TABLE I. Comparison of Human Plasmas in Therapy of Tourniquet Trauma. (Volume equal to 3% body weight.)

Plasma infused	48 hr survival Lived	Died
Fresh (individual donor)	0	35
Shelf stored (pooled, 6 mo at room temperature)	24	11
Reconstituted, lyophilized (pooled, commercial)	26	8

TABLE II. Effect of Aging of Human Plasmas in Therapy of Tourniquet Trauma. (Volume equal to 3% body weight.)

	Plasma infused	48 hr survival Lived	Died
Exp 1	Fresh, pooled	0	18
	Fresh, single donor	0	17
" 2	Pooled, 1 wk old	1	17
	Lyophilized, reconstituted	16	0
" 3	Pooled, 5 wk old	8	9
	Lyophilized, reconstituted	11	7
" 4 & 5	Pooled, 13 and 14 wk old	22	14
	Lyophilized, reconstituted	22	13

lized in therapy of rat tourniquet shock. Fresh human, non-pooled plasma was completely ineffective in protection against this trauma, there being no survivors with the use of this material.

For each type of plasma, rat cells were crossmatched against donor plasma and showed clumping of cells. During one experiment one animal from each group was sacrificed to allow inspection of the serum for hemolysis. In the animals which received fresh and shelf-stored plasma hemolysis was present to a slight degree. In the animals infused with the reconstituted lyophilized plasma a "moderate degree" of hemolysis was present. It would thus seem that, although some hemolysis was present in all groups, this did not interfere with the beneficial therapeutic effect.

The results of the second group of experiments are summarized in Table II. The use of recently collected plasma, whether single donor or pooled, produced no survival after the standard tourniquet trauma, but as the pooled plasma aged, it gradually acquired the ability to protect, so that by 3 months after collection, it was as effective as the lyophilized plasma.

Discussion and summary. The data presented demonstrate that pooled or single donor human plasma immediately after collection exerts no beneficial effect in the therapy of rat tourniquet trauma, but that the aging of this material in some way converts it into a potent therapeutic solution. Whether this is due to neutralization of something harmful or to the conversion of an inactive material into something beneficial has not been determined. Investigations are underway to ascertain whether aged rat plasma is more effective than fresh rat plasma in treatment of this trauma. Sequential electrophoretic studies are also being made.

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Hydroxyproline Excretion in Urine of Patients with Muscular Dystrophy and Other Muscle Diseases.* (28999)

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In some forms of muscular disease, such as muscular dystrophy, the muscle fibers are replaced largely by connective tissue and fat. In muscular dystrophy occurring as an hereditary disease in mice, the muscles affected may have about 25% more collagen than the muscles of controls(1). In the advanced

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TABLE I. Urinary Excretion of Creatine, Creatinine, and Total Hydroxyproline by Patients with Pseudohypertrophic Muscular Dystrophy.

Patient No.	Age Yr	Age Mo	Daily urinary output: Hydroxyproline (mg)	Creatine (mg)	Creatinine (mg)
1	5	10	35	129	166
2	8	6	37	255	194
	9	1	77	340	223
	9	3	73	318	186
	9	6	123	465	127
3	11	8	143	536	220
	12	3	125	538	181
	12	5	118	476	180
	12	8	111	591	173
4	11	5	70	316	223
	12	0	124	638	306
	12	4	132	672	257
5	13	0	170	544	214
	13	0	151	509	195
	13	0	129	555	193
6	14	6	81	725	165
	14	6	77	655	159
7	17	10	29	710	182
	18	5	41	665	171
	18	7	34	611	149
	18	10	33	694	134
8	17	2	36	737	186
	18	0	34	751	154
	18	2	37	745	165
9	17	11	32	700	229
	18	8	27	670	164
	18	9	28	697	173
	18	10	29	719	137

stages of the disease in patients similar alterations may occur. Inasmuch as muscular dystrophy commonly involves most of the muscles of the body, the total accumulation of collagen often is considerable. Collagen metabolism in man may be estimated by the total hydroxyproline excreted in the urine when the subject is on a collagen-free diet (2). We recently reported that the total hydroxyproline excreted by patients with muscular disease who are maintained on a collagen-free diet may be somewhat less than that excreted by normal subjects of the same age(3). We now present a more detailed study of the excretion of total hydroxyproline in patients with pseudohypertrophic muscular dystrophy and other diseases of muscle and compare these results with those of controls.

Materials and methods. Most of the patients who had pseudohypertrophic muscular dystrophy in varying degrees of severity were maintained for long periods in a metabolism ward on a diet free of collagen, creatine, and creatinine(4). Urinary excretion of creatine and creatinine was determined daily(5) to follow the progress of the disease, and urinary hydroxyproline was determined periodically by the method of Prockop and Udenfriend(6). Other patients with muscular dystrophy or other diseases affecting the muscles were similarly maintained but for shorter periods. Creatine and creatinine outputs were determined daily, but hydroxyproline was determined on only one or two 24-hour urine samples.

Results and discussion. Table I shows excretion of total hydroxyproline, creatine, and creatinine by patients with pseudohypertrophic muscular dystrophy for periods from one day to one year. The data in Fig. 1 compare the total hydroxyproline excretion of these patients with that of controls(7) and show that the patients excreted less than did controls of the same age. Patient No. 2 at age 8½ years excreted 37 mg daily; after 7 months this increased to 77 mg, and after 12 months to 123 mg. Normal children in this age period show about the same order of increase in urinary output of hydroxyproline although the absolute amounts are different.

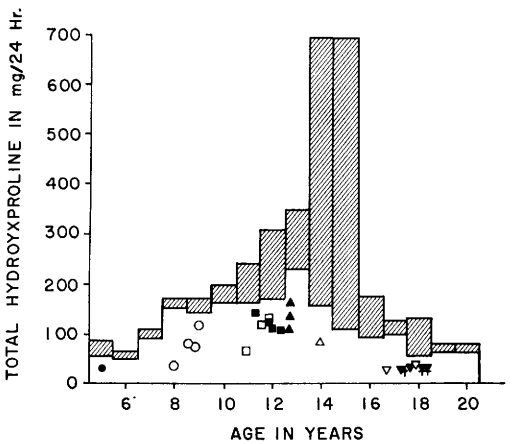

FIG. 1. Comparison of daily urinary output of total hydroxyproline by male patients with pseudohypertrophic muscular dystrophy and normal male subjects. The symbols represent the observed outputs of patients as follows: No. 1, ●; 2, ○; 3, ■; 4, □; 5, ▲; 6, △; 7, ▼; 8, ▽; and 9, +. The shaded areas represent the range of hydroxyproline output by control subjects(7).

TABLE II. Output of Total Hydroxyproline in Diaeases Other than Muscular Dystrophy of Duchenne Type.

Patient No.	Diagnosis	Sex	Age	Total hydroxyproline (mg/24 hr)
10	Facio-scapulo-humeral muscular dystrophy	♀	11	67.4 78.8
11	*Idem*	♀	29	14.3
12	Limb-girdle muscular dystrophy	♀	17	22.0 22.5
13	*Idem*	♀	33	19.8
14	*Myotonia dystrophica*	♂	43	9.0 12.6
15	*Myasthenia gravis*	♀	45	20.9
16	Polymyositis	♂	65	18.7 15.5
17	*Idem*	♀	36	21.4
18	Amyotrophic lateral sclerosis	♂	58	17.8
19	Dermatomyositis	♂	55	76.7 78.5
20	*Idem*	♀	47	20.8

Outputs of controls were: girls aged 14 271, 155, 201
boys " 11 162, 242
" " 17 100, 122 (ref 7)
Outputs of normal adults ranged from 14.5 to 54.6 [average 32.9 (ref 8)].

Excretions of creatine and creatinine of patient No. 2 were compatible with the advanced stage of his disease and changed further as the disability steadily increased. The same order of increase of hydroxyproline was shown by patient No. 4, aged 12, whose output rose from 70 to 132 mg over a period of 11 months. Patient No. 5 already was excreting 170 mg of urinary hydroxyproline when the analyses were begun, and the level decreased to 129 mg during the 2-week period of observation; these are also significantly below the level of controls at age 13. Likewise, in patient No. 6, in an advanced stage of dystrophy, excretion of hydroxyproline was definitely below the normal level at age 14. Similarly, patients Nos. 7, 8, and 9, who were in advanced stages of the disease, showed markedly lower levels than normal subjects at age 18. Urinary excretion of total hydroxyproline increases at puberty; it later drops sharply, but in the late teens it remains at about the same elevated level as in the prepubescent period(7). The results indicate that patients with pseudohypertrophic muscular dystrophy at all ages excrete less total hydroxyproline than controls. The decreased excretion is more marked in absolute amount between ages 11 and 14. Muscular dystrophy of pseudohypertrophic form is a progressive disorder with onset commonly before age 5 and usually incapacitates the patient completely during early puberty. As a rule, therefore, younger patients have less muscle damage than older ones. It seems reasonable to conclude that the total muscle damage is less in the younger patients, for example, No. 6 at age 14, than in the older ones, Nos. 7, 8, and 9 at age 18. The clinical status of these patients supports this concept.

Table II shows total hydroxyproline excretion in muscle diseases other than muscular dystrophy of pseudohypertrophic type. These results are not so well defined. Patients Nos. 12 and 10, girls aged 17 and 11, respectively, apparently had a lower hydroxyproline excretion than controls of these ages. The muscular dystrophy in one patient was the limb-girdle type and in the other facio-scapulo-humeral. All other patients in this group except No. 19, who had dermatomyositis, had fairly normal excretion of hydroxypro-

line. That the urinary output of hydroxyproline is not always elevated in dermatomyositis is evident from the normal excretion of patient No. 20 who from a clinical standpoint had active dermatomyositis at the time of observation. It should be noted that these patients were receiving cortical steroids.

It cannot be assumed that the lower excretion of hydroxyproline in pseudohypertrophic muscular dystrophy is due to accumulation of collagen in the muscles, although the results do not rule out this possibility. A relationship between rate of growth of the individual and excretion of hydroxyproline has been postulated(8). Whether the mechanisms operating during growth are altered in muscular dystrophy is under investigation.

Summary. Endogenous collagen metabolism was studied in 9 patients with pseudohypertrophic muscular dystrophy between 6 and 18 years of age, and in 11 patients with other diseases of the muscles, by measuring urinary total hydroxyproline excretion. The results obtained in pseudohypertrophic muscular dystrophy indicate that excretion of total hydroxyproline is significantly less at all ages than in controls of the same age. Two girls, 11 and 17, one with facio-scapulohumeral muscular dystrophy, and the other with the limb-girdle type of the disease, apparently have a lower excretion of hydroxyproline than controls of the same age. The other patients, all adults, have a normal excretion, with the exception of one patient with dermatomyositis. The significance of these results is discussed.

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Effect of Radioisotope Disintegration on Cell Culture Cell Viability.* (29000)

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Stent and Fuerst(1) have reviewed the applications of the decay of biologically incorporated radioisotopes to genetic and physiological studies in phages and bacteria. By controlled incorporation of a radioisotope into a particular biochemical compound and by using suitable experimental manipulations one may obtain transmutation of one element into another *in situ* and determine the biological consequences of transmutation under varying physiological conditions. If applicable to mammalian cells in culture, studies similar to those reviewed by Stent and Fuerst(1) could be made with animal cells.

The functioning of the blueprint, DNA, for phage synthesis has been examined in detail by Stent and his co-workers(2,3) using ^{32}P decay. They found that the information was transferred to a structure not inactivated by ^{32}P decay after 9 minutes under growing conditions and that protein synthesis was required for this transfer.

These studies have rarely been extended

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