of weight they were given a considerably larger dose of histamine.

Similar gradations of susceptibility in relation to age have been reported for other conditions such as anoxia (Glass[8] *et al.*, Chang[9]) for cardiac sub-endothelial hemorrhages (Visscher[10] *et al.*), for myocardial lesions from digitalis (Barnes[11] *et al.*) and for certain drugs (Bastedo).[12] Moreover, there is evidence[13] that with advancing years the autonomic nervous mechanisms for the control of homeostasis become less efficient resulting in a vagus preponderance in old age.

The senile dogs in this series were 10 years old or older, which age, according to veterinarians[2] is equivalent to a human age of 70 years. The occurrence of peptic ulcers for the first time in humans in this age is not unusual. Klingenstein[14] observed that over one-half of the patients over 50 with proved gastroduodenal ulcer had the first onset of symptoms past the age of 50. Likewise Meyer and Saphir[15] have pointed out that old people are likely to develop acute ulcers and succumb to hemorrhage and perforation even with very low acid levels especially following major surgery, febrile disease, or cardiac disease. These clinical observations would suggest that in the genesis of ulcers, particularly in the aged, factors that have to do with mucosal resistance to digestion are at least as important as acid-pepsin levels. Much the same seems to hold true for these dogs inasmuch as the fasting gastric acidities of aspirated juice while on histamine-in-beeswax stimulation were the same in Groups I, II, and III. (Range 60-100° free acid for all groups).

The present study is of practical importance for those investigators studying the ulcer problem experimentally for it indicates that in using the histamine-in-beeswax technic, or more than likely any other ulcer-producing method, one should compare only dogs of the same approximate age.

Conclusion. There is a gradation on the basis of age in susceptibility to histamine-induced ulcer in the upper gastrointestinal tract of dogs. The oldest dogs exhibit the greatest incidence of ulcers and the pups from 3 to 8 months show the greatest resistance with the dogs of mature age intermediate in their susceptibility to ulcer formation.

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Aminoaciduria in Progressive Muscular Dystrophy.*

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In view of the wasting of muscle which is characteristic of progressive muscular dystrophy, it seemed probable that there might be an increased urinary excretion of minor nitrogenous products in addition to creatine, *e.g.*, free amino acids. On studying the urine from patients suffering from progressive muscular dystrophy we found that there was a generalized aminoaciduria characterized by

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the excretion of a larger number of amino acids than is normally the case, accompanied in many instances by an excessive excretion of amino nitrogen.

Meldolesi[1] studied a number of cases of progressive muscular dystrophy and reported a decreased urinary excretion of nitrogen. However, results of recent studies by Milhorat and Tosconi,[2] and Shank, *et al.*[3] of nitrogen metabolism in progressive muscular dystrophy have failed to demonstrate any significant abnormality. It seems evident, therefore, that any alteration in nitrogen metabolism is not apparent in the measurement of total nitrogen excretion.

The use of partition chromatography to determine the distribution and excretion of urinary amino acids has been developed by Dent.[4] In a study of normal humans he usually found glycine and alanine and occasionally one or two other amino acids. He applied this procedure to the determination of abnormalities in several patients with Fanconi syndrome. In a recent paper,[5] he reported more fully on Fanconi syndrome, in which he found a large number of amino acids and an increased level of amino nitrogen. In 2 of the patients, amino nitrogen excretion was not excessively high but a greater number of amino acids were being excreted.

We have applied this technique in a study of 32 samples** of urine which were obtained from patients who had been diagnosed as suffering from progressive muscular dystrophy or related conditions. A portion of these were obtained by one or more voidings but about one-half represented 24-hour collections of urine. As indicated in Table I some of these patients were receiving therapy. The general diagnosis and an indication of the severity of the condition are given for each patient.

The urines were chromatographed on filter paper employing the strip technique essentially as has been described by Dent.[4] An aliquot of 0.025 ml was placed at a starting point on the strip. The adjacent end of the strip was placed in the solvent and remained there for 15 to 23 hours. The strip was then air-dried, sprayed with a solution of ninhydrin and heated to 100°C for 15 minutes. Colored bands were observed at the points where amino acids were to be found on the strip. Solvents employed were water-saturated solutions of phenol, butanol and a 50-50 mixture of lutidine and collidine. In many cases, strips were run in duplicate with satisfactory correspondence being obtained.

Two methods were used for evaluating the developed paper strips. An arbitrary notation was devised, in which strips which showed a normal number of colored bands were designated as "O"; those which showed a greater number or an unusual distribution were designated by "1"; and strips which showed an excessive number or very unusual distribution were designated as "2." The results of this general analysis agreed with Table I where practically all of the pathological urines indicated excessive amino-acid excretions when compared with normal urines. In addition to this general description, a numerical value was used which was arbitrarily set up as follows: The color intensity of a given band on the strip was visually estimated from "1" to "5" where "1" represented the faintest band observable and "5" represented an intensely colored band. If the color intensity of each band is multiplied by its width in cm, the sum of these products for a given solvent will be designated the *dw* value. It is felt that this *dw* value, although entirely arbitrary, gives a useful estimate of the dispersion and concentration of the individual amino acids appearing as bands on the paper strip. A normal human urine gives *dw* values less than 6 or 8. As may be seen from the data recorded in Table I, in most cases the *dw* values for the patients with progressive muscular dystrophy were greater than 10 and in some

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[3] Shank, R. E., Gilder, H., and Hoagland, C. L., *Arch. Neurol. Psychiat.*, 1944, **52**, 431.

[4] Dent, C. E., *Lancet*, 1946, **2**, 637.

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** These samples were obtained through the courtesy of Dr. A. T. Milhorat of the New York Hospital and Drs. K. E. Mason and L. J. Filer, Jr., of Strong Memorial Hospital, Rochester.

TABLE I.
Urinary Excretion of Amino Acids in Progressive Muscular Dystrophy and Related Conditions.

Diagnosis	No. of patient	Creatine† excretion	*dw* value	Amino-N mg/ml	No. of spots	Degree of aminoaciduria
Progressive Muscular Dystrophy						
Pseudohypertrophic Type						
Far advanced	201*	+	23	1.14	15	High
Advanced	189	+	22	1.39	14	"
	195*	+	21	0.49	9	Moderately high
Moderately Advanced	159*	—	4	0.23	8‡	Normal
	211	+	16	0.29	12	High
	229	+	12	0.42	10	Moderately high
	230	+	11	0.43	10	" "
Early	168*	—	3	0.59	8‡	Normal
Facio-Scapulo-Humeral Type						
Advanced	155*	—	11	0.49	8‡	Moderately high
Moderate	167	+	26	0.85	14	High
Early	157*	—	8	0.39	11‡	Moderately high
	162*	—	7	1.03	10‡	" "
	165	—	5	0.22	8	Normal
Other Types						
Far advanced	197*	+	25	0.51	11	High
Advanced	163*	—	20	1.02	18‡	"
	161	—	21	0.44	14	"
	156*	—	8	0.30	12‡	"
	158	+	15	0.58	9	Moderately high
	154*	—	22	0.48	6‡	" "
Moderately advanced	152	—	22	0.71	13	High
	153	—	16	0.62	13	"
	160	—	7	0.44	13	"
	164	—	15	0.41	8	Moderately high
	166	—	15	0.45	7	" "
	198*	+	9	0.37	4	Normal
	192	+	14	0.22	7	Moderately high
	199*	+	23	0.71	7	" "
Moderate	194	+	8	0.39	9	" "
Dermatomyositis						
Far advanced	190	+	15	1.54	6	Moderately high
Early	193	+	6	0.18	4	Normal
Amyotonia Congenita						
Far advanced	196*	+	6	0.29	11	High
Myotonia Atropica						
Moderate	200*	0	3	0.08	3	Normal
Normal Humans	202	0	7	0.25	5	"
	213	0	5	0.18	1	"
	221	0	3.5	0.16	2	"
	223	0	5.3	0.28	6	"
	225	0	2.4	0.20	4	"

* Patients receiving therapy.

† Samples run after prolonged storage, + indicates creatine excretion; 0, no creatine; and —, no satisfactory determination.

‡ Two-dimensional chromatograms were secured only after several months storage at 0°C under thymol.

cases over 20. Any value over 10 indicates an increased urinary excretion of free amino acids. Normal urines were chromatographed and the amino-acid distribution was similar to that described by Dent.[4]

Two-dimensional partition chromatography was also employed, which resembles the strip method except that a second solvent is run at right angles to the first, giving a two-dimensional distribution of spots on a rectangular sheet of filter paper. We employed phenol saturated with water as the solvent for the first run, followed by 24-hour air drying and subsequent development in a 50-50 mixture of lutidine and collidine saturated with water. The sheets were then dried at room temperature, sprayed with ninhydrin and heated as before. The use of lutidine-collidine

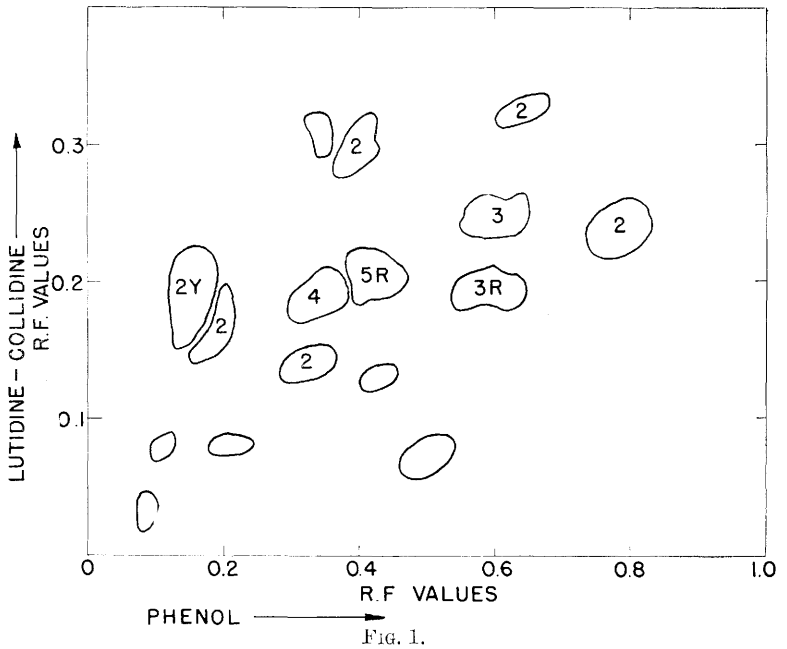

FIG. 1.

Two-dimensional Chromatogram of Urine in Progressive Muscular Dystrophy. The color intensity is indicated by numbers "1" to "5" (deepest color) as described in the text. Unnumbered spots have a color intensity of "1." The spots were purple with the exception of one yellow (Y) and two reddish (R) ones.

in preference to collidine alone was suggested by Dent.[6]

Our results with normal individuals correspond with those obtained by Dent.[4] Normal urine generally yields 2 or 3 spots in the two-dimensional technique and rarely more than 4. As shown in Table I, patients with progressive muscular dystrophy yield numerous spots indicating the excretion of a large number of amino acids. Eight amino acids represents an excretion which is moderately abnormal, and the appearance of 10 or more amino acids indicates a definitely pathological condition. In Fig. 1 is shown a diagram of a two-dimensional chromatogram of urine from a patient with progressive muscular dystrophy. The multiplicity of spots indicates an excessive excretion of amino acids.

In order to estimate the total amount of amino-acid excretion, the determination of amino nitrogen was performed on all urines by the method of Albanese and Irby.[7] This method depends upon the iodometric determination of copper obtained by filtration of the soluble amino acid copper salts. Albanese and Irby reported an amino nitrogen output of 200-700 mg per 24 hours in normal humans. This method yields high values when polypeptides are present and occasionally low values because certain of the amino acid copper salts are comparatively insoluble. As a result only partial correspondence was observed with the data obtained by partition chromatography. However, as may be seen in Table I, many of the values of amino nitrogen excretion are significantly higher than those observed in normal humans. An excretion of 0.50 mg amino nitrogen per ml or greater can be considered as an excessive excretion of amino nitrogen.

Urine of patients suspected of having muscular dystrophy is usually analyzed for creatine content. The finding of creatinuria would substantiate a clinical diagnosis of

[6] Dent, C. E., private communication.

[7] Albanese, A. A., and Irby, V., *J. Biol. Chem.*, 1944, **153**, 583.

dystrophy. In our work, we used a modified creatine determination using the technique of partition chromatography.[8] Those patients who excreted urines which do not show increased amino acid excretion likewise failed to show any significant creatine excretion as measured by this technique.

The amino nitrogen excretion normally represents only 2-4% of the total nitrogen excretion. It is evident that it would take an extremely large increase in amino nitrogen to raise significantly the value for total nitrogen as measured in the usual fashion. On the basis of the samples which are reported it would seem evident that these urines should be considered by all factors cited, that is, general description, *dw* value, number of spots, and milligrams of amino nitrogen excretion per ml. Comparison of the results of these various methods on urines from patients with progressive muscular dystrophy indicates that in many instances a generalized aminoaciduria is present. A general correspondence is observed between the data obtained by partition chromatography and that obtained by chemical amino nitrogen determination, but it is not close enough for the methods to replace one another. The number of amino acids excreted by a patient as indicated by the spots in two-dimensional paper chromatography is probably the most useful of the several determinations. It is possible for a patient to have a normal level of urinary amino nitrogen and still have a urine that would be characterized by an abnormally high excretion of certain amino acids. If the number of amino acids were large but the concentration of each were low, the total amino-nitrogen excretion would remain within normal limits. Details on the excretion of the individual amino acids in urines from humans with progressive muscular dystrophy will appear in a subsequent communication.

Summary. Two methods, partition chromatography and chemical amino nitrogen determination, have been employed in a study of the amino-acid excretions of 32 patients diagnosed as suffering from progressive muscular dystrophy and closely related conditions. In the case of progressive muscular dystrophy, partition chromatography indicated that both the number and the concentration of amino acids excreted were in many cases considerably above the normal limits. In addition, the results of amino nitrogen determinations indicated that some of these patients showed values far above normal limits and the majority were somewhat greater than normal.

Early muscular dystrophies, particularly of the facio-scapulo-humeral type, did not exhibit as marked an excretion of amino acids as the more advanced cases with or without pseudohypertrophic muscle symptoms. Data on the amino acid excretion of 2 cases of dermatomyositis and one each of amyotonia congenita and myotonia atropica were obtained.

On the basis of these observations it is concluded that progressive muscular dystrophy is characterized by a generalized aminoaciduria.

[8] Ames, S. R., and Risley, H. A., to be published.