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.

This work was supported by research grant (B-103) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, U.S. Public Health Service.

Howe Laboratory of Ophthalmology, Harvard University Medical School, Massachusetts Eye and Ear Infirmary, Boston, Mass. (U.S.A.) H. L. KERN

1 R. S. BEAR, *Advances in Protein Chem.*, 7 (1952) 69.
2 J. H. BOWES AND R. H. KENTEN, *Biochem. J.*, 43 (1948) 358.
3 K. H. GUSTAVSON, *The Chemistry and Reactivity of Collagen*, Academic Press, New York, 1956,
4 A. VEIS, J. ANESEY AND J. COHEN, in G. STAINSBY, *Recent Advances in Gelatin and Glue Research*. Pergamon Press, New York, London, Los Angeles, Paris, 1958, p. 155.
5 D. M. G. ARMSTRONG, in G. STAINSBY, *Recent Advances in Gelatin and Glue Research*, Pergamon Press, Oxford, 1958, p. 262.
6 H. L. KERN, *A.M.A. Arch. Ophthalmol.*, 52 (1954) 131.
7 J. E. EASTOE, A. A. LEACH, in G. STAINSBY, *Recent Advances in Gelatin and Glue Research*, Pergamon Press, New York, London, Los Angeles, Paris, 1958, p. 173.
8 J. H. BOWES, R. G. ELLIOTT and J. A. MOSS, *Biochem. J.*, 61 (1955) 143.

Received March 30th, 1960

Biochim. Biophys. Acta, 42 (1960) 345–348

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.

* From a dissertation submitted to the Graduate School of Arts and Sciences of the University of Buffalo in partial fulfillment of the requirements for the Ph. D. degree.

This report describes a study of the diffusion of the imidazole-containing compounds of muscle through cellophane membranes. Frog muscle was used for several reasons. Frog muscle-nerve preparations are known to function over prolonged periods at room temperature, and homogenates of frog muscle carry on oxidative reactions for several hours. The muscle contains high levels of carnosine so that a small amount of suspension provides sufficient compound for study. The muscle can be worked up rapidly and frogs respond satisfactorily to drugs which are known to affect the state of striated muscle. Most importantly, cold-blooded animals can be chilled before sacrifice, reducing post-mortem changes to a minimum.

Dialyses were conducted with an apparatus similar to that of CRAIG *et al.*[6] but modified as described elsewhere[7]. With the CRAIG apparatus, a small volume of sample was dialyzed through Visking dialysis tubing with large surface area and an average diffusion distance of about 1 mm. Duplicate dialyses with different commercial cellophane membranes were conducted on each sample.

Rana pipiens, females, weighing about 70 g were used. Group 1 consisted of four normal animals. Group 2 was made up of three frogs anesthetized with 4 or 8 ml of 25 % $MgSO_4$ injected intraperitoneally 10 min before sacrifice. The muscle from these animals lacked tone and was noticeably soft. Group 3 contained three frogs injected subcutaneously with 2 units of curare (intocostrin, Squibb) in 0.1 ml water. The animals were paralyzed in 20–30 min at which time they were killed. Group 4 consisted of three frogs which were injected intraperitoneally with 1 ml 2 % NaF 20 min before they were sacrificed. At that time the frogs were sluggish but showed no muscular activity or other gross effects of the treatment.

The frogs were brought to 3° at least 15 min before being beheaded, and the muscles from both thighs were pooled for immediate use. The tissue was minced and homogenized with an equal weight of isotonic KCl. The suspension was strained through muslin and a portion was introduced into the sample chamber. All work was conducted in the cold room with equipment pre-cooled to 3°. The dialyses were started 15–20 min after beheading of the animals.

In preliminary experiments, it was found that the relative rate of dialysis of pure carnosine in solution was independent of moderate changes in the volume of sample, the concentration of carnosine, pH (between 6.6 and 7.3), the ionic strength of the bath, and the presence of human serum albumin. Increasing the temperature from 3° to 25° caused a 5-fold increase in the rate of dialysis. Although the rate was not defined by a simple exponential equation, deviation from the theoretical pattern was reproducible, appearing to be a function of the apparatus. Other tests demonstrated that all of the Pauly-positive imidazole compounds of the muscle were free to diffuse into the dialysate.

There is no significant difference between any of the rates for suspensions and those for pure carnosine (Table I). This is especially significant in these studies because of the limited possibility for degradation of the muscle compounds. Approx. 60 % of the "carnosine" diffused from the muscle proteins during 20 min of dialysis, *i.e.* within 40 min of the beheading of the animal.

The data do not support the suggestion that muscle carnosine exists as part of a larger molecule. The results do not exclude this hypothesis, but do impose limitations upon the nature of such a complex compound should it exist. There are three situations in which our data would be consistent with the existence of such a compound.

TABLE I

RATE OF DIALYSIS OF CARNOSINE AND FRESH FROG MUSCLE IMIDAZOLE COMPOUNDS

Source fo sample	*Average amount (as %) of dialyzable imidazole remaining in dialysis bag after*			
	3 min	*8 min*	*20 min*	*80 min*
Carnosine	75	55	37	17
Normal frogs	78	57	38	19
$MgSO_4$-treated frogs	74	54	36	15
Curare-treated frogs	75	53	35	17
NaF-treated frogs	77	56	38	17

1. The presence of a "Pauly positive" compound behaving differently from carnosine might not have been detected if its concentration represented less than 10% of the total diffusible muscle imidazole.

2. A carnosine compound which decomposed within 20 min at physiological pH and at 3°, *i.e.* before the dialysis was started, would not be detected. Attempts to modify the muscle to prevent such decomposition, if it occurred, were not successful. One type of easily hydrolyzable compound meeting this criterion would be the reversible binding product of metal ions and carnosine. In other experiments[7], we have compared the titration curves of carnosine in the presence of several metal ions. No evidence was obtained for a significant degree of chelation between carnosine and Ca^{++}, Mg^{++}, Li^+, K^+ or Rb^+.

3. A complex compound with a diffusion rate the same as that of carnosine would not have been detected in the dialysis experiments. CRAIG AND KING[8] have demonstrated that, in their system, molecular size is the chief factor governing dialysis rates. Thus, the hypothetical compound would have to be of the same general size as carnosine (mol. wt. 226). Further, it would have to be labile to account for the ready isolation of carnosine from muscle.

Our studies are of further interest in view of a report by EGGLETON AND EGGLETON[9], who obtained data suggesting that carnosine cannot move through the membrane of intact muscle cells. In contrast to carnosine, histidine was freely diffusible, even though its size and the p*K*'s of its functional groups are similar to those of carnosine. Since carnosine diffuses rapidly through cellophane membranes, its failure to pass through muscle membranes cannot be due to spontaneous formation of non-diffusible, polymolecular aggregates. Further studies of cell permeability to imidazoles are needed to clarify the status of carnosine in living muscle.

Department of Biochemistry, School of Medicine, University of Buffalo, Buffalo 14, N.Y. (U.S.A.)

FRED G. BOCK*
WILSON D. LANGLEY

1 V. DU VIGNEAUD AND O. BEHRENS, *Ergeb. Physiol. biol. Chem. u. exptl. Pharmakol.*, 41 (1939) 917.
2 D. C. SUTFIN AND H. M. HINES, *Am. J. Physiol.*, 186 (1956) 286.
3 C. J. PARKER, JR. AND W. D. LANGLEY, *Biochim. Biophys. Acta*, 37 (1960) 537.
4 S. E. SEVERIN, E. F. GEORGIEVSKAYA AND V. I. IVANOV, *Biokhimiya*, 12 (1947) 35.

* Present address, Biological Station, Roswell Park Memorial Institute, Springville, New York (U.S.A.).

[5] M. C. GOODALL, *Nature*, 178 (1956) 539.
[6] L. C. CRAIG, T. P. KING AND A. STRACHER, *J. Am. Chem. Soc.*, 79 (1957) 3729.
[7] F. G. BOCK, *Univ. Microfilms*, L. C. Card No. Mic 58-2889 (1958).
[8] L. C. CRAIG AND T. P. KING, *J. Am. Chem. Soc.*, 77 (1955) 6620.
[9] M. G. EGGLETON AND P. EGGLETON, *Quart. J. Exptl. Physiol.*, 23 (1933) 391.

Received December 16th, 1959

Biochim. Biophys. Acta, 42 (1960) 348–351

The uptake of [^{3}H]uridine in normal and filamentous forms of *Escherichia coli* infected with T-2 bacteriophage

HERSHEY[1] and VOLKIN AND ASTRACHAN[2] have shown that although net RNA synthesis almost comes to a complete halt during T-2 phage infection, a small amount of ^{32}P becomes incorporated into RNA nucleotides, indicating the occurrence of some RNA turnover. In the course of investigating sites of RNA synthesis in *E. coli* the existence of a concurrent uptake of uridine was established. Some features of this uptake will be described here.

We first considered the uptake of [^{3}H]uridine (obtained from New England Nuclear Corp.) by a culture of *E. coli* B in minimal A-1 medium[3], aerated at 37°, and infected with approximately 20 T-2 bacteriophages per cell. [^{3}H]uridine, at a concentration of 50 μC/ml (specific activity, 680 mC/mmole) was added to the culture 3 min after infection. The final cell concentration was $3.5 \cdot 10^7$ cells/ml. Colony counts following 10^4 dilution of the aliquots of this culture revealed that at this point less than 1 % of the cells were non-infected. Samples (5 μl in volume) from the culture were taken at regular intervals and placed on stainless-steel planchets in a 10 % aq. solution of formalin containing a high concentration of unlabeled uridine to reduce adsorption of label to the metal surface. The cells were fixed for 10 min, dried, fixed again for 10 min in Carnoy solution, transferred to distilled water through graded alcohols, and dried again. Counts were made in a windowless flow Geiger counter. A correction was made for background due to adsorption of label to the planchet and other causes.

Fig. 1 shows that the uridine content of the cells rises at a linear rate for about 10 min, then reaches a plateau for the next 20 min. The initial rate of uptake is much higher than that expected for the non-infected cells present at that time. It must be concluded therefore that this uptake takes place in the infected cells. It has been shown that in normal cells 90 to 95 % of the incorporated [^{3}H]uridine appears as RNA uridine and cytidine, the rest appearing as DNA deoxycytidine[4]. Treatment with RNAase of the phage-infected cells removed approx. 90 % of the label. It is, therefore, inferred that [^{3}H]uridine uptake is an indication of RNA synthesis or turnover. One difference should be noted between our results and those of VOLKIN AND ASTRACHAN[2]. They found that in minimal medium phosphorus turnover in RNA continues throughout the entire latent period, while in our experiment the

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.