deduced for the rotation of a small cylinder about a transverse axis through its center. It is $\overline{\theta^2} = 24\,kTt/\pi\eta l^3$, where k is the Boltzmann constant, T is the absolute temperature, η is the coefficient of viscosity of the medium, l is the length of the cylinder, and t is the time interval between observations of the angle θ through which the sphere rotates. It must be understood that $\overline{\theta^2}$ represents the resultant mean-square angular displacement due to a very large number of nudges from impacts of the substrate molecules. In all observations on rotational Brownian motion, however, the projection of the angle on a fixed plane is measured, so that the foregoing equation should be divided by 3 in applying it to experimental data.

Consider a rod-like bacterium 1.5 μ long and 0.5 μ in diameter in a medium at a temperature of 27°. Set η equal to 1 cpoise, t to 1 sec and k to $1.38 \cdot 10^{-16}$ erg/°. Then the ratio of the mean-square angle of rotation about a transverse axis to the time interval for observations equals $24 \times 1.38 \times 10^{-16} \times 300 \times 0.01 \times (1.5)^3 \times 10^{-12}$ or 9.3 radians^2 sec. On the average, therefore, the bacterium would rotate through approximately 175°, almost a complete about face, during a 1-sec time interval. Thus locomotion in a given direction long enough to measure the speed, or even to allow the bacterium to go anywhere, is practically impossible unless provision is made to reduce the effects of rotational Brownian motion. A flagellum or flagellar tress several tens of μ long would prevent undue rotations; in effect, it would act as a stabilizer for the bacterium. If the over-all length is increased by a factor of 10 the root-mean-square angle is reduced by a factor of 1/30. REICHERT's observations that, by and large, small bacteria have longer flagella than large bacteria[10] support this hypothesis.

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Masked condition of ionic residues in collagen

Physico-chemical studies on collagen have emphasized the importance of hydrogen bonds in the structure. However, the state of the ionized residues in this protein is still obscure, although there is evidence from electron microscopy that they may have a special orientation in the fibril[1]. Also, analysis of titration curves reveals unusual behavior in that there is slight reactivity over the range pH 6–10, and a low alkali-neutralizing capacity compared to the content of basic amino acids[2]. This and other evidence mentioned by GUSTAVSON suggests the existence of salt-like

crosslinks in collagen[3]. Recent observations dealing with the interaction of collagenous materials with salt are those of VEIS *et al.*[4], who found that acid-precursor gelatin bound about 200 μmoles NaCl/g from 0.1 *M* NaCl solution. This corresponds to about 20% of the protein's capacity, judged from the content of ionic residues. At neutral reaction, ARMSTRONG[5] detected no binding of K^+ by insoluble collagen, but noted a binding of about 65 μmoles/g of acid from solutions of low ionic strength.

The distribution of NaCl between collagen* and external salt solutions was examined in order to obtain additional information on the reactivity of the ionic residues. In these experiments, 50–100 mm^3 pieces of collagen were equilibrated with various concentrations of NaCl (1–2 l) for several days at 3°. Next, they were removed and blotted with paper tissues or pressed mechanically, weighed, dried *in vacuo* over P_2O_5, and reweighed to obtain the water content and dry weight. The dry protein was extracted several times with 2-ml portions of 0.1 *N* HNO_3 (see ref. 6), and sodium and chloride were determined in the combined acid extracts and in the medium.

When insoluble collagen is equilibrated with a 1000-fold excess of 0.15 *M* NaCl, it is reasonable to assume that Na^+ and Cl^- are the only mobile ions present in significant amount in the protein phase. Since the concentrations of both these ions in the collagen phase have been determined, the net charge, which is available for interaction with salt, can be obtained by application of the condition of electroneutrality. This may be represented as

$$(X^+) = (Cl^-)_c - (Na^+)_c,$$

where (X^+) is the net charge and $(Cl^-)c$ and $(Na^+)c$ are the concentrations of Na^+ and Cl^- ions in the protein phase. The magnitude of this available charge, (X^+), should be independent of the nature of the interaction of the salt with the macromolecules.

If the protein is compressed mechanically after equilibration with a salt solution, Na^+ and Cl^- ions will be removed in equivalent amount, and the net charge should remain unaltered. The results of such an experiment are presented in Table I. The protein was subjected to hydraulic forces while sandwiched between cotton cloths and cellulose tissues in order to remove entrained solution, and analyzed for the mobile ions remaining. The original water content of 54% decreased to 26% at an applied force of 6–10 tons. The chloride concentration in the collagen phase increased (Column 2) as solution was expressed by the force applied, illustrating that there was interaction of chloride with cationic groups of the protein. On the other hand, the sodium concentration in the collagen phase decreased significantly as solution was removed from the protein, suggesting that there was no interaction of sodium and protein ions. This is concluded because, if there were appreciable interaction, the concentration of Na^+ remaining in the collagen phase should increase as progressive removal of water increases the concentration of immobile charged anionic groups. The amount of sodium found in the collagen subjected to a force of 6 tons or greater was only 25 μmoles/g. Table I shows that the value of X, the content of available cationic sites, was remarkably constant under the experimental conditions. Since no interaction with Na^+ was detected, there appear to be negligibly few available free anionic sites compared to the number of available cationic sites in collagen.

* Steerhide collagen was generously supplied by Dr. F. O'FLAHERTY of the University of Cincinnati. It was prepared by extraction with 8% NaCl and dehydration with alcohol and acetone.

TABLE I

CONSTANCY OF AVAILABLE CHARGE OF COLLAGEN AS CONTENT OF ENTRAINED SOLUTION IS VARIED AFTER EQUILIBRATION WITH 0.15 *M* NaCl

Force tons	$(Cl^-)_c$* *mM*	$(Na^+)_c$* *mM*	*Available charge(X)*,** μmoles/g dry wt.*
0	201 ± 2	100 ± 3	118 ± 3
0.5	248 ± 3	96 ± 9	104 ± 7
1	270 ± 3	80 ± 7	107 ± 2
2	301 ± 7	73 ± 6	106 ± 1
4	331 ± 6	70 ± 8	105 ± 2
6	367 ± 5	70 ± 5	109 ± 4
8	373 ± 6	68 ± 1	109 ± 1
10	364 ± 6	67 ± 1	108 ± 3
		Average	108 ± 3

* Mean values for triplicate samples ± average deviation.
** Available charge on collagen obtained from

$$X = ((Cl^-)_e - (Na^+)_e)\ (\text{G entrained } H_2O)/\text{dry wt.}$$

The influence of the concentration of NaCl on the nature of the collagen-NaCl interaction was also studied. The media had final pH 5.0–6.0, and the data are summarized in Table II. It is evident that there was always a marked excess of chloride and deficit of sodium in the collagen phase compared to the medium phase. Again, this is the expected result if the protein has a net positive charge. The magnitude of the charge shown in column 4 agreed well with the values obtained in 0.15 *M* NaCl except when the NaCl concentration was less than 19.5 mM. The last column in Table II gives the excess of chloride associated with the collagen over and above that predicted by the Donnan relationship. This excess decreased with increasing concentrations of NaCl in the medium. No adequate explanation for this can be offered at the present time. However, it does not seem to be an osmotic effect on the

TABLE II

DISTRIBUTION OF NaCl BETWEEN COLLAGEN AND MEDIUM

Concentration (mM)			*μmoles/g dry weight**	
Medium phase $(Na^+)_m$, $(Cl^-)_m$	*Collagen phase* $(Cl^-)_c$	*Collagen phase* $(Na^+)_c$	*Net charge(X)***	*Excess chloride(E)****
2.5	51	0.6	79 ± 1.3	65 ± 4.8
9.8	64	3.1	94 ± 3.0	51 ± 4.1
19.5	79	7.7	107 ± 2.4	43 ± 3.4
49	102	29	106 ± 1.3	27 ± 2.7
97	147	67	110 ± 2.0	10 ± 5.4

* Mean values for 6 samples ± standard deviation.
** Method of computation of X given in notes under Table I.
*** Excess of chloride in collagen phase obtained from

$$E = ((Cl^-)_c^{\text{Observed}} - (Cl^-)_c^{\text{Donnan}})\ (\text{G entrained } H_2O)/\text{dry weight}$$

where

$$(Cl^-)_c^{\text{Donnan}} = (Na^+)_m\ (Cl^-)_m/(Na^+)_c.$$

collagen structure since the water content of the protein decreased only from 61 % in 2.5 m*M* NaCl to 58 % in 97 m*M* NaCl. To summarize, the data in Table II show that, even at salt concentrations in which the system deviated appreciably from an ideal Donnan distribution, the net charge on the collagen remained constant at the level found in 0.15 *M* NaCl, where a simple Donnan relationship prevailed.

These results may be considered in connection with the amino acid composition of native bovine collagen. The excess of basic over acidic groups is about 120 μmoles/g[7,8]. It may be noted that the available charge found in this study is close to this value. Therefore, the data suggest that only the excess basic residues in collagen are available for interaction with NaCl. Since the sum of the free basic and acidic protein groups is about 1700 μmoles/g[7], it is concluded that approximately 95 % of the ionic sites in steerhide collagen most probably exist in some sort of internal compensation.

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Dialysis of imidazole compounds from frog-muscle suspensions*

It has been suggested that in striated muscle, carnosine forms part of a more complex, biochemically active compound[1]. This hypothesis was prompted by the failure of many workers to recognize a function attributable to carnosine[1-3] and it has been supported by reports that carnosine phosphates exhibit pronounced effects *in vitro*[4,5]. However, no carnosine-containing compound has been isolated from muscle extracts, whereas free carnosine has been obtained repeatedly from the muscle of several species. The isolation methods have required a substantial period of time and, in many cases, considerable chemical manipulations. Thus, it remains possible that complex carnosine compounds have been decomposed during experimental manipulation.

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