Table 2. The correlation coefficients (r) between functions measured in the males of the drinker strain in the F_7 generation. The limit value of the statistical significance at $P = .05$ is .25; at $P = .01$ it is .32. Functions measured are absolute alcohol and total fluids in milliliters per 100 g of body weight per day, total calories per 100 g of body weight per day, weight at beginning of period, and growth in grams.

Functions	1	2	3	4	5
1 Absolute alcohol	1.00				
2 Total fluids	.65	1.00			
3 Total calories	.43	.58	1.00		
4 Weight	− .19	− .26	− .43	1.00	
5 Growth	.34	− .17	.16	.24	1.00

take of the drinkers is significantly higher than that of the nondrinkers. The same applies to fluid intake. The greater fluid intake is probably due to the diuretic effect of alcohol. And with the same standard food, greater consumption of food usually leads to higher fluid intake; this may reflect a metabolic difference between strains. With respect to weight, such a marked difference between the strains may also signify an inherited metabolic difference, which is associated with alcohol consumption (*6*). The sexes differ in the rate of alcohol elimination, which is higher in females (*6*). Subsequent experiments have indicated that these strains do not differ in the rate of alcohol elimination or in alcohol dehydrogenase activity. The difference between the sexes which is conspicuous in the drinker strain, is not so apparent in the nondrinker strain. In both strains the difference between sexes is greater for alcohol consumption than for alcohol preference. The alcohol consumption as a percentage of total fluid intake has generally been adopted as a measure of preference. In my experiments, the regression of preference values of alcohol intake per unit weight was determined for the F_7 generation, of which there were 210 animals. The regression model accounted for as much as 94 percent of the variance, the regression coefficient being .85. The two measures show a high correlation, but the preference value is at a disadvantage, because it does not vary freely but depends on total fluid intake. From the same data, the correlations of other variables with alcohol consumption were also calculated. Because the four-peaked curve of variation does not permit analysis of the total data, I have chosen drinker males as an example (Table 2), especially because their range of intake was wide but the range of other variables, within individuals, was slight during the experimental period. Consequently, the possible association of some variables with alcohol consumption is easier to discern. The results confirm the validity of the sex and interstrain differences (Table 1), in that calorie intake, fluid intake, and alcohol consumption constitute a positively correlated group of variables. In contrast, the interstrain difference in initial weight shows only a weak negative correlation with alcohol consumption, and the difference is possibly fortuitous.

Rodgers and McClearn (*4*) have adopted the working hypothesis that alcohol consumption is an additively inherited polygenic trait. However, they have not constructed a heritability model, although they have shown that females exert a greater effect on the offspring. Construction of a heritability model on the basis of the hypothesis of self-selection requires that the inheritance be additive, that there be no dominance, that the quantitative effects of the genetic units involved be approximately equal, and that there be no linkage. The experiments of Reed (*1*) reveal large deviation within inbred strains with regard to drinking behavior. These results suggest that the role of inheritance is slight, either because of an inadequate phenotypic measure or of a predominating role of the environment. The model constructed here shows the importance of genetic factors in determining selection of alcohol, although the determining power of the environment still seems stronger. The model will undoubtedly be improved by an elaboration of measuring techniques.

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10 October 1967

Hemoglobin Rainier (β^{145} Tyrosine → Histidine): Alkali-Resistant Hemoglobin with Increased Oxygen Affinity

Abstract. *Hemoglobin Rainier, a new hemoglobin variant associated with erythrocytosis, was found in six members of a Caucasian family. Structurally, it represents substitution of histidine for the invariant residue H23 tyrosine in the β-hemoglobin polypeptide chain (β^{145} tyrosine → histidine). Hemoglobin Rainier is the first example of a single amino acid substitution in adult human hemoglobin, causing increased resistance to alkali denaturation.*

Thus far, more than 50 examples of chemical abnormalities in the human hemoglobin polypeptide chains have been reported. For the most part, they represent single amino acid substitutions which usually have no measurable effect on the physiological property of the hemoglobin molecule as a respiratory pigment. Recently, abnormal hemoglobins with a functional abnormality consisting of increased oxygen affinity have been reported (*1, 2*). In heterozygotes for these abnormal hemoglobins, lower volumes of oxygen per red cell are released to the tissue. The consequent relative tissue hypoxia stimulates erythropoietin secretion, producing erythrocytosis. We report the biochemical findings of a new abnormal hemoglobin, associated with increased affinity to oxygen, designated as hemoglobin Rainier.

Hemoglobin Rainier was detected in a 47-year-old Caucasian female with erythrocytosis (hematocrits 57 to 59 percent) of unknown etiology and high normal values of urinary erythropoietin. Five other members of her family had this abnormal hemoglobin accompanying erythrocytosis. Hemoglobin-oxygen dissociation of blood samples from individuals with hemoglobin Rainier indicated increased affinity of hemoglobin to oxygen and decreased heme-heme interaction ($n = 1.2$). the pH of red cells and the Bohr effect were normal (*3*).

On electrophoresis, hemoglobin Rainier did not separate from hemoglobin A when hemolyzates of heterozygotes were studied on starch gels with phosphate buffer (*p*H 6.2 and 7.0), tris-EDTA-borate buffer, *p*H 8.6 or tris-HCl buffer, *p*H 9.5. However, electrophoretic separation was achieved on agar gels when 0.05*M* citrate buffer at *p*H 6.2 was used. Hemolyzates from heterozygotes showed increased alkali resistance (from 12.0 to 16.0 percent), although fetal hemoglobin was not elevated by either starch-gel or agar-gel electrophoresis.

Further characterization of hemoglobin Rainier was carried out after isolation on a carboxymethyl-Sephadex column (*4*). The concentration of Hb Rainier after column chromatography in one heterozygote was 30 percent. Study of alkali denaturation rate of isolated hemoglobin Rainier (*5*) showed rapid denaturation of approximately 40 percent of the hemoglobin in the first 30 to 60 seconds of the reaction. However, the remainder of the denaturation curve was relatively flat, and at 15 minutes, approximately 40 percent of Hb Rainier was still undenatured. By extrapolation to zero time the alkali resistant component was estimated to 60 percent. Purified Hb Rainier showed a single sedimentation boundary by analytical ultracentrifugation. The sedimentation constant ($s_{20,w}$) measured in 0.05*M* Na_2HPO_4–KH_2PO_4 buffer (*p*H 6.8) containing 0.2*M* NaCl was 4.1*S*, indicating the usual tetrameric form of the hemoglobin. Electrophoresis after hybridization of isolated hemoglobin Rainier with canine hemoglobin (*6*) showed that the hybrid consisting of β-chains from hemoglobin Rainier and α-chains from canine hemoglobin migrated more slowly than the corresponding hybrid obtained from β-chains from hemoglobin A and α-chains from canine hemoglobin. The finding indicated that the lesion in hemoglobin Rainier was located on the β-chain.

Globin was prepared from Hb Rainier by acid-acetone precipitation (*7*), and α- and β-chains were isolated by carboxymethyl-cellulose column chromatography in 8*M* urea (*8*). The β-chain was aminoethylated (*9*) and digested by trypsin. The tryptic peptides were separated by chromatography on Dowex 50-X2 column (*9*). The elution patterns (Fig. 1, A and C) indicated that in hemoglobin Rainier the peptide βT-15 (Tyr-His, residues 145 and 146) was absent and a new peptide (marked X) was eluted between βT-12b and βT-7,8. Amino acid analysis of peptides X and βT-15, together with the chromatography of authentic histidylhistidine (Fig. 1B), revealed that tyrosine at position No. 145 of β-hemoglobin chain was replaced by histidine in Hb Rainier. There was no indication of other substitutions, although this has not been determined with absolute certainty.

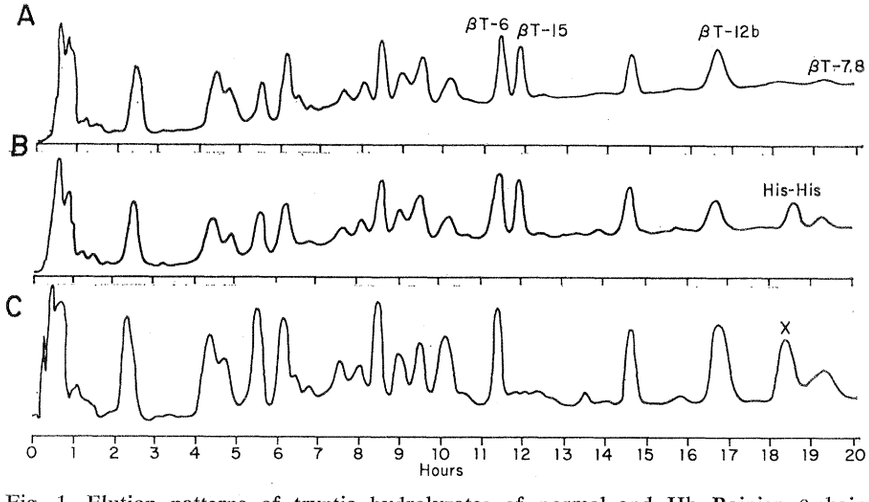

Fig. 1. Elution patterns of tryptic hydrolyzates of normal and Hb Rainier β-chains from a Spinco type 15-A resin column (0.9 by 16 cm). The peptides were eluted with a gradient produced with the aid of a six-chamber varigrad. Chambers 1, 2, and 3 each contained 100 ml of *p*H 3.1, 0.02*M* pyridine–acetic acid buffer and 4, 5, and 6 each contained 100 ml of *p*H 5.0, 0.2*M* pyridine–acetic acid buffer. The flow rate was 30 ml/hr at 50°C. The effluent stream was split; about one-fifth was used for continuous detection by reaction with ninhydrin, and the remaining was collected in a fraction collector. (A) Peptide patterns of hydrolyzates of normal β-chain (about 3 mg); (B) Peptide patterns of hydrolyzates of normal β-chain (about 3 mg) plus histidylhistidine; (C) Peptide patterns of hydrolyzates of Hb Rainier β-chain (about 8 mg). Peptides are numbered in accordance with Jones (*9*).

The substitution, according to the helical numbering proposed by Perutz (*10*), involves residue 23 in the H-helix. Tyrosine in residue H23 is one of the nine so-called invariant amino acids, that is, amino acids occurring at structurally identical sites in all the myoglobins and hemoglobins so far investigated. In Perutz's hemoglobin model (*10*), H23 tyrosine is given some importance in determining the tertiary structure of the polypeptide chain; it has been implicated in cross-linking helices G and H through a hydrogen bond, with the alpha carbonyl of residue FG5 at the corner of the FG nonhelical region. In hemoglobin Rainier, histidine in position H23 would not form the interhelical bond with FG5 valine. Residue FG5 is in the proximity of the heme plane; the substitution in hemoglobin Rainier might indirectly alter the relation of this residue to heme iron. Alternatively, as suggested by Jones in the case of Hb Yakima (*2*), the substitution could alter the nature of the interactions between the hemoglobin chains and could favor the oxyhemoglobin quarternary structure.

These molecular alterations, particularly the possible abnormal interaction between chains, might be correlated with the increased resistance to alkali found in Hb Rainier; however, no plausible mechanism can be proposed at present. Human fetal hemoglobin ($\alpha_2\gamma_2$) and several animal hemoglobins are resistant to denaturation by alkali; however the structural basis of this property of hemoglobin is not clear. Hemoglobin Rainier is the first example of a single amino acid substitution in one of the adult (human) hemoglobin polypeptide chains which renders the hemoglobin resistant to denaturation by alkali. Since residue H23 is invariant (and therefore occupied by tyrosine in alkali-resistant hemoglobins with known sequence), it is not the change in the primary structure but rather the consequent changes in tertiary or quarternary structure which make hemoglobin Rainier alkali resistant. A relative increase in alkali resistance has been recorded in another, presumably adult, hemoglobin variant (*11*); however, the exact molecular lesion in this variant is not known.

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5. Hemoglobin denaturation by 0.06*N* NaOH was studied by following, in a Beckman DU spectophotometer, the rate of decrease in absorbance at 430 mμ at 28°C as a function of time for 15 minutes; the reaction was completed by warming the samples at 37°C for 30 minutes. The proportion of undenatured hemoglobin at a given time was calculated according to J. H. P. Jonxis and T. H. J. Huisman, *Blood* **11**, 1009 (1956).
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12. Supported by NIH research grants HD-00061, HD-02497, GM-15253, and HE-06242.

27 November 1967

Myotatic Reflex: Its Input-Output Relation

Abstract. *The dynamic properties of the myotatic reflex and of its components were determined by a systems-analysis approach. The gain and phase relations between an applied stretch, which initiates the reflex, and the output of the primary muscle spindles, which impinge upon α-motoneurons, are not further changed by the properties of the motoneurons. The dynamic relation between motoneuron activity and the resultant muscle tension balances these changes in gain and phase; the result is a flat gain and nearly zero phase difference between stretch and tension produced by the myotatic reflex. Moreover, the distribution of activity in multiple channels extends the range in which the overall reflex is linear.*

The myotatic reflex comprises at least the following events: (i) generation of impulse activity by the primary endings of muscle spindles as a consequence of the stretch applied to the muscle, (ii) excitation of α-motoneurons, and (iii) development of muscle tension resulting from α-motoneuron activation. The dynamic behavior of the systems responsible for these events is largely unknown, although some data are available (*1–3*). Once these systems are known, one can predict the behavior of the reflex under various conditions and can study the functional influence of supraspinal systems on spinal motoneurons.

Study of the myotatic reflex also enables one to inquire into the functional significance of neuronal systems composed of multiple channels having similar information content. Indeed, the primary endings of muscle spindles converge upon single motoneurons and they seem to behave rather uniformly in response to muscle stretch, since the rate of impulse activity generated in these endings is nearly the same for all amounts of sustained stretch (*4*). Moreover, their dynamic behavior is similar (*2*), and the unloading produced by the activation of even one, or of a few motor units, should affect all the spindles similarly, if one assumes that the muscle fibers belonging to each motor unit are not highly segregated within the muscle (*5*). By use of a preparation in which differences in spindle behavior due to γ-motoneurons are abolished by cutting of the ventral roots, one can examine the result of the convergence, upon single motoneurons, of information conveyed by parallel channels. Moreover, one can compare the behavior of single motoneurons with the behavior of the motoneuron population to see what properties may arise from handling of similar information by multiple units, on the assumption that the input distribution and the properties or the functional state (or both) of the individual motoneurons belonging to a uniform population are not the same.

The dynamic behavior of the overall myotatic reflex (stretch input and active-tension output) was measured in decerebrate cats (Fig. 1). The tendon was stretched as it would be for movements of the ankle joint as much as 15 deg about the steady-state position (foot at 90-deg angle from leg). The gain (*6*) of the dynamic length-tension relation was about flat (Fig. 2A, curve *b*), and only a small but constant phase difference was present between input and output (Fig. 2B, curve *b*), within the range of frequencies and amplitudes used. This behavior can be predicted from the dynamic characteristics of the systems responsible for the reflex.

Behavior of the primary endings of muscle spindles was measured in decerebrate cats (ventral roots cut or uncut) and in cats under barbiturate anesthesia (pentobarbital, 35 to 40 mg/kg). The modulation component of the impulse activity in a single primary ending, evoked by sinusoidal stretches of different amplitudes and frequencies, was determined by a reported technique (*7*). The results yielded by use of slightly different resting lengths and different average firing frequencies (between 18 and 30 impulses per second) can be fitted by a single curve (Fig. 2A, solid circles). The behavior ceased to be linear for displacements above 50 to 100 μ (Fig. 3B). Within these limits the percentage modulation increased linearly with increasing displacement amplitude, and the modulation component was sinusoidal. At the larger amplitudes the distribution of impulse activity was no longer sinusoidal, and saturation occurred at approximately 300 μ (Fig. 3A).

The second step in the myotatic reflex, the processing of afferent information by α-motoneurons, involves at least two separate processes: demodulation of information carried by afferent impulses (synaptic processes), and re-

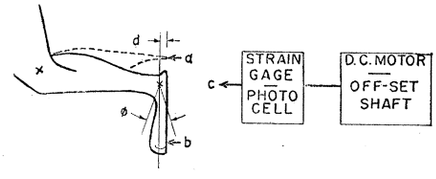

Fig. 1. Stimulator. The hind leg is fixed at the distal ends of the femur and tibia with pins. The steady-state value of muscle displacement was chosen to correspond to the limb position shown—foot approximately 90 deg from leg. The amplitude of sinusoidal displacement (*d*) of the extensor muscles is that due to displacement of the foot through angle φ (up to 15 deg). Stimulus applied at *a* displaces triceps surae only. Stimulus applied at *b* displaces both flexor and extensor muscles. Sinusoidal displacements were generated by a feedback-controlled, variable-speed d-c motor that turned a variable-offset shaft. Displacement was measured by a photocell, and tension was measured by a strain gage on the output shaft. The sinusoidal displacement at *c* was applied at *a* or *b*, appropriate adjustments in amplitude being made by variation in the amount of offset.