Collagen Deficiency in Rectus Sheath of Patients with Inguinal Herniation (35582)

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Herniation through the groin is by far the commonest type of rupture seen in man. Occurring almost always in the male, its high incidence (one in seven) has been related to intrauterine passage of the testicle into the scrotum with persistence of the spermatic cord introducing a locus minoris resistentiae in the abdominal wall. Two types are recognized: indirect inguinal herniation with retention of the process vaginalis and a direct form without any congenital peritoneal protrusion. However, certainly in the adult an acquired factor of fascial weakness presumably due to aging has been proposed (1). Nevertheless, direct evidence for such an aponeurotic atrophy has not been presented until recently (2), when the observation was made that the conjoined oblique and transversus abdominis aponeuroses in direct hernia were thinner than when indirect herniation was present. This observation was confirmed by weighing biopsy samples of constant area. The results could not be related to age.

The thinness and the decrease in weight of the rectus sheath in direct herniation prompted us to study the biochemical makeup of this tissue to determine if certain connective tissue components might be involved in the cause of herniation. We therefore conducted chemical compositional analyses of several fascial samples taken at operation. This communication presents our data, which strongly suggest that inguinal herniation is a collagen defect.

Materials and Methods. Biopsy samples of the anterior rectus sheath (fascia) were obtained one fingerbreadth below the anterior superior iliac spine close to the midline from patients operated on at the Veterans Administration for direct and indirect inguinal hernia. Samples designated as "controls" were taken from patients without hernia but on whom other surgical procedures were performed. Each sample was 2.27 cm^2 in area and was cut with a punch.

Defatting. The tissue was briefly rinsed with physiological saline to remove blood and was extracted in 5 ml of ether:acetone (3:1; v/v) at 37° for 1 hr. After decanting the solvent, the sample was washed with acetone and dried *in vacuo* at 70° for 16 hr. The residue thus obtained was designated "defatted dry tissue".

Proteolytic digestion. The method of Mier and Wood (3) was used with slight modifications. The defatted dry tissue was hydrolyzed with papain (2× crystallized, Worthington Biochemical Corporation) in a final volume of 1.0 ml of 0.2 *M* sodium acetate buffer containing 2.0 mg of cysteine hydrochloride (pH 7.0). The ratio of tissue:enzyme was 15:1 (w/w). Incubation was carried out at 65° for 48 hr which allowed complete disintegration of the tissue. The reaction mixture was treated with 0.2 ml of cold 50% trichloroacetic acid (TCA). The resulting precipitate and supernate were collected by centrifugation at 3000 rpm for 20 min. The precipitate was washed once with 0.5 ml of distilled water and centrifuged as above. The supernates were combined and the precipitates were dissolved in 1.0 ml of 0.1 *N* NaOH. The TCA supernates and the precipitate solutions were used for chemical analyses.

Chemical analyses. Hydroxyproline was determined by the method of Woessner (4) after hydrolysis with 6 *N* HCl for 4 hr at 130°. Hexose was determined by the phenol–sulfuric acid method (5) employing galactose

TABLE I. Composition of Anterior Rectus Sheath from Control and Indirect or Direct Hernia Patients.

Variable	Control	Hernia	
		Indirect	Direct
Age (years)	62.2 ± 4.45 (5)[b]	53.7 ± 2.16 (51)	56.6 ± 1.75 (52)
Defatted dry wt (mg/biopsy)	42.5 ± 3.60 (5)	43.9 ± 2.05 (57)	43.7 ± 1.55 (56)
Hydroxyproline (mg/biopsy)	3.84 ± 0.36 (5)	3.53 ± 0.16 (55)	3.23 ± 0.15 (56)[d]
(μg/mg ddw[a])	90.1 ± 1.49 (5)	81.9 ± 1.72 (55)	74.5 ± 2.28 (56)[e]
Hexose (μg/biopsy)	714 ± 76.00 (5)	702 ± 30.50 (57)	686 ± 23.60 (55)
(μg/mg ddw)	16.7 ± 0.60 (5)	16.1 ± 0.28 (57)	15.7 ± 0.34 (55)
Uronic acid (μg/biopsy)	193 ± 6.94 (5)	223 ± 12.96 (56)	217 ± 9.85 (55)
(μg/mg ddw)	4.5 ± 1.06 (5)	5.1 ± 0.20 (56)	4.9 ± 0.13 (55)
Hexosamine (μg/biopsy)	—[c]	284 ± 23.80 (21)	285 ± 13.50 (23)
(μg/mg ddw)	—	6.4 ± 0.20 (21)	6.2 ± 0.23 (23)

[a] ddw = defatted dry weight.

[b] Mean ± SE of the mean; number of observations is given in parentheses.

[c] Not determined.

[d] t values: control vs indirect = 0.56; control vs direct = 1.21; and indirect vs direct = 1.36.

[e] t values: control vs indirect = 1.43; control vs direct = 2.00 ($p < .05$); and indirect vs direct = 2.60 ($p < .02$).

as the standard. Uronic acid was quantitated by the procedure of Bitter and Muir (6) using glucuronolactone as the standard. Hexosamine was measured by the method of Gatt and Berman (7) after hydrolysis with 2 *N* HCl for 16 hr at 100°. Glucosamine hydrochloride was used as the standard. The hexosamine content was expressed as glucosamine (free base). Total protein was measured by the method of Lowry *et al.* (8) using human serum albumin as the standard. The conditions of hydrolysis used for hexosamine and hydroxyproline determinations were established by kinetic studies.

Results. Data relative to the composition of the anterior rectus sheath from controls and patients with direct or indirect inguinal hernia are presented in Table I. The values reported for hexose, uronic acid, and hexosamine were obtained from the analyses of the TCA supernate fractions while those for hydroxyproline were obtained after direct acid hydrolysis of the defatted dry tissue. When the TCA supernates were hydrolyzed and analyzed for hydroxyproline, it was observed that all (95–100%) of the hydroxyproline present in the biopsy sample could be recovered in the TCA supernate. We could not detect hydroxyproline, uronic acid, or hexosamine in the TCA precipitate. A trace amount of hexose (1–2% of hexose occurring in the TCA supernate) appeared in this fraction. Protein content of the TCA precipitate as measured by the method of Lowry *et al.* (8) represented about 5–7% of the tissue used for the proteolytic digestion.

There was no difference in the defatted dry weights of the tissue among the patients studied. The most striking change was observed in the hydroxyproline content of the samples. The amount of hydroxyproline per milligram of defatted dry tissue appeared to be the least among patients with direct hernia. Also the indirect hernia patients showed lower amounts of hydroxyproline when compared with the controls. These differences were significant (Table I). The total hydroxyproline in the biopsy samples showed a similar trend. This is expected since the defatted dry weights were similar. Since hydroxyproline is a measure of collagen, we conclude, therefore, that the amount of collagen present in the fascia of direct and indirect hernia patients is lower than those in controls. Also the values for indirect hernia patients fell between those of controls and direct hernias.

The amount of hexose, uronic acid, and

hexosamine were not significantly affected among all patients, indicating that glycoproteins and mucopolysaccharides may not be directly related to herniation.

Correlation analyses of age versus all the parameters studied revealed that age was not related to the changes discussed.

Discussion. An earlier study from this laboratory (2) provided evidence that in inguinal herniation the rectus sheath unrelated to the defect is thinner than normal. It was shown that the mean wet weights of the rectus sheath among patients with inguinal hernia were in the magnitude: direct < indirect < control. Since the delipidated and dehydrated weights of this tissue are similar it is evident that the total amount of fat and moisture would have the reverse magnitude.

From the fact that the total amount of collagen decreased among patients with direct and indirect hernia as compared to controls and that the amount of glycoprotein and mucopolysaccharide component did not significantly alter, we reason that the difference could be accounted for by an increase in noncollagenous proteins among hernia patients. Also, the lower amount of fat and moisture may be a reflection of decreased hydration of the rectus sheath in herniation. Thus the lower amount of collagen, accompanied by an increase in noncollagenous-type proteins and decreased hydration may be factors contributing toward the weakness of the fascia in inguinal herniation.

The decreased amount of collagen in the rectus sheath may be the result of decreased rate of biosynthesis or increased rate of catabolism. These studies sugegst that the adult with inguinal herniation suffers from collagen defect. Further work is in progress to study the nature of this defect at the cellular level.

Summary. Chemical composition of rectus sheath from normal, and indirect and direct inguinal hernia patients has indicated that in inguinal hernia the rectus sheath contains decreased amount of collagen as compared to controls. Total hexose, uronic acid, and hexosamine were not significantly altered.

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