Low Free Serum Histidine Concentration in Rheumatoid Arthritis

A MEASURE OF DISEASE ACTIVITY

DONALD A. GERBER

From the Department of Medicine of the State University of New York Downstate Medical Center, the Kings County Hospital, and the State University Hospital, Brooklyn, New York 11203

ABSTRACT A study of sera from 285 patients with definite or classical rheumatoid arthritis (including 37 patients receiving no anti-inflammatory drugs) and sera from 67 healthy subjects has confirmed 10 published reports of a statistically significant decreased blood histidine concentration in patients with rheumatoid arthritis. Contrastingly, in sera from 231 patients with a variety of acute and chronic illnesses other than rheumatoid arthritis, no statistically significant hypohistidinemia was observed either in the group as a whole or in association with the administration of aspirin, prednisone, indomethacin, phenylbutazone, or dextropropoxyphene.

In the patients with rheumatoid arthritis there was a statistically significant correlation between the serum histidine concentration and the following: Westergren sedimentation rate ($r = -0.33$, $P < 10^{-9}$), grip strength ($r = 0.26$, $P < 10^{-9}$), hematocrit ($r = 0.23$, $P < 10^{-9}$), duration of morning stiffness ($r = -0.14$, $P = 10^{-5}$), walking time ($r = -0.13$, $P = 10^{-4}$), latex titer of rheumatoid factor ($r = -0.11$, $P = 0.001$), and the duration of arthritis ($r = -0.06$, $P = 0.05$). There was no statistically significant association between the serum histidine concentration and the duration of rheumatoid arthritis in the 151 patients with disease of 0–10-yr duration ($r = 0.02$, $P = 0.5$), the sex of the patient, or the presence of antinuclear antibody ($r = 0.007$, $P = 0.9$). The serum histidine concentration was less in rheumatoid patients receiving steroids ($P = 0.00001$), gold ($P = 0.009$), and aspirin ($P = 0.15$) than in rheumatoid patients not receiving these drugs. This study indicates that histidine determinations on properly preserved casual serum samples can be helpful in the diagnosis of rheumatoid arthritis and in the evaluation of the activity of the disease.

Dr. Gerber is a Career Scientist of the Health Research Council of the City of New York.

Received for publication 17 July 1974 and in revised form 21 January 1975.

INTRODUCTION

The free blood histidine concentration has been reported to be statistically significantly decreased in patients with rheumatoid arthritis in 10 (1–10) of 11 (1–11) reports. In two reports (3, 9), patients with more active rheumatoid arthritis had smaller serum histidine concentrations than patients with less active disease. The present report confirms and extends these observations.

METHODS

The concentration of free histidine in serum was determined with a method in which histidine and *o*-phthaldialdehyde react in alkali to form a fluorescent product which is measured in a fluorescence spectrometer (12). The method is linear in the range used (12). Venous blood samples were collected without regard to time of day, meals, drugs, or activity. No subject had received L-histidine therapeutically for at least 1 mo before the time blood was obtained. Serum was separated from clotted blood within 4 h after venipuncture and frozen immediately at $-20°C$ to prevent an otherwise misleading increase in the concentration of histidine in the specimen. Hematocrits were measured in heparinized microhematocrit tubes centrifuged at 625 *g* for 30 min. The Westergren sedimentation rate (millimeters per hour) was used (13). Rheumatoid factor was measured in serum by tube dilution using latex particles (14) and was considered to be present when the test was positive in a dilution of at least 1 to 10. Antinuclear antibody was measured by immunofluorescence using rat liver (15). Grip strength was measured as described by McCarty (16). Walking time was seconds required to walk 50 ft. Patients with rheumatoid arthritis had either definite or classical disease (17). Of the patients receiving anti-inflammatory steroids, 77% were taking prednisone. To

TABLE I

Serum Histidine Concentration in Healthy Subjects, Patients with Illnesses Other Than Rheumatoid Arthritis, and Patients with Rheumatoid Arthritis (RA)

	Number of subjects*	Serum histidine		P		
		Mean	SE	Vs. healthy‡	Vs. sick§	Men vs. women
		mg/100 ml				
Healthy						
Men and women	67	1.85	0.05	—	0.6	
Men	30	1.89	0.08	—	0.1	0.4
Women	37	1.82	0.05	—	0.4	
Sick, excluding RA						
Men and women	231	1.83	0.02	0.6	—	
Men	109	1.78	0.03	0.1	—	0.03
Women	122	1.87	0.03	0.4	—	
Rheumatoid arthritis						
Men and women	285	1.39	0.02	10^{-14}	10^{-47}	
Men	67	1.38	0.03	10^{-6}	10^{-14}	0.9
Women	218	1.39	0.02	10^{-8}	10^{-30}	
Rheumatoid arthritis (no anti-inflammatory drugs‖)						
Men and women	37	1.56	0.05	10^{-5}	10^{-5}	
Men	14	1.59	0.09	0.02	0.05	0.5
Women	23	1.53	0.05	0.0006	10^{-5}	

* Multiple determinations averaged for each subject.

‡ Comparison with healthy subjects of the same sex.

§ Comparison with sick subjects of the same sex.

‖ E.g., no aspirin, indomethacin, phenylbutazone, steroid, gold, chloroquine, or immunosuppressive drugs.

simplify the calculations, the steroid doses for the 23% of patients receiving anti-inflammatory steroids other than prednisone were converted to the equivalent doses of prednisone.

Student's *t* test was used to compare means (18). Tests of significance were two sided. In the preparation of Tables II–IV, each serum value was considered as a separate statistical unit.

RESULTS

Statistically significant hypohistidinemia was present in patients with rheumatoid arthritis but not in patients with other diseases (Table I). The average free serum histidine concentration in 67 healthy subjects was 1.85 mg/100 ml (SE = 0.05); in 231 subjects with a variety of acute or chronic illnesses or abnormal conditions, not including rheumatoid arthritis, the serum histidine concentration was not statistically significantly different ($P = 0.6$), averaging 1.83 mg/100 ml (SE = 0.02). The mean serum histidine concentration in 285 patients with rheumatoid arthritis, however, was 1.39 mg/100 ml (SE = 0.02), and this value was statistically significantly ($P < 10^{-13}$) less than the mean serum histidine concentration in both the healthy subjects and the sick nonrheumatoid subjects (Table I). The mean serum histidine concentration in patients with rheumatoid arthritis receiving no anti-inflammatory drugs (e.g., no aspirin, indomethacin, phenylbutazone, steroid, gold, chloroquine, or immunosuppressive drugs) was also statistically significantly subnormal (Table I).

Tables II–IV and Figs. 1–8 summarize an analysis of 1,235 serum samples from 285 patients with rheumatoid arthritis with respect to associations between the degree of hypohistidinemia and the activity and other clinical characteristics of the arthritis. The following supplements the material in these tables and figures.

Sedimentation rate (Tables II and III and Fig. 1). The strongest correlation was that between the serum histidine concentration and the Westergren erythrocyte sedimentation rate. The sedimentation rate was most abnormal (81.3 mm/h) in association with the 17 sera from the 16 patients whose serum histidine concentrations were most subnormal, i.e., between 0.40 and 0.80 mg/100 ml. The sedimentation rate was closest to normal (32.8 mm/h) in association with the 141 sera from the 74 patients with the largest serum histidine concentration, i.e., greater than 1.80 mg/100 ml. Between these two extremes there was a virtually linear relationship

TABLE II

Correlation between the Serum Histidine Concentration (mg/100 ml) and Characteristics of Patients with Rheumatoid Arthritis

	Number of sera	Number of patients	Correlation coefficient	Slope of regression	P
ESR*	1,043	218	−0.33	−27.3 mm/mg/100 ml	$<10^{-9}$
ESR* (no anti-inflammatory drugs‡)	81	37	−0.43	−34.8 mm/mg/100 ml	10^{-4}
Grip	1,079	206	0.26	47.8 mm/mg/100 ml	$<10^{-9}$
Grip (no anti-inflammatory drugs‡)	74	32	0.41	109.3 mm/mg/100 ml	10^{-4}
Hematocrit (men and women)	963	213	0.23	3.27%/mg/100 ml	$<10^{-9}$
Hematocrit (no anti-inflammatory drugs‡)	70	32	0.33	7.17%/mg/100 ml	0.004
Hematocrit (men)	206	52	0.38	6.00%/mg/100 ml	10^{-8}
Hematocrit (women)	757	161	0.18	2.17%/mg/100 ml	10^{-6}
Morning stiffness (>0.5 h)§	886	177	−0.14	−20.6%/mg/100 ml	10^{-5}
Morning stiffness (>0.5 h)§ (no anti-inflammatory drugs‡)	63	27	−0.06	−9.5%/mg/100 ml	0.6
Walking time (50 ft)	1,045	199	−0.13	−3.31 s/mg/100 ml	10^{-4}
Walking time (50 ft) (no anti-inflammatory drugs‡)	71	30	−0.22	−4.28 s/mg/100 ml	0.07
Rheumatoid factor	993	225	−0.11	−1.58 tubes/mg/100 ml	0.001
Rheumatoid factor (no anti-inflammatory drugs‡)	74	35	−0.18	−2.75 tubes/mg/100 ml	0.12
Age	1,141	231	−0.10	−0.0025 mg/100 ml/yr	0.001
Prednisone dose (all patients)	1,122	226	−0.10	−6.9 μg/100 ml/mg/day	0.001
Aspirin dose (all patients)	1,044	241	−0.07	−0.28 g/mg/100 ml	0.02
Duration of arthritis (all patients)	1,122	226	−0.06	−0.0027 mg/100 ml/yr	0.05
Time of day	956	146	−0.03	−0.0037 mg/100 ml/h	0.4
Duration of arthritis (0–10 yr only)	723	151	0.02	0.0027 mg/100 ml/yr	0.5
Prednisone dose‖	419	75	0.02	1.1 μg/100 ml/mg/day	0.7
Antinuclear antibody	409	89	0.007	0.72%/mg/100 ml	0.9
Aspirin dose‖	864	213	−0.005	−0.0026 g/mg/100 ml	0.9

* ESR = Westergren sedimentation rate.

‡ E.g., no aspirin, indomethacin, phenylbutazone, steroid, gold, chloroquine, or immunosuppressive drugs.

§ Analyzed by testing for a linear trend in proportions (18).

‖ Only patients receiving the drug are included in the analysis.

between each histidine subgroup's average serum histidine concentration and average sedimentation rate (Fig. 1). 82% of the sera from patients with a sedimentation rate greater than 40 mm/h had a histidine concentration of less than 1.60 mg/100 ml, 62% had a histidine concentration of less than 1.40 mg/100 ml, and 34% had a histidine concentration of less than 1.20 mg/100 ml.

There was considerable scatter in the data. For example, in the subgroup with the largest number of sera (serum histidine concentrations between 1.20 and 1.40 mg/100 ml), the average sedimentation rate was 50.9 mm/h (SD = 26.5 mm/h, SE = 1.68 mm/h), the 95% confidence limits for the mean were 47.6 and 54.2 mm/h, but the 95% confidence limits for a single value of the sedimentation rate were 0 and 103.2 mm/h and the coefficient of variation (SD/mean) was 0.52.

The correlation coefficients for the relationship between the serum histidine concentration and the Westergren sedimentation rate were also computed separately for each patient and then combined (19). The weighted combined correlation coefficient thus obtained ($r = -0.24$, $P = 10^{-9}$) was not statistically significantly different ($P = 0.06$) from the correlation coefficient ($r = -0.33$) obtained in the usual manner by using the values for all the patients in one calculation. The correlation coefficients computed separately for each patient were statistically significantly ($P = 0.0001$) heterogeneous by chi square analysis (18). There was also a statistically significant ($P = 0.02$) correlation between the Wintrobe sedimentation rate and the serum histidine concentration ($n = 22$, $r = -0.50$).

Grip strength (Table II and Fig. 2). Of the clinical, as opposed to laboratory, characteristics of patients with rheumatoid arthritis, grip strength was most closely correlated with the serum histidine concentration ($r = 0.26$, $P < 10^{-9}$). The relationship between mean grip strength and the mean serum histidine concentration for each subgroup of serum histidine concentrations in

TABLE III

Relationship between the Westergren Sedimentation Rate and Serum Histidine Concentration in Patients with Rheumatoid Arthritis

Sedimentation rate	Total number of sera	Concentration of histidine in serum, *mg/100 ml*			
		<1.0	<1.2	<1.4	<1.6
mm/h		*% of sera**			
0–19	123	2.4	13.0	30.1	49.6
20–39	264	3.0	14.0	38.3	62.5
40–59	200	8.0	26.5	54.5	78.0
60–79	151	13.9	37.1	64.9	86.8
80–99	76	15.8	36.8	67.1	80.3
100–132	45	33.3	55.6	77.8	91.1

* The values listed are the percentages of sera with histidine concentrations less than 1.0, 1.2, 1.4, and 1.6 mg/100 ml. To illustrate, 36.8% (28 sera) of the 76 sera from patients with sedimentation rates between 80 and 99 mm/h had histidine concentrations less than 1.2 mg/100 ml.

Fig. 2 was virtually linear (test for linearity [20]: $P = 0.3$). In contrast to the linear relationship of grip strength to serum histidine concentration, the similarly calculated relationship of grip strength to sedimentation rate (Fig. 9) was not linear (test for linearity: $P = 10^{-6}$).

Hematocrit (Table II and Fig. 3). There was a statistically significant difference between men and women both with regard to the slopes ($P = 0.005$) and the correlation coefficients ($P = 10^{-4}$) of the relationship between hematocrit and the serum histidine concentration.

Morning stiffness (Table II and Fig. 4). 100 sera were obtained from 48 patients who, when blood was obtained, reported morning stiffness "all day." Since data containing "all day" values cannot be readily converted into a Gaussian distribution of durations of morning stiffness, the percent of patients whose morning stiffness exceeded ½ h and the median duration of morning stiffness were calculated for each patient subgroup of serum histidine concentrations (Fig. 4). Statistical analysis was performed by testing for a linear trend in the percent of patients with stiffness greater than ½ h (18).

Aspirin dose (Tables II and IV and Fig. 7). In the 24 patients (45 sera) with Westergren sedimentation rates greater than 100 mm/h there was a statistically significant positive correlation ($r = 0.40$, $P = 0.005$) between the serum histidine concentration and the patient's aspirin dose. That is, in this subgroup, the larger the aspirin dose, the less abnormal the serum histidine concentration. No correlation coefficient for any other sedimentation rate subgroup (i.e. 0–19, 20–39, . . . 80–99) was greater than 0.07, less than −0.08, or statistically significant.

Duration of arthritis (Table II and Fig. 8). In the 28 patients (52 sera) with rheumatoid arthritis of less than 6-mo duration, the average serum histidine concentration (1.43 mg/100 ml) was the same as in the 285 patients with rheumatoid arthritis of all durations (also 1.43 mg/100 ml).

Age (Table II). There was a strong correlation ($r = 0.85$, $P < 10^{-6}$) between age and the duration of arthritis.

Time of day (Table II and Fig. 10). The most abnormal histidine concentrations were present in samples collected between 11 A.M. and 1 P.M. (1.39 mg/100 ml); the least abnormal were from specimens collected between 8 and 9 A.M. (1.48 mg/100 ml) and after 4 P.M. (1.46 mg/100 ml). There were no statistically significant differences between the mean serum histidine concentrations in any of the time intervals in Fig. 10 (analysis of variance and Student's *t* test [18]).

Antinuclear antibody (Tables II and IV). Of the 409 sera (89 patients) selected at random for antinuclear antibody tests, 16% were positive. Of these 89 patients, 7% had a positive antinuclear antibody test in

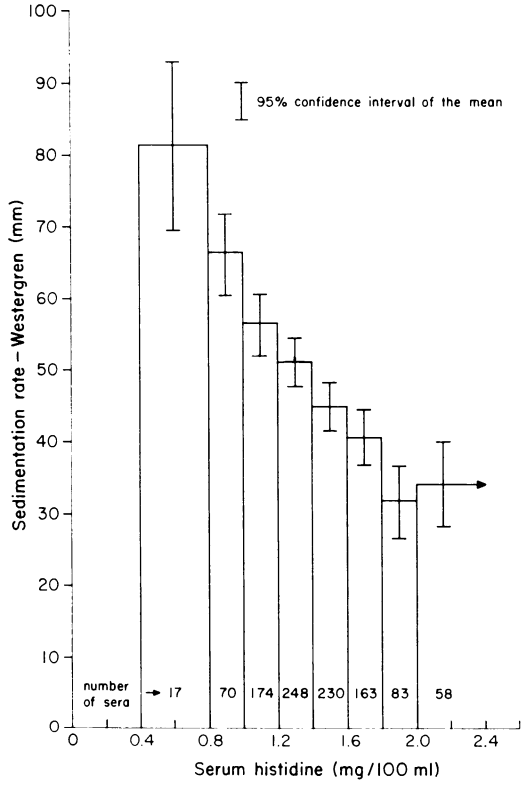

FIGURE 1 Association between the Westergren sedimentation rate and the serum histidine concentration in 1,043 sera from 218 patients with rheumatoid arthritis. $r = -0.33$; $P < 10^{-6}$.

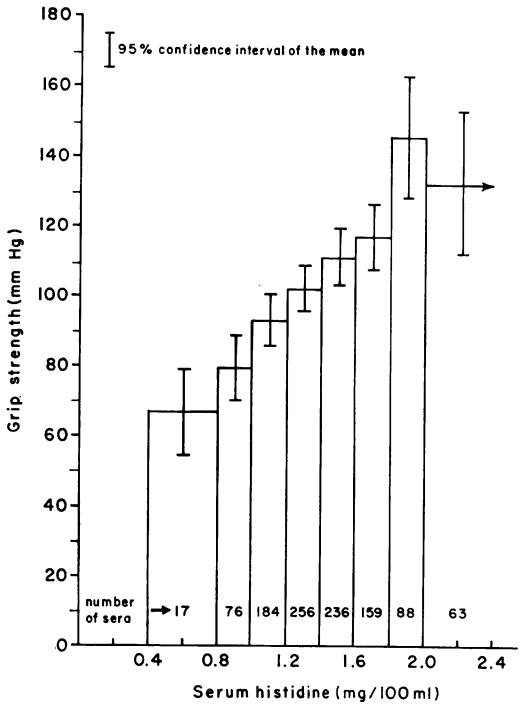

FIGURE 2 Association between the grip strength and the serum histidine concentration in 1,079 sera from 206 patients with rheumatoid arthritis. $r = 0.26$; $P < 10^{-9}$.

all samples studied, and 22% had a positive antinuclear antibody test in one or more, but not all, samples studied.

Anti-inflammatory steroids (Tables II and IV). The average sedimentation rate for the patients receiving steroids was 50.2 mm/h (SD = 28.2). The average sedimentation rate for the patients not receiving steroids was 46.5 mm/h (SD = 27.6). The difference was statistically significant ($P = 0.04$).

Gold (Table IV). The correlation between the serum histidine concentration and the sedimentation rate was virtually the same in the 85 blood samples from the 27 patients receiving gold ($r = -0.35$) as in the 980 blood samples from the 204 rheumatoid patients not receiving gold ($r = -0.34$).

Relative clinical status at time blood was obtained. At each visit patients were asked whether they felt better, worse, or the same as on the previous visit. The average serum histidine concentration in the 283 sera from those patients reporting "better" was 1.49 mg/100 ml (SD = 0.34); in the 244 sera from those reporting "same" it was 1.44 mg/100 ml (SD = 0.35); and in the 375 sera from those reporting "worse" it was 1.40 mg/100 ml (SD = 0.34). The difference in the serum histidine concentrations between those feeling "better" and those feeling "worse" was significant ($P = 0.0008$). In the 48 patients reporting more than one "better" and "worse" during the study, the average difference ("better" minus "worse") between the serum histidine concentrations at these times was 0.09 mg/100 ml (SD = 0.25) ($P = 0.02$).

Subjects free of rheumatoid arthritis. In subjects free of rheumatoid arthritis, both sick and well, there was no statistically significant (i.e., $P < 0.05$) correlation between the serum histidine concentration and the following subject characteristics (n = number of sera): Westergren sedimentation rate ($n = 130$, $r = -0.10$), Wintrobe sedimentation rate ($n = 58$, $r = -0.15$), hematocrit ($n = 112$, $r = 0.07$), prednisone dose ($n = 360$, $r = 0.09$), aspirin dose ($n = 337$, $r = -0.00005$), grip strength ($n = 42$, $r = -0.10$), walking time ($n = 25$, $r = 0.12$), rheumatoid factor ($n = 307$ [24 sera containing rheumatoid factor and 283 sera with no rheumatoid factor], $r = -0.01$), serum albumin ($n = 39$, $r = -0.20$), serum globulin ($n = 37$, $r = -0.03$). In 21 anemic nonrheumatoid patients (hematocrit less than 37% in women and less than 40% in men) the average serum histidine concentration was normal (1.90 mg/100 ml, SE = 0.11). The mean serum histidine concentration in 24 sera containing rheumatoid factor (average titer 1:359) from 24 patients free of rheu-

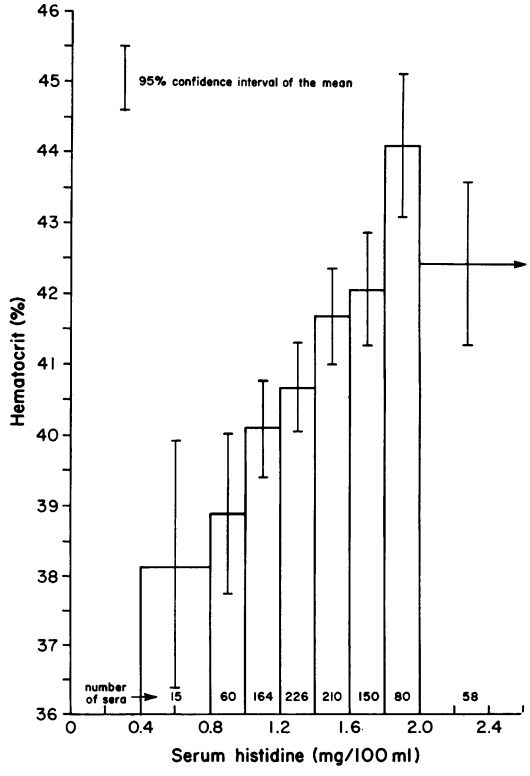

FIGURE 3 Association between the hematocrit and the serum histidine concentration in 963 sera from 213 patients with rheumatoid arthritis. $r = 0.23$; $P < 10^{-9}$.

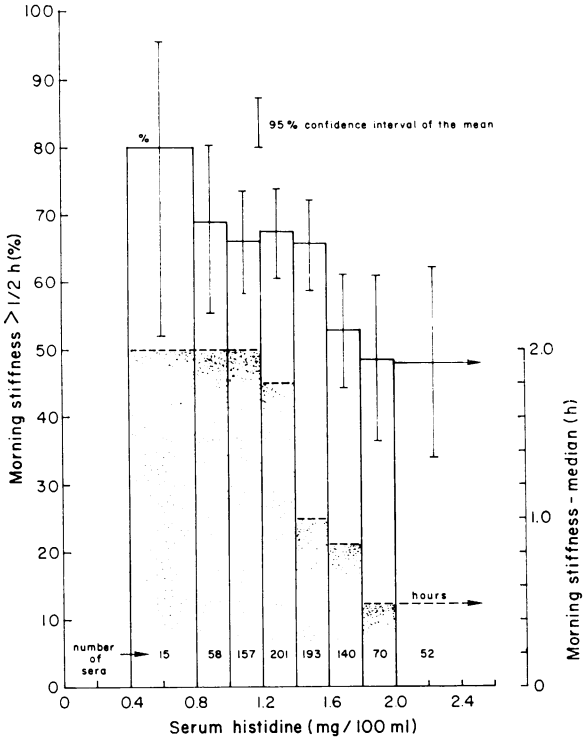

FIGURE 4 Association between the morning stiffness and the serum histidine concentration in 886 sera from 177 patients with rheumatoid arthritis. The shaded area indicates the median morning stiffness in hours. The complete bars refer to the percent of patients with stiffness lasting more than $\frac{1}{2}$ h. The correlation coefficient was obtained by testing for a linear trend in proportions (18). $r = -0.14$; $P = 10^{-5}$.

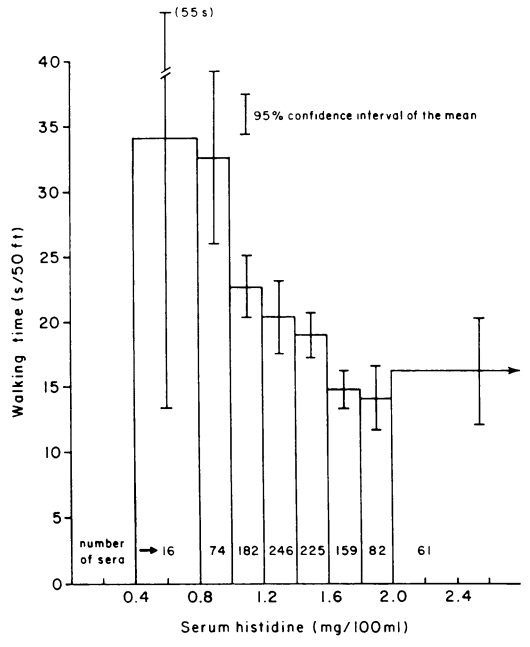

FIGURE 5 Association between the 50-ft walking time and the serum histidine concentration in 1,045 sera from 199 patients with rheumatoid arthritis. $r = -0.13$; $P = 10^{-4}$.

matoid arthritis was also normal (1.83 mg/100 ml, SE = 0.06).

No statistically significant hypohistidinemia was observed in patients free of rheumatoid arthritis in association with the following anti-inflammatory and analgesic drugs (n = number of sera, h = average serum histidine concentration [mg/100 ml] ±SE): aspirin (average dose 2.0 g/day) ($n = 44$, $h = 1.78 \pm 0.05$), prednisone (average dose 38 mg/day) ($n = 16$, $h = 1.86 \pm 0.09$), indomethacin ($n = 4$, $h = 2.31 \pm 0.25$), phenylbutazone ($n = 3$, $h = 2.19 \pm 0.18$), dextropropoxyphene ($n = 17$, $h = 1.81 \pm 0.10$).

DISCUSSION

The foregoing data indicate that the serum histidine concentration is decreased in rheumatoid arthritis but not in sickness in general and that there is a relationship between the activity of rheumatoid arthritis and the degree of hypohistidinemia. Several aspects of the relationship deserve added comment.

Anti-inflammatory drugs. Three observations favor the thesis that the correlation between the serum histidine concentration and disease activity is not caused by

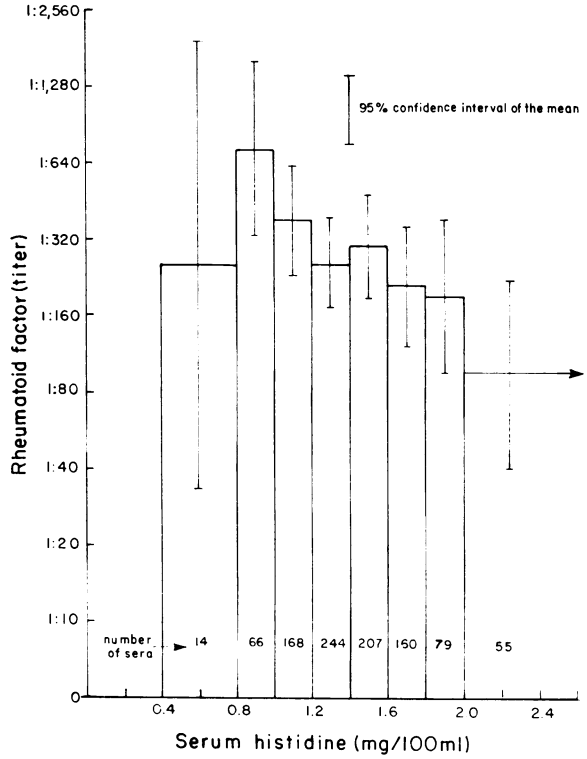

FIGURE 6 Association between the titer of rheumatoid factor and the serum histidine concentration in 993 sera from 225 patients with rheumatoid arthritis. $r = -0.11$; $P = 0.001$.

TABLE IV

Serum Histidine Concentration in Patients with Rheumatoid Arthritis

	Number of sera	Number of patients	Serum histidine			P
			Average	SD	SE	
			mg/100 ml			
Men	283	67	1.46	0.33	0.02	0.06
Women	952	218	1.42	0.34	0.01	
No aspirin	180	76	1.47	0.32	0.02	0.15
Aspirin*	864	209	1.43	0.34	0.01	
No steroid	703	151	1.46	0.35	0.01	0.00001
Steroid‡	419	75	1.37	0.31	0.02	
No gold treatment	980	204	1.45	0.34	0.01	0.009
Gold treatment	85	27	1.35	0.31	0.03	
No rheumatoid factor	348	115	1.49	0.35	0.02	0.002
Rheumatoid factor	645	136	1.41	0.33	0.01	
ANA§ absent	342	81	1.49	0.37	0.02	0.98
ANA§ present	67	26	1.49	0.34	0.04	

* Average dose 1.89 g/day, range 0.3–6 g/day.

‡ Average dose 8.40 mg/day, range 1.0–30.0 mg/day.

§ ANA = antinuclear antibody.

drugs: (*a*) The serum histidine concentration is statistically significantly subnormal in patients taking no anti-inflammatory drugs (Table I). (*b*) The correlation coefficients and slopes for the relationship between the serum histidine concentration and disease activity (sedimentation rate, grip strength, walking time, rheumatoid factor, hematocrit, but not morning stiffness) were statistically significant in the patients with rheumatoid arthritis receiving no anti-inflammatory drugs and also

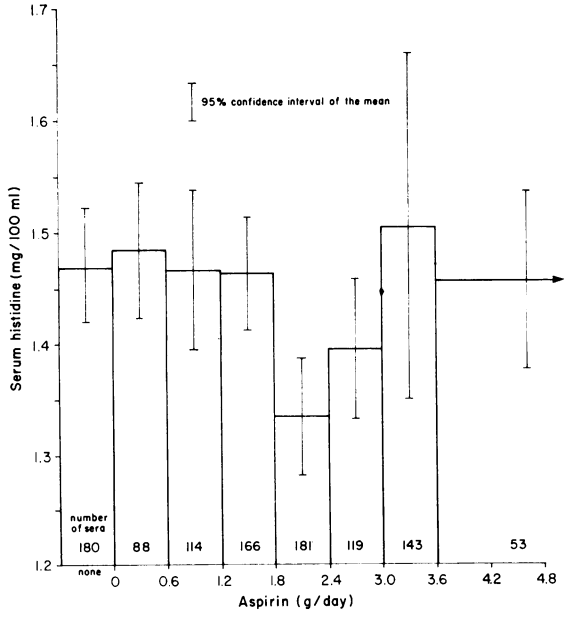

FIGURE 7 Association between the serum histidine concentration and the aspirin dose in 1,044 sera from 241 patients with rheumatoid arthritis. For example, the interval between 0.6 and 1.2 g aspirin/day refers to patients taking > 0.6 and ≤ 1.2 g/day. $r = -0.07$; $P = 0.02$.

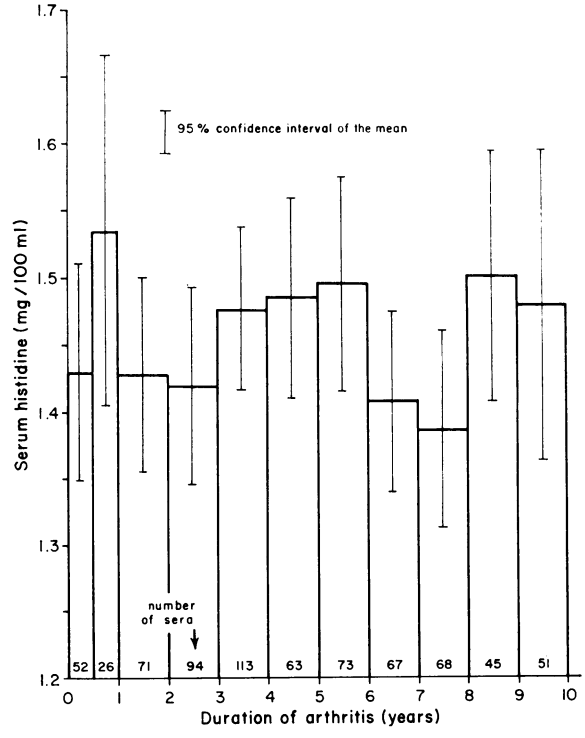

FIGURE 8 Association between the serum histidine concentration and the duration of rheumatoid arthritis in 723 sera from 151 patients with disease for 10 yr or less. $r = 0.02$; $P = 0.5$.

greater in these patients than in patients consisting also of those receiving these drugs (Table II). (*c*) The administration of prednisone, aspirin, phenylbutazone, indomethacin, and dextropropoxyphene to the nonrheumatoid subjects in this study was not associated with hypohistidinemia. Others have observed no effect of cortisone or ACTH on the serum histidine concentration in patients with rheumatoid arthritis (21) or of dexamethasone (22) or ACTH (2) in normal subjects.

It is unlikely that *anemia* is responsible for the hypohistidinemia of rheumatoid arthritis: In this study the serum histidine concentration was normal in anemic nonrheumatoid subjects, and there was no correlation between the serum histidine concentration and the hematocrit in nonrheumatoid subjects. Armstrong and Stave (23) observed no statistically significant correlation between the plasma histidine concentration and the blood hemoglobin concentration in either healthy men, women, boys, or girls.

Time of day. Blood samples obtained from patients with rheumatoid arthritis between 8 and 10 A.M. and after 4 P.M. were the least hypohistidinemic (Fig. 10). This is probably because patients were allowed to select the hour of their appointment. As a consequence, working patients tended to come before or after work and patients with profound morning stiffness rarely came early in the day.

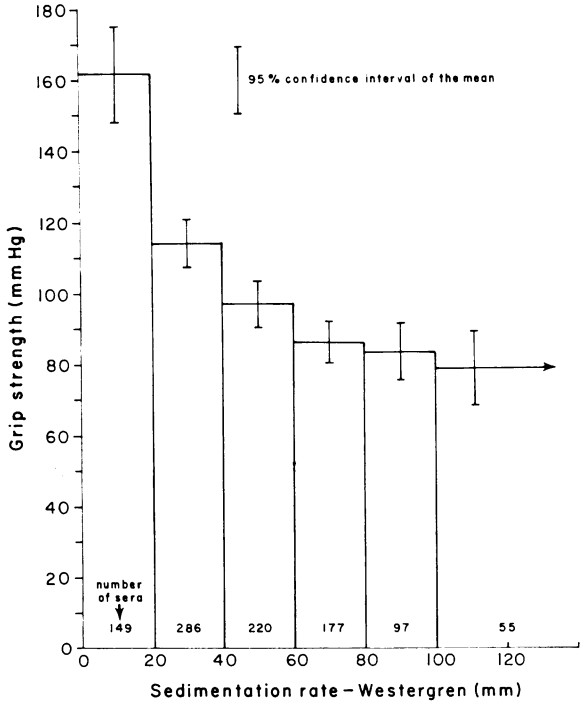

FIGURE 9 Association between the grip strength and the sedimentation rate in 984 visits by 169 patients with rheumatoid arthritis. $r = -0.36$; $P < 10^{-9}$.

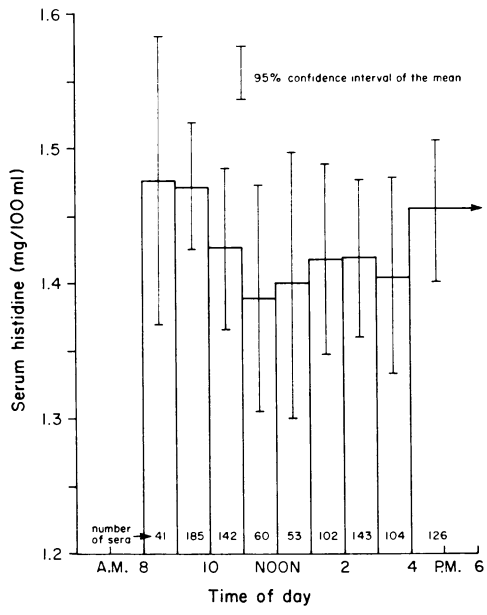

FIGURE 10 Association between the serum histidine concentration and the time of day the blood was obtained in 956 sera from 146 patients with rheumatoid arthritis. $r = -0.03$; $P = 0.4$.

Duration of arthritis. Characteristics of rheumatoid arthritis such as deformity, muscle atrophy, restricted activity, debility, and nodules are more common in patients with disease of long duration. There was, however, no correlation ($r = 0.02$, $P = 0.5$) between the serum histidine concentration and duration of disease in patients with disease of 0–10-yr duration. Moreover, the serum histidine concentration was as abnormal in patients with disease of less than 6-m duration as in the entire group of rheumatoid patients. This suggests that the characteristics of rheumatoid arthritis referred to above are probably not responsible per se for the hypohistidinemia of rheumatoid arthritis.

Usefulness of a single determination of the serum histidine concentration. A single measurement of the serum histidine concentration correlated only roughly with the sedimentation rate and other measures of disease activity (Tables I and II). There are several possible reasons for this. First, all measures of disease activity to which the serum histidine concentration has been compared in this study are subject to technical error and inherent extraneous variability (16). Thus, the interpretation of a single grip strength determination depends to considerable extent on the patient's motivation and effort and on a comparison with the patient's usually unknown premorbid grip strength. The sedimentation rate is affected by coexistent disease, hematocrit, and technical factors such as deviation of the Westergren tube from a vertical position. Second, the serum histidine concentration itself is subject to technical

error and extraneous variability. Among the technical factors involved are the period of time before separation of the clot from the serum and the period of time the serum is left unfrozen. Differences in temperature between the fluorescing solutions used in the assay procedure also affect the results obtained. Nevertheless, measurement of the serum histidine concentration in casual samples, properly declotted and stored, particularly if the determination is repeated several times, appears to be helpful in the evaluation of patients with rheumatoid arthritis.

Attempts to restore the serum histidine concentration to normal by the long-term administration of L-histidine, by mouth, to patients with rheumatoid arthritis have been associated with clinical improvement and more normal laboratory values (24, 25).

It has been proposed that hypohistidinemia, because of an associated low synovial fluid histidine concentration (26), contributes to the pathogenesis of rheumatoid arthritis by permitting the sulfhydryl-dependent hyaluronate-augmented aggregation of synovial fluid gamma globulin, generating in the joint fluid an inflammatory and antigenic substance (aggregated gamma globulin) (27, 28). The observations in the present study are consistent with this hypothesis.

ACKNOWLEDGMENTS

This study was supported by Public Health Service Research Grant R01 AM10370 from the National Institute of Arthritis, Metabolism, and Digestive Diseases; the Kayser Foundation of Houston, Texas; the Houston Endowment Inc.; the Health Research Council of the City of New York (U-1507); the New York Chapter of the Arthritis Foundation; and the Joint Awards Council/University Awards Committee of the Research Foundation of the State University of New York.

REFERENCES

1. Borden, A. L., E. B. Wallraff, E. C. Brodie, W. P. Holbrook, D. F. Hill, C. A. L. Stephens, Jr., L. J. Kent, and A. R. Kemmerer. 1950. Plasma levels of free amino acids in normal subjects compared with patients with rheumatoid arthritis. *Proc. Soc. Exp. Biol. Med.* **75**: 28–30.
2. Borden, A. L., E. C. Brodie, E. B. Wallraff, W. P. Holbrook, D. F. Hill, C. A. L. Stephens, Jr., R. B. Johnson, and A. R. Kemmerer. 1952. Amino acid studies and clinical findings in normal adults and rheumatoid arthritis patients treated with ACTH. *J. Clin. Invest.* **31**: 375–379.
3. Trnavská, Z., and Š. Sitaj. 1960. Die Veränderungen freier Aminosäuren im Serum und im Harn von Kranken mit primär chronischer Polyarthritis. *Z. Rheumaforsch.* **19**: 125–130.
4. Nettelbladt, E., and B. Sandell. 1963. Amino-acid content of serum in rheumatoid arthritis. *Ann. Rheum. Dis.* **22**: 269–272.
5. Georgescu, C. 1966. Protein and amino acid metabolic disturbances in certain collagen diseases. *Microchem. J.* **11**: 384–397.
6. Gerber, D. A., and M. G. Gerber. 1967. Decreased concentration of histidine in the serum of patients with rheumatoid arthritis. A new diagnostic aid. *Clin. Res.* **15**: 294. (Abstr.)
7. Dormidontov, E. N. 1971. Characteristics of amino acid metabolism in different forms of infectious nonspecific polyarthritis [Russian]. *Ter Arkh.* **43**(1): 52–55.
8. Kurmaeva, M. E., A. A. Zotov, and I. V. Rosin. 1971. Data from a complex study of metabolism in several vitamins, free amino acids, and biogenic amines in patients with infectious nonspecific (rheumatoid) polyarthritis [Russian]. *Ter Arkh.* **43**(11): 98–101.
9. Smithwick, E. M., and D. A. Gerber. 1972. Serum histidine levels in juvenile rheumatoid arthritis. *Pediatr. Res.* **6**: 402. (Abstr.)
10. Roux, H., R. Aquaron, B. Laurent, L. Veron, and A. M. Recordier. 1972. Les acides aminés basiques du sérum au cours de la polyarthite rhumatoïde. *Rev. Rhum. Mal. Osteo-articulaires.* **39**: 677–680.
11. Briscoe, A. M., H. Lackland, and C. Ragan. 1968. Blood serum arginine in rheumatoid arthritis. *Fed. Proc.* **27**: 485. (Abstr.)
12. Gerber, D. A. 1970. Determination of histidine in serum with o-phthaldialdehyde. *Anal. Biochem.* **34**: 500–504.
13. Fischel, E. E. 1967. The erythrocyte sedimentation rate. *In* Laboratory Diagnostic Procedures in the Rheumatic Diseases. A. S. Cohen, editor. Little, Brown and Company, Boston. 1st edition. 52–69.
14. Singer, J. M., and C. M. Plotz. 1956. The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis. *Am. J. Med.* **21**: 888–892.
15. Friou, G. J. 1967. The LE cell factor and antinuclear antibodies. *In* Laboratory Diagnostic Procedures in the Rheumatic Diseases. A. S. Cohen, editor. Little, Brown and Company, Boston. 1st edition. 114–167.
16. McCarty, D. J. 1972. Methods for evaluating rheumatoid arthritis. *In* Arthritis and Allied Conditions. J. L. Hollander and D. J. McCarty, Jr., editors. Lea & Febiger, Philadelphia. 8th edition. 419–438.
17. McEwen, C. 1972. The diagnosis and differential diagnosis of rheumatoid arthritis. *In* Arthritis and Allied Conditions. J. L. Hollander and D. J. McCarty, Jr., editors. Lea & Febiger, Philadelphia. 8th edition. 403–418.
18. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames, Iowa. 6th edition. 104–105, 185–188, 246–248, 258–267.
19. Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, New York. 350–351.
20. Dixon, W. J., and F. J. Massey, Jr. 1969. Introduction to Statistical Analysis. McGraw-Hill Book Company, New York. 3rd edition. 200–202.
21. Stephens, C. A. L., Jr., E. B. Wallraff, A. L. Borden, E. C. Brodie, W. P. Hollbrook, D. F. Hill, L. J. Kent, and A. R. Kemmerer. 1950. Apparent free histidine plasma and urine values in rheumatoid arthritis treated with cortisone and ACTH. *Proc. Soc. Exp. Biol. Med.* **74**: 275–279.
22. Zischka, R., E. Orti, and S. Castells. 1970. Effects of short-term administration of dexamethasone on urinary and plasma free amino acids in children. *J. Clin. Endocrinol. Metab.* **31**: 95–97.
23. Armstrong, M. D., and U. Stave. 1973. A study of plasma free amino acid levels. V. Correlations among the amino acids and between amino acids and some other blood constituents. *Metab. Clin. Exp.* **22**: 827–833.

24. Gerber, D. A. 1969. Treatment of rheumatoid arthritis with histidine. *Arthritis Rheum.* **12**: 295. (Abstr.)
25. Pinals, R. S., E. D. Harris, Jr., J. Frizzell, and D. A. Gerber. 1973. Treatment of rheumatoid arthritis with histidine-A double-blind trial. *Arthritis Rheum.* **16**: 126–127. (Abstr.)
26. Stuhlsatz, H. W., L. Plagemann, and H. Greiling. 1973. Die freien Aminosäuren in Synovialflüssigkeit und Serum bei der rheumatoiden Arthritis. *Z. Rheumaforsch.* **32**:388–393.
27. Gerber, D. A. 1974. Copper-catalyzed thermal aggregation of human gamma globulin. Inhibition by histidine, gold thiomalate, and penicillamine. *Arthritis Rheum.* **17**: 85–91.
28. Gerber, D. A. 1975. Sulfhydryl-dependent thermal aggregation of human gamma globulin. Augmentation by hyaluronic acid. *Arthritis Rheum.* **18**: 59–66.