cells were resorbed. Enlarged blood vessels remained after the other modified structures were no longer present in the pocket wall.

1. Campbell, J. A., *Physiol. Rev.*, 1931, v11, 1.
2. Wright, A. W., *Am. J. Path.*, 1930, v6, 87.
3. Pfalz, J. A., *Frank. Z. fur Pathol.*, 1958, v69, 53.
4. Rahn, H., Canfield, R. E., *Wright Air Development Command, Tech. Report*, 1955, No. 55357, 395.
5. Selye, H., *Arch. Int. Pharcodyn.*, 1954, v97, 379.
6. ———, *Rev. Canad. de Biol.*, 1957, v15, 328.
7. ———, *J. Nat. Cancer Inst.*, 1958, v20, 581.
8. Van Liew, H. D., in press.
9. Henderson, Y., Henderson, M. C., *Arch. Int. Med.*, 1932, v49, 88.
10. Rahn, H., *Fed. Proc.*, 1957, v16, 685.

Received September 14, 1961. P.S.E.B.M., 1962, v109.

Lobster Shell and Hemolymph Composition Under Normal and Acidotic Conditions.* (27124)

DORA K. HAYES,† LEON SINGER AND W. D. ARMSTRONG
Department of Physiological Chemistry, Medical School, University of Minnesota, Minneapolis

The calcified portion of the shell of the American lobster, *Homarus americanus*, contains primarily calcium carbonate, with smaller amounts of sodium, potassium, phosphate and other ions present. The mammalian skeleton participates in homeostatic regulation of body-fluid ionic concentration but the effects of disturbances in acid-base balance upon the shell mineral are not clear. Collip(1) and Tilgner-Peter and Tilgner(2) suggested that the carbonate of the exoskeletons of molluscs and intermolt crustaceans could serve as a source of buffer during such disturbances. In the present study, the carbon dioxide tension of the artificial sea water environment of the lobster was increased. This permitted a study of the effects of alterations of acid-base balance on the composition of the shell and hemolymph.

Methods. Live male lobsters weighing 400-550 g, obtained from a local market, were allowed to equilibrate at least 24 hours at 8-10°C in an aerated bathing solution composed of 3.4% commercial sea salt in distilled water. The animals were not fed during the experiments.

The concentrations of several components of the hemolymph and shell of the control animals were determined. Citrate was estimated by a modification of the method of Carlsson and Hollunger(3), and the procedure of color formation developed by Taussky and Shorr(4) was employed. A step was introduced in which the heptane extract, containing pentabromoacetone obtained by oxidation of citric acid, was washed with saturated sodium carbonate solution. Hemolymph chloride was estimated as described by Schales and Schales(5). The method of Caster, MacDonald and Armstrong(6) was employed for determination of shell chloride.

Weighed portions of hemolymph and dry shell were ashed at 450-500°C and the ash was dissolved in a known volume of dilute hydrochloric acid. The sodium and potassium contents of aliquots of these ash solutions were determined, using a Coleman flame photometer. Calcium content of the ash solutions was determined as described by Clark and Collip(7). The method of Fiske and Subbarow(8) was used to estimate phosphorus. Analysis of ash solutions indicated the quantity of total phosphate, while a trichloroacetic acid filtrate was employed in determination of inorganic hemolymph phosphate.

The pH of the hemolymph in the pericardial sinus and the arthrodial joints was determined *in vivo* with a pointed quinhydrone electrode (Beckman electrode 1190-12). To estimate total hemolymph CO_2 concentration, a sample of this fluid was with-

* Supported by grant from U.S. Public Health Service.
† Trainee supported by Biochemistry Training Grant, U. S. Public Health Service.

TABLE I. Composition of Hemolymph and Shell of Control Lobsters.

Ion	Hemolymph,* meq/l ± S.E.	Dry shell,† meq/g carapace ± S.E.	meq/g claw ± S.E.	P‡ diff. claw and carapace
Na^+	438 ±10.1 (8)†	.25 ± .0084 (12)	.28 ± .013	<.01
K^+	8.5 ± .68 (8)	.014 ± .0011 (12)	.021 ± .0016	<.01
Ca^{++}	34.5 ± .87 (8)	12.6 ± .139 (10)	11.9 ± .12	<.05
Total	481	12.9	12.2	
Cl^-	475 ± 7.4 (8)	.107 ± .0061 (6)	.163 ± .0053	<.01
PO_4§	.55 ± .069 (8)	1.29 ± .070 (14)	1.99 ± .214	<.02
HCO_3^-	10.8 ± 1.79 (6)*			
$CO_3^=$		12.7 ± .46 (4)	11.1 ± .76	<.2
Citrate$^=$	.16 ± .022 (8)	.0215± .0052 (10)	.012 ± .0019	<.05
Total	487	14.1	13.3	

* Hemolymph analyses, with exception of HCO_3^-, from same group of animals. Fourteen lobsters served as sources of shell, but all analyses were not completed on every shell sample. Carapace and claw from the same animal were always analyzed.

† Numbers in parentheses indicate No. of animals serving as source of tissue.

‡ The probability that differences were significant was evaluated by means of the t-test. The carapace and claw of each animal were considered to be one pair of variates.

§ Phosphate is expressed as meq $PO_4^{-1.8}$/l in hemolymph and as meq PO_4^{-3}/g in shell.

drawn from the arthrodial joint with a syringe coated with mineral oil immediately after the pH had been measured. Total CO_2 content of this sample was determined at once. When subsequent samples of hemolymph were to be obtained within a 3 hour period, a fresh arthrodial joint was punctured. The micromethod of West, *et al.*(9), was used to determine total CO_2 in both hemolymph and shells.

When gas mixtures containing carbon dioxide were administered, an animal was placed in a 5-liter portion of the artificial sea water in which it had been maintained. Either 10% CO_2-90% air or 5% CO_2-95% O_2 was bubbled into the sea water at low pressure while temperature was maintained at 10°C. Periods of exposure varied from 20 to 180 minutes. Hemolymph pH was determined before and after exposure to the gas mixtures. The composition of the hemolymph of 3 animals before and after administration of 5% CO_2-95% O_2 was examined and the composition of the shells was also determined. The effect of increased CO_2 tension on inorganic phosphate concentration in the hemolymph of an additional 2 lobsters was determined.

Results. The composition of the hemolymph and of the shell of the control animals is summarized in Table I. Values reported for inorganic ions in the hemolymph are similar to those tabulated by Florkin and Mason (10). The carapace and claw shell are similar in composition. When the data are evaluated statistically the dry claw, except for calcium and carbonate, contains a significantly greater concentration of inorganic ions. Citrate is present in greater concentration in the carapace, however.

The pH of the hemolymph of control animals, maintained under laboratory conditions, is somewhat variable. Six measurements of arthrodial joint pH in 4 animals ranged from 7.30-7.65. In the same animals 6 measurements of pericardial sinus pH varied from 7.10 to 7.60. The pH of the sea water was 7.30-7.50. The data in Table II

TABLE II. Effect of 10% CO_2-90% Air on Hemolymph pH of Lobsters.

Lobster treatment	Bathing solution	Hemolymph: 5th arthrodial joint	Hemolymph: Pericardial sinus
1* Before CO_2-air	7.50	7.55	7.45
CO_2-air; 20 min.	6.30	7.30	7.20
4 days later	7.95	7.55	7.50
CO_2-air; 50 min.	6.60	6.55	6.75
2† Before CO_2-air	7.55	7.10	7.40
CO_2-air; 60 min.	6.00	6.50	6.60
CO_2-air; 180 "	6.00	6.40‡	6.30

* Animal died 12 hr after 2nd exposure to CO_2-air.

† Animal died 10 min. after gas bubbling discontinued.

‡ From 4th arthrodial joint.

TABLE III. Effects of 5% CO_2-95% O_2 on Hemolymph of Lobsters.

Hemolymph component	Lobster 10		Lobster 13		Lobster 14	
	Before*	After 100 min.*	Before	After 140 min.	Before	After 180 min.
Cation, meq/l						
Na^+	402	417	400	390	376	406
K^+	7.8	7.8	9.7	10.7	8.4	7.9
Ca^{++}	41.3	34.6	32.5	31.8	32.8	35.3
Total	451.1	459.4	442.2	432.5	417.2	449.2
Anion, meq/l						
Cl^-	447	449	430	405	430	450
PO_4†	.85	1.02	.68	1.76	.51	1.23
HCO_3^-	13.2	13.1	6.1	16.5	13.9	28.3
Citrate	.38	.0	.08	.09	.18	.41
Total	461.4	463.1	436.9	423.4	444.6	479.9
pH, 5th joint	7.25	6.70	7.30	6.90	7.05	6.90
pH, bathing sol.	7.10	6.10	7.50	6.50	7.25	6.65
H_2CO_3, mM/l	1.0	3.5	.4	2.8	2.1	4.7
pCO_2, mm Hg	17	57	7	46	34	78

* Immediately before and after exposure to CO_2-O_2 gas mixture.
† Valence calculated from pH of hemolymph and pK of $H_2PO_4^-$.

indicate that a 10% CO_2-90% air mixture, administered for 60 minutes, results in a decrease of 0.6-1.0 unit in hemolymph pH. This decrease appears to be close to the maximum possible, since an additional 120 minutes of exposure resulted in a further decrease of only 0.1 pH unit (animal 2). This gas mixture was toxic, since after one to 3 hours of exposure, the animals did not recover.

The effects of 5% CO_2-95% O_2 upon the electrolyte composition of the hemolymph of lobsters are found in Table III and Fig. 1. HCO_3^- and H_2CO_3 concentrations were calculated from total CO_2 content and pH of the hemolymph by mathematical operations similar to those of Hastings and Sendroy(11). The pK(12) of carbonic acid at 10°C and infinite dilution was extrapolated to the pK′ at 10°C and an ionic strength of 0.51, the calculated ionic strength of lobster hemolymph. The value obtained, 6.12, was then substituted in the Henderson-Hasselbalch equation and the calculations were completed.

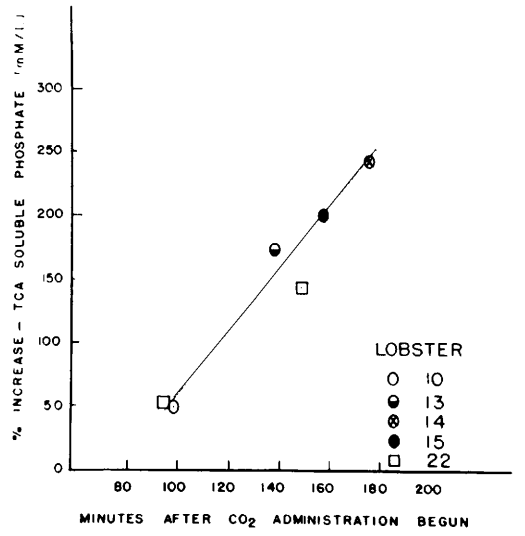

FIG. 1. Effect of administration of 95% CO_2-5% O_2 on trichloroacetic acid soluble phosphate concentration of hemolymph.

From the calculated carbonic acid concentration, the pCO_2 was calculated, according to the formula:

$$pCO_2 = (H_2CO_3) / a$$

a is a constant relating the dissolved CO_2 in mM/l to the partial pressure of this gas. At 10°C and an ionic strength of 0.51, a was estimated from the data given by Edsall and Wyman(13) to be 0.06 rather than the more familiar 0.03.

In Fig. 1, the percent increase in mMoles per liter of inorganic phosphate in the hemolymph during administration of 5% CO_2-95% O_2 is plotted as a function of time. These alterations occurred with no change or a slight decrease in total hemolymph phosphate.

Discussion. In agreement with the observations of Creac'h(14), little correlation was

found between calcium carbonate content and citrate content in the shells of crustaceans. The high hemolymph calcium concentration cannot be accounted for by sequestration by citrate owing to the low concentration of the latter in hemolymph.

Comparison of the composition of the shells of lobsters treated with 5% CO_2-95% O_2 with that of the shells of control animals (Table I) demonstrated that this treatment had no effect upon the sodium, potassium, calcium or phosphorus contents of the shell. These results indicate that the shell does not effectively participate over short periods of time in acid-base balance regulation. The failure of the hemolymph calcium to increase as pH decreases further suggests that the shell is not a source of readily available base. The changes observed in sodium and chloride concentrations in the hemolymph could be interpreted as gain or loss of hemolymph ions across the gill membrane.

There is no regular pattern apparent for compensation for respiratory acidosis. Lobster 13 appears to have compensated somewhat for the increase of 10 meq/l of bicarbonate ion by a loss of 23 meq/l of other anions and 12 meq/l cations, so that the anion-cation balance remains constant. Lobster 14, on the other hand, appears to have compensated for the increase of 14 meq/l in bicarbonate by an increase of 32 meq/l of cations and 21 meq/l of anions, with the balance between the two again remaining constant.

The increased hemolymph phosphate concentration could result from hydrolysis of phosphate esters in the hemolymph. Brown and Prasad(15) obtained similar results when CO_2 tension was increased in the blood of dogs.

Summary. The effects of increased CO_2 tension on the hemolymph and shell of fasting intermolt lobsters were examined. The citrate and calcium contents of the hemolymph were not increased. The pattern of distribution of ions observed in the dry shell of control lobsters was not altered by an increased environmental and body fluid CO_2 tension but a decrease in hemolymph pH occurred. After 100 minutes of exposure to CO_2, an increase in hemolymph inorganic phosphate was observed, and, after 140 minutes, an increase in hemolymph bicarbonate occurred. It can be concluded that, although exposure to an aqueous environment with an increased CO_2 tension results in changes in the concentration patterns of some hemolymph ions, little effect is produced on the calcified tissues of the lobster.

1. Collip, J. B., *J. Biol. Chem.,* 1921, v49, 297.

2. Tilgner-Peter, A., Tilgner, S., *Zool. Jahrb. Abt. Allgem. Zool. Physiol. Tiere,* 1958, v68, 369.

3. Carlsson, A., Hollunger, G., *Acta Physiol. Scand.,* 1954, v31, 317.

4. Taussky, H. H., Shorr, E., *J. Biol. Chem.,* 1947, v169, 103.

5. Schales, O., Schales, S. S., *ibid.,* 1941, v140, 879.

6. Caster, W. O., MacDonald, R., Armstrong, W. D., *J. Lab. Clin. Med.,* 1955, v46, 910.

7. Clark, E. P., Collip, J. B., *J. Biol. Chem.,* 1925, v63, 461.

8. Fiske, C. H., Subbarow, Y., *ibid.,* 1925, v66, 375.

9. West, E. S., Christensen, B. E., Rinehart, R. E., *ibid.,* 1940, v132, 681.

10. Florkin, M., Mason, H. S., *Comparative Biochemistry,* Vol. II, Academic Press, New York, 1960, p446.

11. Hastings, A. B., Sendroy, J., Jr., *J. Biol. Chem.,* 1925, v65, 445.

12. *Stability Constants, Part II,* Chemical Soc., (London) Special Publication No. 7, 1958, Table 23, pp42-44.

13. Edsall, J. T., Wyman, J., *Biophysical Chemistry,* Academic Press, New York, 1958, p556.

14. Creac'h, P. V., *Compt. Rend.,* 1953, v237, 944.

15. Brown, E. B., Jr., Prasad, A. S., *Am. J. Physiol.,* 1957, v190, 462.

Received September 18, 1961. P.S.E.B.M., 1962, v109.