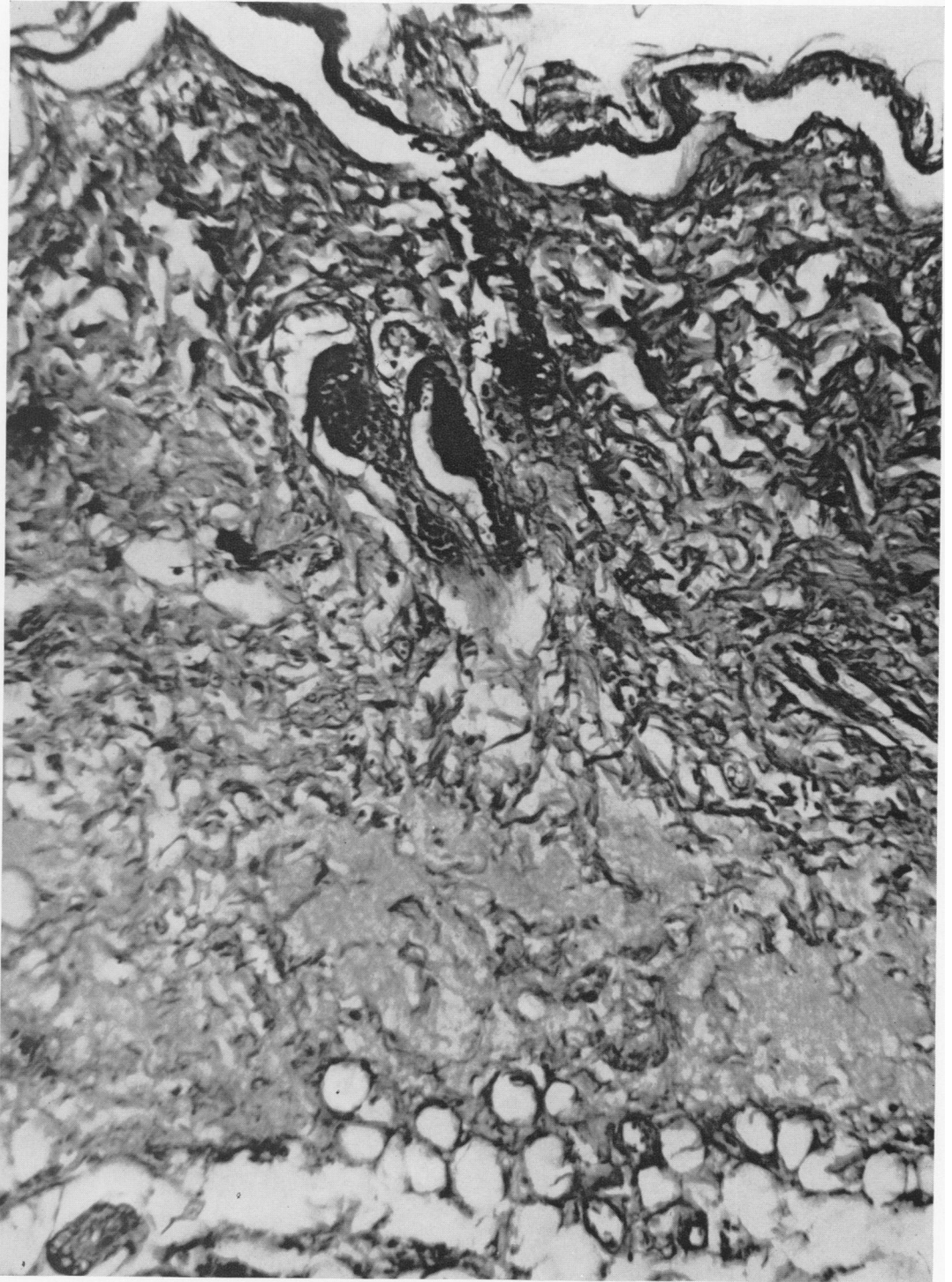


FIG. 7. Experimental extracted skin. After extraction, a great deal of the dermal collagen is absent, in contrast to the similarly treated normal tissue (Fig. 6). Wilder's reticulin stain ($\times 115$).

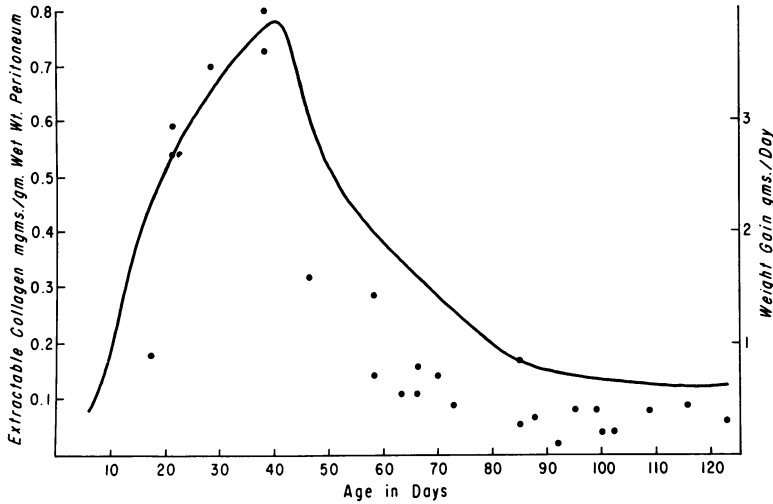


FIG. 8. Comparison of the rat growth rate with extractable collagen concentration of peritoneum. The solid line shows the average weight gain in grams per day. Extractable collagen concentrations superimposed on this curve represent three pooled peritoneums at each point.

greater in skin from young lathyritic rats than was that previously described, and is a reflection of the extremely high values of skin-extractable collagen previously reported,⁸ and of similarly high values of the peritoneum and hernial sacs which are described here. The results are in very good agreement with those of Van den Hooff

et al.,⁷ who showed similar changes in lathyritic chick embryo tissue.

Extractable Collagen. From Figure 8 it can be seen that the rate of growth of Long-Evans rats is maximal at 35 days, and that at this time the concentration of extractable collagen in the peritoneal tissue from normal rats is also higher than at other

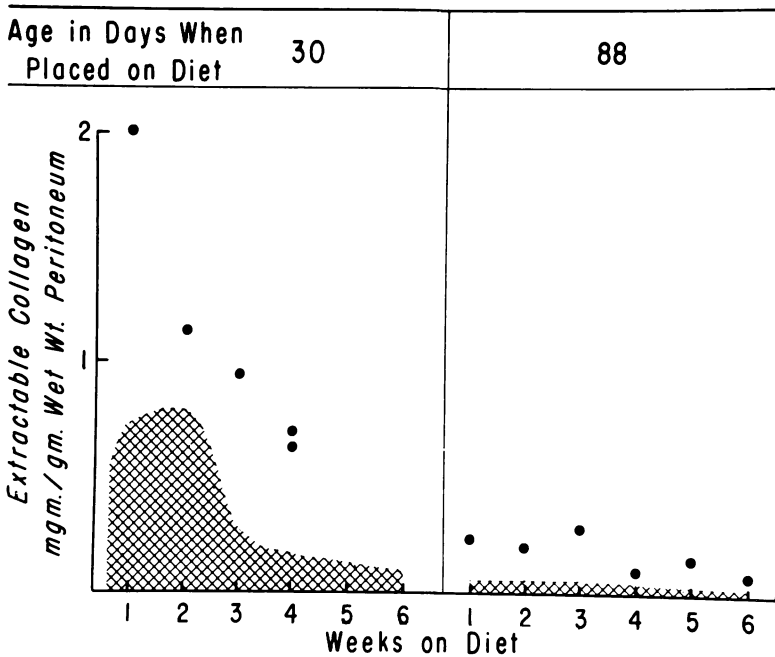


FIG. 9. The extractable collagen of the peritoneum of normal rats and experimental rats placed on the lathyritic diet at 30 days of age and at 88 days of age. The shaded area represents the range seen in normal rats.

times. This correlation is in excellent agreement with previously reported work on the extractable collagen of rat skin.⁹ The extractable collagen concentration of similar tissue from the lathyritic litter mates of the controls is presented in Figure 9, and it can be seen that much higher concentrations are found when young rats are fed the lathyrus diet than when older animals are so treated. This is also in good agreement with the results from skin.

It now becomes evident that the lathyrus-induced defects in peritoneal tissue, which occur in young animals, coincide with grossly elevated extractable collagen concentrations within these tissues. When the concentration of this protein is lower, as in rats placed upon the diet at 88 days of age, hernias are not seen. These results support the contention that the elevation of extractable collagen concentration, which is so typical of lathyrism, is due to a failure in aggregation or ageing of newly formed collagen molecules rather than to break-down of mature fibers. Either of these mechanisms could, of course, produce a mechanical weakening which would lead to tissue rupture in areas of stress, but if mature fibers were to become disaggregated, then older animals would be expected to develop hernias and increased extractable collagen concentrations along with the younger group.

On the basis of these results it is interesting to speculate on the etiology of hernia generally. When hernia occurs in the young human, it is usually considered a congenital defect, and since hernias never occur in the authors' stock colony of Long-Evans rats, the high incidence of hernia incidence of hernia in lathyrus-fed rats of this strain is of particular interest. The occurrence of hernias in lathyrism can definitely be related to a defect in collagen metabolism, and it is thus possible that other types of hernias, i.e. congenital hernias, may also be related to a similar collagen maturation defect.

Summary

The occurrence of hernias in lathyrus-fed rats is directly related to concentration of extractable collagen in these tissues, and to the growth rate of the rats. A simple technic for the histological demonstration of high values of extractable collagen is also described.

Acknowledgments

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