Serum and Urine Polyamines in Normal and in Short Children

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ABSTRACT The serum and urine polyamines putrescine, spermidine, and spermine were measured in 112 normal subjects from 0 to 70 yr of age, and in three groups of short children from 7 to 20 yr: 21 growth hormone (GH) deficient patients, 20 normal variant short stature children, and 9 girls with 45, X Turner's syndrome. Urine polyamines were expressed as micromoles per gram of creatinine or per kilogram body weight, and serum polyamines were expressed as nanomoles per milliliter.

In normals, the three polyamines were highest in urine and serum at birth. The mean levels declined progressively with age, the rate of change decreasing with age. The mean for the normal subjects, and its 95% confidence and prediction intervals, were estimated from birth to age 70 for each serum and urine polyamine.

In GH-deficient children, serum and urine values were significantly lower ($P < 0.05$) than the age-specific normal values (with the exception of serum spermidine and spermine), averaging 25–55% below normal. This abnormality was corrected during 1 wk of treatment with human GH.

In Turner's syndrome, serum and urine values were significantly reduced ($P < 0.05$), averaging 35–80% below age-specific normals. GH treatment had no corrective effect.

In 6 of 20 normal variant short stature children, polyamine levels were significantly ($P < 0.01$) subnormal, averaging 50–80% below age-specific normals in both serum and urine. Treatment with GH had no corrective effect.

These data show that levels of polyamines in serum and urine are correlated with linear growth primarily during the first decade of life. Subnormal polyamine levels are generally associated with growth retardation.

INTRODUCTION

Polyamines are nitrogenous organic bases, 80–300 mol wt. The three principal polyamines in man are putrescine (Pu),[1] spermidine (Sd), and spermine (Sm); their structures and mode of synthesis are known (1). The biosynthetic pathway begins with ornithine and is carried out by four enzymes: ornithine decarboxylase (ODC), S-adenosyl-L-methionine decarboxylase, Sp synthetase, and Sm synthetase.

Except for Sm in bacteria, the three polyamines are present in all living cells, where they are largely bound by noncovalent forces to DNA and RNA (2). The cellular concentration of polyamines increases during cell growth and proliferation not only in animals but also in plants and bacteria (1). Examples are: regenerating rat liver (3), embryonic and neoplastic tissues (1), and logarithmically dividing *Escherichia coli* (4). The increment of polyamines generally parallels the accumulation of RNA (5, 6). Polyamines stimulate RNA synthesis (7, 8) and growth (9, 10) in some bacteria, plants, and animal cell cultures. The growth-promoting action is believed to be related to their interaction with nucleic acids. Polyamines, when they complex with DNA in chromosomes or RNA in ribosomes, increase the thermal stability of the nucleic acids (11, 12), enhance the activity of DNA-dependent RNA polymerase and the efficiency of ribosomal protein synthesis (12), promote the attachment of ribosomes to endoplasmic reticulum (12), and inhibit the susceptibility of ribosomal RNA to degradation by ribonucleases (13).

The rate-limiting step in the biosynthesis of polyamines is ODC. This enzyme declines in activity in

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[1] *Abbreviations used in this paper*: ADH, antidiuretic hormone; BW, body weight; GH, growth hormone; GHD, GH deficient; hGH, human GH; NVSS, normal variant short stature; ODC, ornithine decarboxylase; Pu, putrescine; Sd, spermidine; Sm, spermine; TS, Turner's syndrome; TSH, thyroid-stimulating hormone.

both liver and kidney after hypophysectomy, as do the polyamine concentrations in these organs. These reductions are corrected by growth hormone (GH) (14, 15). Therefore, it has been proposed that polyamines mediate some growth-promoting actions of GH. That the biosynthesis of the polyamines is not under the sole control of GH, however, is demonstrated by the rises in ODC activity caused by other hormonal factors (2, 16).

Polyamines can be quantified in human serum and urine. Although these levels have been thoroughly investigated in cancer (17–26), they have not been examined in relation to GH status. The objective of this study was to measure serum and urine polyamines in children with growth failure caused by GH deficiency, before and during treatment with human GH (hGH). For comparative purposes, similar observations were made with children of short stature from other causes, and with normal children and adults.

METHODS

Subjects

The study population consisted of four groups (Table I).

Normal group. 112 normal individuals from 0 (newborn) to 70 yr. Five were newborns and an additional eight were below 1 yr in age.

GH-deficient (GHD) group. 21 children, deficient in GH, age 6–21. Diagnostic criteria, including average plasma immunoreactive GH <2 ng/ml during four provocative tests, have been given previously (27). GH deficiency was idiopathic in 14 and isolated in 7; it was secondary to craniopharyngioma in 7 and associated with deficiency of corticotropin (ACTH), thyroid-stimulating hormone (TSH), or antidiuretic hormone (ADH) in 14. In patients deficient in TSH, ACTH, or ADH, replacement treatment with thyroxine, hydrocortisone, or ADH had been given for at least 6 mo before treatment.

Normal variant short stature (NVSS) group. 20 children with NVSS, age 6–15. Diagnostic criteria have been given elsewhere (28) and can be summarized as: present height <3rd percentile; predicted adult height <3rd percentile; average plasma immunoreactive GH >12 ng/ml during two insulin/arginine provocative tests (28) after pretreatment with 2.5 mg stilbesterol twice a day for 2 d; birth weight >2.5 kg;

and no organic or psychosocial explanation for the short stature. One or both parents of eight NVSS children were below the 3rd percentile in height.

Turner's syndrome (TS) group. Nine girls, age 8–18, with Turner's syndrome (45, X karyotype). The diagnostic criteria used to identify this condition have been described elsewhere (29). There was no evidence of mosaicism by peripheral lymphocyte karyotype.

Except for thyroxine, hydrocortisone, or ADH, no medications had been taken for 1 wk before the plasma and urine analyses for polyamines. No patient had received gonadal steroids during the preceding 4 mo.

The normal children were generally relatives of GH or TS children who are under care in this clinic. The project was done with the approval of Emory University's Clinical Trials Committee and with informed consent of the subjects or their parents, as appropriate.

Experimental design

Phase A. Phase A was concerned with methodology. Within the normal subjects, we estimated the intra-assay variability using duplicate fasting serum polyamines (24) and 24-h urine polyamines (30) from four subjects. The estimated coefficients of variation (estimated standard deviation/mean of duplicates × 100) ranged from 2.7 to 11.4%. The intra-individual variability in fasting serum polyamines (24) and in 24-h urine polyamines (30) was estimated in four normal subjects taken over 3 d. The estimated coefficients of variation ranged from 8.0 to 19.6%. To estimate the degree of diurnal variability in the urinary values, urine polyamines were measured in four successive 6-h samples in six subjects. The estimated coefficients of variation were 4.9, 13.0, and 18.5% for Pu, Sd, and Sm, respectively. The estimated intra-assay variability was generally 2–3 times smaller than the estimated intra-individual variability, whereas the diurnal variability was similar in magnitude to the intra-individual variability. Each of the above sources of individual variability was considerably (10–400 times) smaller than the estimated inter-individual variability.

Technical details of polyamine analysis were as follows: (a) 5–10 ml of plasma (2 ml below age 3) was precipitated with an equal volume of 10% trichloracetic acid that contained 1,6-hexanediamine as internal standard. Recovery of the internal standard averaged 72% in plasma and 86% in urine. The supernate was extracted three times with an equal volume of water-saturated ether and dried under a stream of air at 60°C. Residue was dissolved in 5 ml of 6N HCl and hydrolyzed for 15 h at 110°C in sealed tubes. (On the average, 75% of polyamines are conjugated in serum and 50%

TABLE I

Age and Sex Distribution of the Four Groups Studied

Group	Subgroup	Number (M/F)	Age						
			0–9	10–19	20–29	30–39	40–49	50–59	60–70
N (normal)		112 (56/56)	43	26	11	10	7	7	8
GHD		21 (9/12)	2	19	0	0	0	0	0
	Idiopathic	14 (6/8)	0	14	0	0	0	0	0
	Craniopharyngioma	7 (3/4)	2	5	0	0	0	0	0
NVSS		20 (12/8)	6	14	0	0	0	0	0
TS		9 (0/9)	2	7	0	0	0	0	0

in urine. Free and conjugated polyamines are recovered in the 5% trichloracetic acid filtrate of serum in the same ratios as they are present in untreated serum, as shown by data with an alternate method of analysis [24] whereby whole serum is hydrolyzed and polyamines are then measured in the butanol extract.) (b) 5 ml of concentrated HCl was added to 5 ml of urine prefiltered through a 0.2 micromillipore filter and hydrolyzed for 15 h at 110°C in sealed tubes that contained 1,6-hexanediamine as internal standard. (c) Plasma or urine hydrolysates were evaporated to dryness on a Buchler rotary evaporator (Buchler Instruments Div., Searle Diagnostics Inc., Fort Lee, N. J.) and reconstituted in 1 ml of water. The hydrolysates were chromatographed on a Beckman 121 amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) equipped with a column, packed with 5-cm Beckman PA-35 resin topped with 15 disks, and an expanded range recorder. The column was operated at 68 ml/h at 60°C and eluted with 0.35 M sodium citrate buffer that contained 0.35 M NaCl for 15 min, then changed to 0.35 M sodium citrate buffer that contained 2 M NaCl.

Phase B. 24-h urine samples were collected at 0°C in all subjects >7 yr old while they ate an ad lib. diet, and then stored at −20°C until analysis. Pu (24), Sd (24), Sm (24), and creatinine (31) were measured. In 69 individuals, fasting serum polyamines (24) (overnight fast) were measured on the same day. From these data, we calculated 24-h urine polyamines, urine polyamines per gram of creatinine, and 24-h urine polyamines per kilogram body weight (BW).

Phase C. In phase B, we found that serum and urine polyamines were depressed in certain short children. Phase C was designed to measure the effect of a standard dose of hGH on the urine and serum levels in these children. 17 subjects (6 GHD, 6 NVSS, and 5 TS) were admitted to the metabolic research unit and fed a previously described (33) standard diet for 21 d. After 3 d of adaptation, 24-h urine polyamines and creatinine and fasting serum polyamines were measured at 4- to 5-d intervals during the next 18 d. During the last 10 d, the patient received 0.532 U/kg BW$^{3/4}$ of hGH i.m. daily.[2]

RESULTS

Normals. Levels of all polyamines in both serum and urine were age dependent (Fig. 1). Values were highest at birth, declining less rapidly per year as the age of the subject increased. A regression model was used to express the relationship for each polyamine level (serum or urine) as a function of age (logarithm$_e$ [level] = α + β [age + ¾] + γ logarithm$_e$ [age + ¾] + ϵ).

For each polyamine, the parameters α, β, and γ were estimated using standard regression techniques (35). The estimated regression equations with their coefficients of multiple determination (R^2) are given in Table II. 95% confidence intervals[3] were obtained for

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[2] BW$^{3/4}$ was used as the unit of reference because of the evidence that it is more closely related to metabolic mass than is BW or surface area.

[3] A confidence interval portrays the precision associated with predicting the mean polyamine level at a given age, whereas the prediction interval portrays the precision associated with predicting an individual's polyamine level at a given age.

the mean of the normals for each age and are shown in Fig. 1. In addition, 95% prediction intervals[3] are also given in Fig. 1.

Residual analysis revealed a good fit for the normal group and various degrees of subnormal polyamine levels for the three subgroups. The residuals were standardized by dividing each by their estimated standard deviation ($\sqrt{\text{MS Residual}}$). The frequency distributions of the standardized residuals for the plasma and urine polyamines for each of the groups are plotted in Fig. 2 in standard deviation units. Note especially the Gaussian-shaped residual distributions for the normal group, the bimodal appearance of the NVSS group, and the subnormal GHD and TS groups.

Pearson product-moment correlation coefficients among plasma and urine polyamines and among polyamines within each group of subjects revealed a significant ($P < 0.05$) degree of linear association (36). Therefore, we conclude that there is a significant degree of linear interdependence among the polyamine levels of Pu, Sd, and Sm expressed either in plasma or urine. In both fluids, the ratios Sm:Sd and Sd:Pu varied significantly with age, whereas the ratio Sm:Pu was age independent.

GHD children. Serum Pu concentrations within this group averaged 25% less ($P < 0.05$) than those of age-specific normals. Urinary excretion, expressed either per gram of creatinine or per kilogram BW for each polyamine, was also low for the group, averaging 25–55% below age-specific normals ($P < 0.05$). Ratios of Pu:Sp:Sm did not differ significantly from normal in either serum or urine.

NVSS. For the entire group, serum and urine polyamines did not differ significantly from normal. Within this group, 6 had serum and urine polyamines below the 95% prediction interval of normals (these individuals are circled in Fig. 1). Consequently the distribution of polyamine levels in the NVSS group was bimodal (Fig. 2). In the latter six children, the ratios between the serum or urinary Pu, Sd, and Sm values did not differ from normal. In four of the six NVSS children with depressed polyamines, one or both parents was <3rd percentile in height, as compared with 4 of the 14 children with normal polyamines.

TS. In all nine girls with 45, X karyotype, both serum and urine polyamines were severely depressed below normal, averaging 35–80% below age-specific normals ($P < 0.05$). The majority of the serum and urine polyamine levels were below the 95% prediction interval for normals. Figs. 1 and 2 clearly show the TS group to be more severely depressed than the GHD group.

Effect of hGH. In GHD children, hGH caused significant increases in serum and urine polyamines (P
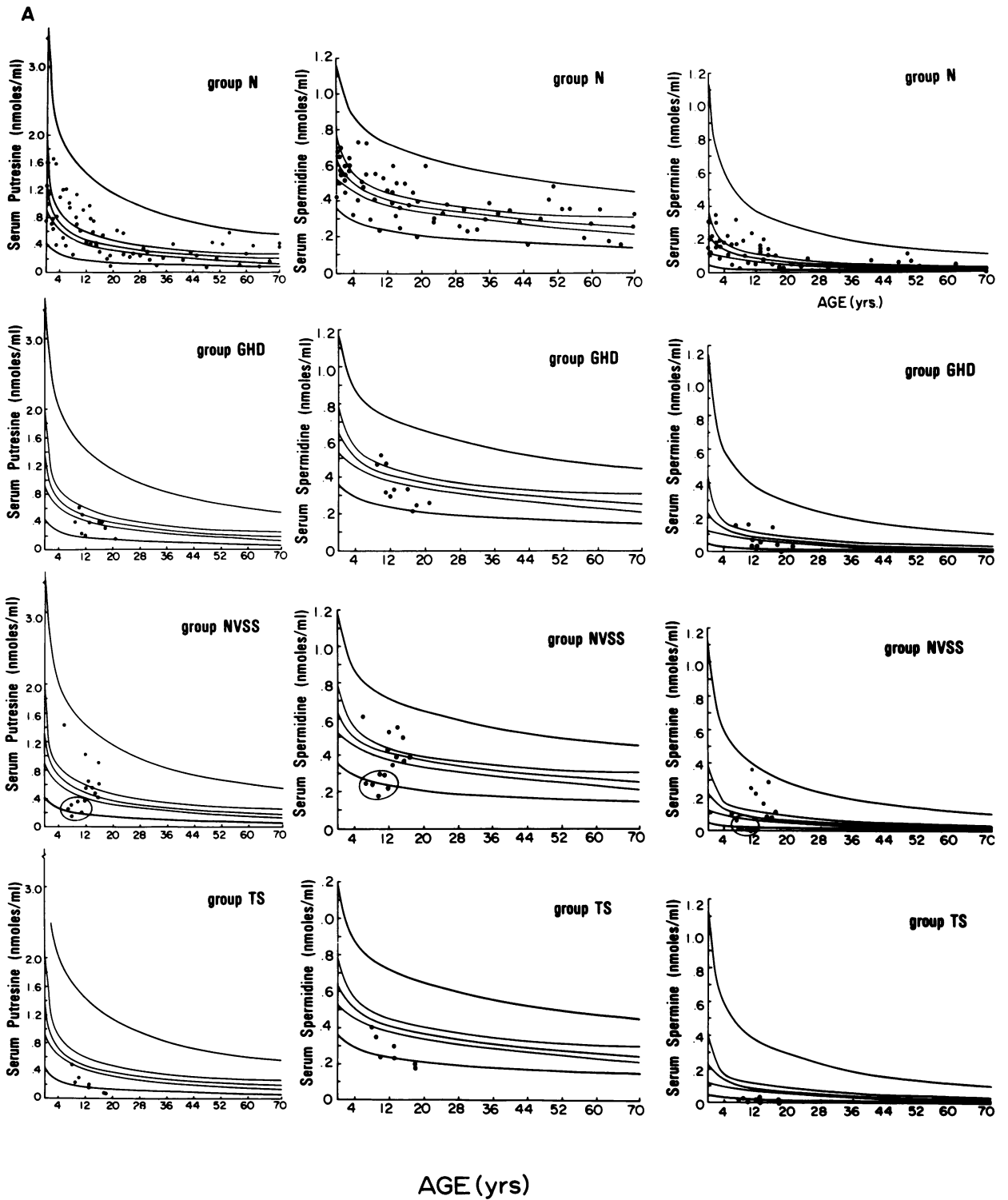
A

FIGURE 1 Relations between age and serum (A) or urine polyamines (B). In each graph, center line is the mean; nearest lines above and below center line define the 95% confidence interval; highest and lowest lines define the 95% prediction interval. In the NVSS group, the circle indicates the six children with depressed serum and urine polyamine levels (see text). N, normal.
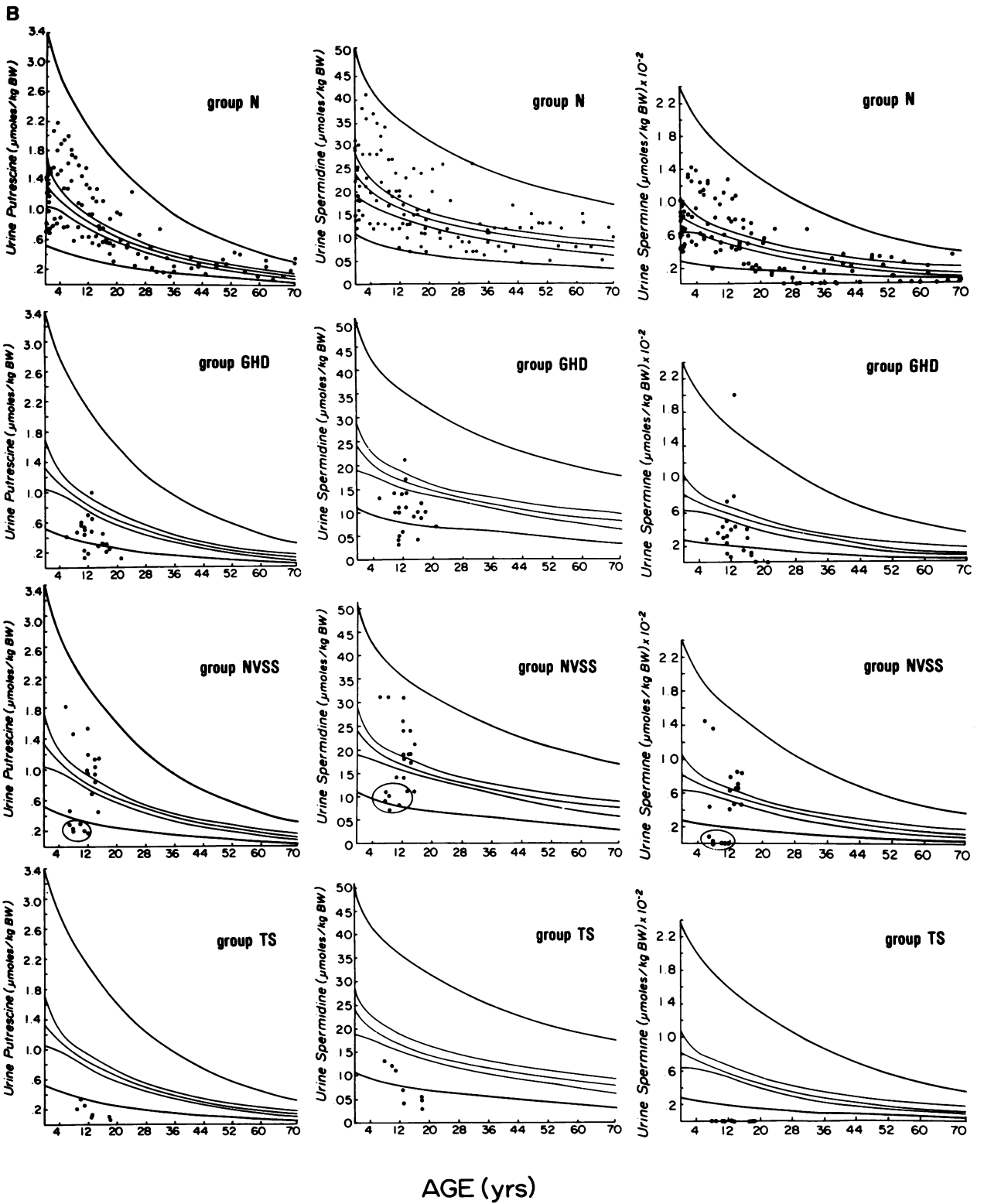
B

AGE (yrs)

TABLE II

TABLE II

Estimated Relationships between Polyamines (Serum or Urine) and Age for Normals

Multiple R^2	
0.53	ln (Pu serum) = 0.206 − 0.008 (age + ¾) − 0.320 ln (age + ¾)
0.48	ln (Sd serum) = 0.473 − 0.004 (age + ¾) − 0.142 ln (age + ¾)
0.45	ln (Sm serum) = −1.532 − 0.016 (age + ¾) − 0.296 ln (age + ¾)
0.77	ln (Pu urine/kg BW) = 4.773 − 0.024 (age + ¾) − 0.285 ln (age + ¾)
0.66	ln (Sd urine/kg BW) = 3.016 − 0.006 (age + ¾) − 0.322 ln (age + ¾)
0.61	ln (Sm urine/kg BW × 10^{-2}) = −0.300 − 0.016 (age + ¾) − 0.297 ln (age + ¾)
0.68	ln (Pu urine/g creatinine) = 0.300 − 0.013 (age + ¾) − 0.031 ln (age + ¾)
0.59	ln (Sd urine/g creatinine) = −1.454 − 0.012 (age + ¾) − 0.071 ln (age + ¾)
0.46	ln (Sm urine/g creatinine) = 4.787 − 0.027 (age + ¾) − 0.013 ln (age + ¾)

< 0.10) (Table III). Before treatment, average serum and urine polyamines of the five GHD children had been below the 95% confidence interval of the normal group; during hGH treatment, the average values were within this range.

In TS girls, hGH had no appreciable effect (P >0.05) on the severely depressed polyamine levels in serum and urine.

The six NVSS children who had been found to have subnormal serum and urine polyamines (Figs. 1 and 2) were also treated with hGH. The hormone had no appreciable effect ($P > 0.05$) on polyamines in either fluid.

DISCUSSION

Polyamine contents were depressed in serum and urine of GHD children, and were restored to normal by administration of hGH. These findings concur with earlier reports (15) on the reduced activity of ODC and depleted content of polyamines in liver and kidney of the hypophysectomized rat, and the rapid increases in ODC within 4 h after injection of GH (14, 15). However, the serum and urine levels in the GHD children were reduced only to ~70% of the normal mean; the individual values were rarely below the 95% prediction interval defined by normal subjects. This indicates that other factors besides GH act to maintain polyamine production, and that polyamine biosynthesis can proceed at a substantial rate even in the absence of endogenous GH.

The three polyamines were also depressed in two other varieties of children with growth failure, in whom
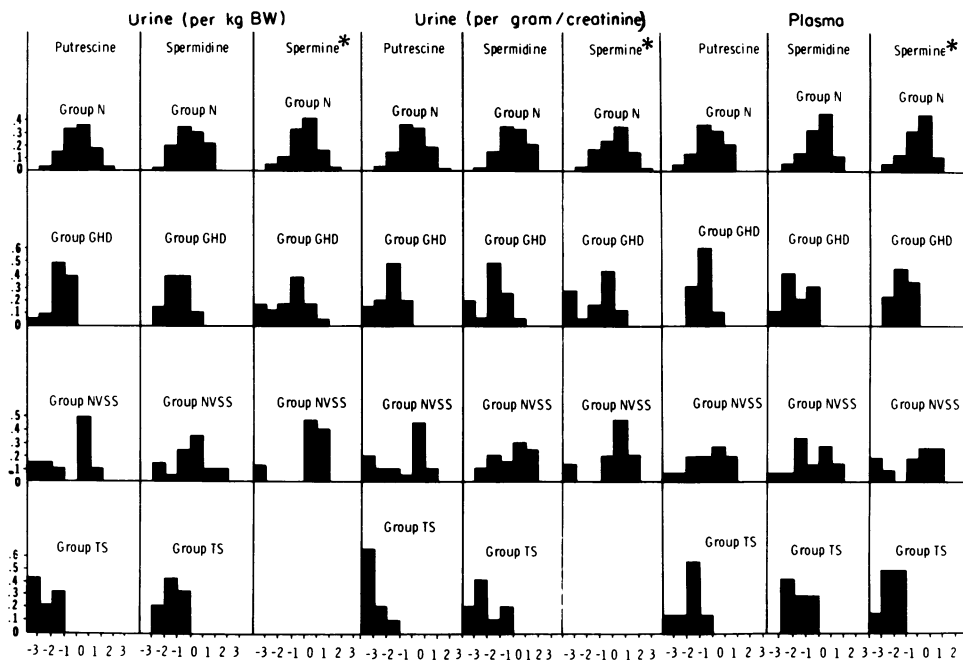

FIGURE 2 Relative frequency distributions of residuals, expressed in numbers of standard deviations from the mean (horizontal axis). Zero levels of Sm are not included in the graph. Number of zero values in urine was: normal group, 15 (all age >26); GHD, 1; NVSS, 5; TS, 9. Zero values in serum occurred in 6 of the normal group (N) (all age >29), 1 GHD, 2 NVSS, and 3 TS.

TABLE III
GHD Group Means*

	T_1 (before treatment)		T_2 (during treatment)			
	Time 1	Time 2	Time 3	Time 4	n	T_1 vs. T_2
Serum Pu	0.41	0.43	0.71	0.67	6	$(P < 0.01)$
Serum Sd	0.38	0.41	0.49	0.48	6	$(P = 0.10)$
Serum Sm	0.09	0.10	0.18	0.20	6	$(P < 0.05)$
Urine Pu	30.5	28.0	42.8	47.5	6	$(P < 0.01)$
Urine Sd	7.0	9.3	11.3	11.5	6	$(P = 0.10)$
Urine Sm	0.28	0.30	0.52	0.53	6	$(P < 0.01)$

* Similar analyses were done for the NVSS group and the TS group. However, no significant treatment effects were found within each of these groups.

endogenous GH was normal, namely in 6 of the 20 NVSS children, and in all girls with the 45, X karyotype. In both of these groups, polyamine levels were more severely reduced than in GH deficiency, and exogenous GH failed to correct the abnormality. Here is further evidence that biosynthesis of polyamines is modulated not only by GH but by other endogenous mechanisms as well.

What is responsible for the reduction of polyamine levels in TS and in one-fifth of the "short normals," all with normal endogenous GH? A few speculations can be offered. Because a substantial proportion of newly synthesized polyamines is oxidized to CO_2 or converted to acidic products (37), a reduced level in the body fluids could reflect accelerated degradation rather than decreased production. Because the ratios of serum or urine Pu:Sd:Sm were closely similar to normal in GHD, NVSS, and TS groups, a retarded production would probably be determined at the ODC step in the biosynthetic pathway. In TS, the polyamine deficiency must be caused directly or indirectly by the lack of the X chromosome. Thus a gene which influences polyamine production or degradation may be located on this chromosome. In four of the six NVSS children with subnormal polyamine levels, one or both parents were abnormally short. This familial pattern suggests that a genetic cause for polyamine deficiency might obtain in some of these children as well. In general, the polyamine depletion cannot be explained as a secondary effect of growth retardation, because four-fifths of NVSS children, all below the 3rd percentile in height, had normal polyamine values.

Besides hormonal and genetic mechanisms, nutritional factors could also be responsible for depressed polyamine levels in some short children. Concentrations of polyamines and activities of their biosynthetic enzymes rise markedly in the tissues of underfed animals during nutritional repletion (38, 39).

Our findings lead to these generalizations: (a) GH deficiency lowers, and hGH treatment in GHD children restores, polyamine levels. (b) Unidentified factors, some of which may be genetic, are quantitatively more important determinants of serum and urine polyamines than is endogenous GH. (c) Growth rate is subnormal whenever polyamine levels are subnormal, but normal polyamine levels do not guarantee a normal growth rate (Fig. 2).

ACKNOWLEDGMENTS

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