each chain being used. This value was corrected for the difference in absorption of the three chains. At 280 nm, the absorbances of gamma and beta chains in this system were found to be, respectively, 2 and 1.5 times that of an equal amount of alpha chain. Specific activity of the gamma chain was further corrected for its small difference in the molar content of leucine (γ, 17; α, 18; and β, 18) (*5*). Figure 2 presents the corrected specific activity ratios of the gamma to alpha and beta to alpha chains in the five cord bloods that were examined. The ratios of gamma to alpha chains of the affected newborn and the normal newborns were similar to each other and considerably lower than the ratios of beta to alpha chains of the normal newborns. This indicates that the synthesis of gamma chains in the affected newborn was declining at a rate similar to that in the normal newborns. In the four normal newborns, acceleration of the synthesis of beta chains at birth was indicated by the fact that the ratio of beta to alpha chains was higher than that of gamma to alpha. However, in the newborn affected with beta thalassemia trait, the ratio of beta to alpha chains was approximately one-half that of normal. The reduction of this ratio in the affected newborn was not simply the result of immaturity because no difference was found in the four normal newborns who varied in gestation from 35 to 41 weeks and in birth weight from 4½ to 8 lb.

We conclude from this study that the synthesis of beta chains of this newborn with beta thalassemia trait was decreased by a factor of approximately 2. Therefore, it was possible by this technique to detect the presence of a single beta thalassemia gene at birth, even though routine studies did not suggest the diagnosis. We have not yet had the opportunity to study a newborn affected with homozygous beta thalassemia, but on the basis of this study and of similar examinations performed in older individuals with homozygous beta thalassemia and with beta thalassemia trait (*1*), it is reasonable to assume that detection of such homozygotes would be possible with this method. The homozygous state can also be detected by morphologic methods that are reliable and simple (*6*).

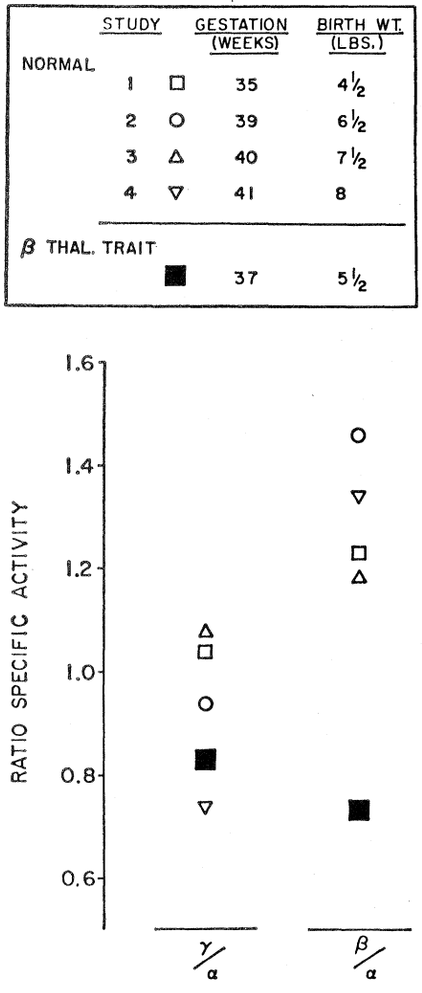

Fig. 2. Ratios of the specific activities of the gamma to alpha and the beta to alpha chains of the patient compared with those of four normal newborns. Gestational periods and birth weights are given in the upper part of the figure.

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8. Drs. H. C. Rosen, J. Smith, R. Barker, and H. Judd provided the samples of cord blood. Dr. W. C. Mentzer drew our attention to the patient.

21 May 1968

Regulation of Segment-Building during the Postembryonic Development of a Common Milliped

Abstract. *Starvation of larvae of the milliped* Narceus annularis *results in the formation of a smaller than normal number of body segments in the early stadia. However, this reduced number is compensated for by subsequent formation of a larger than normal number of body segments in the later stadia, or by the addition of extra segment-building stadia. Apparently there is a mechanism whereby the milliped "keeps count" of the number of body segments produced and can regulate the production of these segments from a proliferative region so that the total number of body segments at sexual maturity is within the normal range for the species.*

A characteristic feature of the postembryonic development of Diplopoda is the addition of new body segments and new limbs during each stadium (*1*). For example, larvae of the common spiroboloid milliped *Narceus annularis* (Raf.) have only seven body segments when they hatch, whereas the adults have 51 to 59 segments (*2*). Most normal individuals pass through nine segment-building stadia plus two maturational stadia, reaching sexual maturity at the eleventh ecdysis. The new segments added at each ecdysis differentiate from a structurally distinct proliferation region located between the penultimate segment and the telson (anal segment) (*3*). The new segments are legless when they first appear; their legs form during the next stadium and are exposed at the next ecdysis, when additional legless segments are also added. This process of segment and limb increase during larval development is basically the same in all orders of Diplopoda studied (*1*, *3*, *4*).

Previous observations (*2*) indicated that young larvae reared under conditions of near starvation add fewer body segments per ecdysis than larvae reared with an optimum food supply. This raised the question of whether semistarved larvae would develop into adults with fewer body segments than normal, or whether the millipeds possess a regulatory system that will alter the later stages of development in a manner that will insure the acquisition of a full complement of body segments before maturity is reached.

Four hundred and fifty larvae hatched from eggs laid by caged females were placed in 15 boxes (30 ani-

mals per box) with an abundant supply of leaf litter for food. Another 450 larvae were distributed in 15 boxes but were given only fecal pellets for food for the first 6 months; after 6 months, the diet was modified to include a small amount of leaf litter (*5*). Larvae from both the well-fed and semistarved groups were examined in each stadium for body length and width, number of ocelli, gonopod development, and number of body segments (*6*). In addition, the duration of each stadium and the number of stadia passed through to sexual maturity were recorded.

Table 1 presents data on the number of segments added at each stadium for both the well-fed and semistarved larvae (*7*). Because very early stadia remain inside the egg capsule in which the eggs are enclosed (*8*), starvation could not be begun until the larvae emerged from the capsules; hence no dietary effects would be expected before the fourth ecdysis. At ecdyses 4, 5, and 6, the well-fed larvae added more body segments than the semistarved ones (t-test, $P<.001$). However, at ecdysis 7 a reversal occurred, and the semistarved larvae added more body segments than the well-fed ones ($P<.001$); the same tendency was apparent at ecdyses 8 and 9, though the difference was not statistically significant ($P<.3$ and .5).

Table 2 shows six different maturational sequences observed in *N. annularis*. The most frequent sequence found for both the well-fed and the semistarved animals was type 3. However, more than 25 semistarved animals went through one additional segment-building stadium (sequences 4 or 5), and seven went through two extra segment-building stadia (sequence 6); no semistarved animals followed sequences 1 or 2. By contrast, only three well-fed animals went through sequence 4, and none went through sequences 5 or 6.

These results indicate that environmental factors, such as the amount of food available, can influence the number of body segments produced at each ecdysis by the embryonic tissue of the proliferative region. They indicate, further, that the developing milliped somehow "keeps count" of the number of new body segments it has produced; if the number is below normal by the time it reaches the seventh stadium, it may compensate by building more than the normal number of segments in the later stadia (even though it may still be on a semistarvation diet), or by going through additional segment-building stadia (or by doing both). When the semistarved animals reached maturity, all had body segment numbers within the normal range (51 to 55, compared with the normal of 51 to 59), but the average (53.7) was lower than the normal average (55.1) for the species (*2*).

Environmental influence on the number of developmental stages is not uncommon in other arthropods (*9*). For example, temperature affects the number of instars of *Tenebrio molitor* (*10*), *Dermestes* (*11*), and *Pieris brassicae* (*12*). Starvation can increase the number of ecdyses of the German cockroach (*13*), and decrease the number of ecdyses of some lepidopterous larvae (*14*). In all these cases, the number of ecdyses is primarily related to growth in size. In millipeds, however, size [which is exceedingly variable in *N. annularis* (*2*)] seems less important than the number of body segments; when the animals reached maturity, semistarved ones were much smaller than the well-fed ones (width 31 to 42 mm for semistarved, 42 to 75 mm for well-fed). Apparently the millipeds can mature sexually when still much smaller than normal, but there seems to be a species-typical range of body segment number that must be attained before sexual maturation can occur. How the animal "keeps count" of the number of new body segments remains an unanswered but intriguing question.

Table 1. Body segments added per stadium (well-fed, +; semistarved, −).

Stadium	Ecdysis	Mean segment increase	Standard deviation	Standard error of mean	Range limits	Animals (No.)
3 +	2	14.33	0.65	0.19	14–16	12
3 −	2	14.0	.0	.0	14	13
4 +	3	5.58	.57	.11	4–6	24
4 −	3	5.25	.83	.17	4–6	24
5 +	4	5.88	.47	.09	5–7	25
5 −	4	4.67	.25	.05	4–5	24
6 +	5	6.21	.70	.13	5–7	28
6 −	5	4.90	.46	.10	4–6	20
7 +	6	5.83	.36	.02	5–7	30
7 −	6	5.23	.78	.13	4–6	35
8 +	7	4.17	.38	.26	4–5	23
8 −	7	5.15	.36	.08	5–6	27
9 +	8	3.55	.67	.14	3–5	20
9 −	8	3.79	.41	.10	3–4	19
10 +	9	2.58	.74	.14	1–4	26
10 −	9	2.76	1.08	.26	1–5	17

Table 2. Maturational sequences of *Narceus annularis*; +, segments are being constructed during this stadium; −, no segments are being constructed during this stadium.

Sequence type	Stadium					
	8	9	10	11	12	13
1	+	−	Mature			
2	+	+	−	Mature		
3	+	+	−	−	Mature	
4	+	+	+	−	Mature	
5	+	+	+	−	−	Mature
6	+	+	+	+	−	Mature

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7. New body segments develop from the proliferative region during the stadium preceding the ecdysis at which they first become outwardly visible. Thus, for example, new segments exposed by the ecdysis (ecdysis 4) to stadium 5, actually developed during stadium 4.
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7 May 1968

Collagen: Relatively Invariant (Helical) and Variable (Nonhelical) Regions

Abstract. *The structural identity of certain helical regions of collagen from human and rat skin equals or exceeds that of other homologous proteins. In contrast, the short nonhelical sequences in the two proteins, although homologous, differ appreciably in structure. The requirements of the collagen helix and the numerous intermolecular interactions characteristic of collagen may restrict the number of functionally acceptable amino acid replacements occurring during evolution.*

Studies of the structural changes which occur during evolution indicate that the number of amino acid replacements in homologous proteins roughly parallels interspecial phyletic distances, as estimated by morphological taxonomic criteria (*1*). The relatively invariant regions which exist are frequently involved in a specialized function of the protein, but intramolecular side-chain interactions associated with a specific tertiary structure also serve to select against amino acid substitutions. However, as suggested by the comparative biochemistry of globin chains (*2*), selective pressures at a functional level appear to be exerted toward preserving the overall conformation of a protein rather than a unique amino acid sequence.

In keeping with the structure proposed for collagen (*3*), the interior of the highly asymmetric cylindrical molecule is occupied by the helical backbone of the three polypeptide chains, whereas all amino acid side chains are directed exteriorly. The formation of the triple-stranded collagen helix requires that every third amino acid residue be glycine, and, in order for the structure to be stable in vivo, a minimum pyrrolidine (proline plus hydroxyproline) content is necessary (*4*). The side chains of the remaining 45 percent of the amino acids do not appear to participate significantly in intramolecular interactions. If there are no mutational "cold spots" in the DNA coding for collagen, amino acid residues which are not critical to the function of the protein may be subject to change by mutation, and the positions occupied by these amino acids would be expected to show interspecial variation. By extension of this reasoning, intraspecial polymorphism could result from the formation of allotypic collagen chains produced by numerous allelic genes.

To investigate this matter, the amino acid composition and partial sequence of portions of the $\alpha 1$ and $\alpha 2$ chains of human skin collagen (HSC) were determined in three subjects. There were no differences in composition in the isologous proteins. In addition, with the exception of a short sequence near the amino terminus the composition and sequence (insofar as determined) of HSC and rat skin collagen (RSC) were very similar.

Specimens of skin were obtained at autopsy from three infants who had died a respiratory death shortly after delivery. Pathologic examination revealed no abnormalities in connective tissues. Rat skin was obtained from male Sprague-Dawley rats weighing 100 to 150 g. Collagen was successively extracted from skin with 1*M* NaCl, 0.5*M* acetic acid, and 5*M* guanidine, and purified as described (*5*). Single-chain (α) and double-chain (β) components were separated by carboxymethyl (CM) cellulose chromatography (*5, 6*). Isolated $\alpha 1$ and β_{12} components were cleaved nonenzymatically at methionyl residues with cyanogen bromide (CNBr) (*5, 7*), and the resulting fragments were separated by chromatography on CM-cellulose at *p*H 4.8 (*7*) and on phosphocellulose (*5*). Individual CNBr-produced fragments were cleaved with trypsin or chymotrypsin, and the enzymatic digests were separated by column chromatography on Bio-Gel P-2 or phosphocellulose. Peptide maps of tryptic digests were obtained by two-dimensional chromatography and electrophoresis (*8*). Amino acid analyses were performed with a Beckman 120C analyzer modified for high-speed single-column gradient elution (*9*).

The CM-cellulose and phosphocellulose elution patterns of CNBr digests of the $\alpha 1$ chain of HSC and RSC were very similar. The position of elution of only one peptide, $\alpha 1$-CB1, was clearly different in digests of the two proteins. Three peptides, $\alpha 1$-CB1, $\alpha 1$-CB2, and $\alpha 1$-CB3 were further compared largely because of their relative ease of purification. The phosphocellulose elution patterns of CNBr digests of β_{12} (the $\alpha 1$-$\alpha 2$ dimer) from HSC and RSC differed only in the position of elution of the cross-linked peptide, β_{12}-CB1. Peptide $\alpha 2$-CB2 was purified and analyzed.

Homologous peptides in RSC and HSC were identified by similarities in elution from phosphocellulose and by amino acid composition. The amino acid compositions of $\alpha 1$-CB2 from HSC and RSC were the same with the exception of the degree of hydroxylation of proline (Table 1). The chromatographic properties and amino acid compositions of the chymotryptic fragments of the two peptides were also the same. The compositions of $\alpha 2$-CB2 (Table 1) and $\alpha 1$-CB3 (Table 2) were extremely similar in the two proteins.

Table 1. Amino acid compositions of some CNBr-produced fragments from rat and human skin collagen. Results are given as residues per peptide. Values are the average of three or more determinations. Lack of a number indicates that the amino acid was either entirely absent or present as less than 0.1 residue. Residues in parentheses are fractional residues thought to be impurities.

Amino acid	$\alpha 1$-CB1		$\alpha 1$-CB2		$\alpha 2$-CB2	
	HSC	RSC*	HSC	RSC*	HSC	RSC
4-Hydroxyproline			5.5	4.9	2.9	2.8
Aspartic acid	1.1	1.0			1.9	2.8
Threonine	1.0					1.0
Serine	2.9	2.0	1.8	2.2	1.8	1.1
Homoserine†	0.9	0.9	1.0	1.0	0.8	0.9
Glutamic acid	2.1	1.0	3.9	4.0	1.3	1.1
Proline	2.1	1.8	6.0	6.9	2.9	3.1
Glycine	4.2	3.2	11.4	12.1	9.7	9.4
Alanine	(0.2)	1.1	2.1	2.1	3.2	2.1
Valine	1.2	1.8			1.1	1.0
Isoleucine	1.1					
Leucine	1.0		1.0	1.0	1.1	1.0
Phenylalanine			1.0	0.9		
Tyrosine	1.8	1.2				
Lysine	0.9	1.0				
Arginine			1.1	1.0	2.8	3.0

* Data from Bornstein and Piez (*5*). † Includes homoserine lactone.