Role of Citrate, Lactate, Pyruvate, and Fatty Acids in Albumin-Induced Bone Resorption[1] (36280)

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Certain serum albumins stimulate living fetal rat bone incubated *in vitro* to undergo resorption with marked loss of calcium and matrix (1). This phenomenon cannot be attributed to a higher calcium-binding capacity of the active albumins, nor do the active substances act indirectly by lowering the pH of the medium. One of the albumins which was inactive at a concentration of 1 mg/ml was a commercially available fraction V bovine serum albumin preparation which had been treated with activated charcoal. This albumin stimulated bone resorption prior to the charcoal treatment.

The tendency of albumins to bind a variety of substances is well known (2). It has been shown by Chen (3) and by Hanson and Ballard (4) that a number of these bound substances are removed by treating the albumin with activated charcoal. Conceivably, one of these substances could be producing or promoting the resorption. Although it would be impractical to test the effects of all substances bound to albumin, it seemed reasonable to try adding back substances shown to be removed by charcoal treatment. The bone-resorbing activities of citrate, lactate, pyruvate, and fatty acids were therefore tested.

Methods. Radius + ulna pairs from 19-day rat fetuses were incubated for 72 hr in a modified culture medium BGJ (5) containing 1 mg/ml "fatty acid-free" fraction V bovine serum albumin (lot 10, Pentex). Calcium lactate (Fisher), sodium citrate (Mallinckrodt), sodium pyruvate (Sigma), oleic acid (Sigma), palmitic acid (Sigma), and stearic acid (Sigma) were added directly to the culture medium. The fatty acids were first dissolved in absolute alcohol. All media were adjusted to pH 7.45 at the start of incubation. The bones had been prelabeled with ^{45}Ca by administration of 200 μCi of the isotope to the pregnant mother 2 days previously. Resorption was quantified in terms of release of ^{45}Ca from the cultured bones into the medium. This release is expressed as the percentage of bone ^{45}Ca released, calculated as follows:

$$\%\ \text{bone}\ ^{45}\text{Ca released} = \frac{\text{medium}\ ^{45}\text{Ca} \times 100}{\text{medium}\ ^{45}\text{Ca} + \text{bone}\ ^{45}\text{Ca}}.$$

Values are given as means ± standard errors. Albumins were obtained from Pentex. The fatty acid content of various albumins was determined by the method of Dole and Meinertz (6).

Results. The effects of lactate, citrate and pyruvate on ^{45}Ca release are shown in Table I. Sodium pyruvate, added to the culture medium at concentrations of 0.01, 0.1, or 1.0 m*M* did not significantly alter ^{45}Ca release. Calcium lactate was inactive at 0.01 and 0.1 m*M*. Sodium citrate had no significant effects at concentrations of 0.01 and 0.1 m*M*. A small increase in ^{45}Ca release was obtained with 1.0 m*M* citrate. However, the morphological appearance of the bones was not that of resorption. With 1.0 m*M* citrate, the entire bone appeared more translucent. Thus, this may be a calcium chelating effect.

Effects of the fatty acids are shown in Table II. None of the fatty acid salts altered ^{45}Ca release when present at a concentration of 0.1 m*M*. However, both oleate and stearate significantly enhanced ^{45}Ca release at 0.3 m*M*. The enhanced ^{45}Ca release was accompanied by histological resorption, which was

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most marked with stearate. A significant increase in ^{45}Ca release was seen when both 0.01 m*M* citrate and 0.1 m*M* stearate were added to the culture medium. However, the response was not greater than that with 0.1 m*M* stearate alone.

To determine whether there was a correlation between bone resorbing activity and fatty acid content, the fatty acid content of several of the albumins used previously was measured (Table III). It was noted that all of the commercial albumins examined had very low fatty acid content. Added oleate was effectively recovered, indicating that these low values were not due to loss during extraction. Activated charcoal treatment did simultaneously lower both the bone resorbing activity and the fatty acid content (from 0.0026 to 0.0012 m*M*) of one albumin (fraction V, lot 72). However, in another case (fraction V, lot 80), the fatty acid content was also markedly reduced by activated charcoal treatment, yet the bone-resorbing activity was only slightly diminished. A relatively inactive albumin (crystalline bovine serum, lot 16) had a fatty acid content of 0.0134 m*M*, whereas much more active albumins (fraction V, lot 66; fraction V, lot 12) had fatty acid contents of 0.0019 and 0.0012 m*M*, respectively. Finally, without exception, the fatty acid concentrations of the active albumins were much lower than the amounts required to produce resorption.

TABLE I. Effects of Pyruvate, Lactate, and Citrate on ^{45}Ca Release from Fetal Rat Bone *in Vitro*.[a]

Expt. no.	Conditions (m*M*)	*N*	^{45}Ca released (%)	*p*
145	No additions	5	16.4 ± 1.0	
	Pyruvate, 0.01	5	16.8 ± 0.6	ns
	Lactate, 0.01	5	17.2 ± 0.8	ns
	Citrate, 0.01	5	20.0 ± 1.5	ns
146	No additions	4	15.3 ± 0.8	
	Pyruvate, 0.01	4	16.5 ± 2.0	ns
	Lactate, 0.01	4	15.7 ± 1.0	ns
	Citrate, 0.01	4	16.8 ± 2.2	ns
140	No additions	4	19.3 ± 1.0	
	Lactate, 0.1	4	18.5 ± 0.9	ns
	Citrate, 0.1	4	19.6 ± 1.7	ns
144	No additions	5	13.5 ± 0.9	
	Pyruvate, 0.1	5	12.6 ± 0.6	ns
	Pyruvate, 1.0	5	14.5 ± 0.8	ns
	Citrate, 1.0	5	16.5 ± 0.2	<.05

[a] Prelabeled bones, incubated 72 hr; values given as means ± standard errors; *p* values based upon comparison with ^{45}Ca released (%) with no additions to culture medium.

Discussion. Hanson and Ballard (2), in assaying various lots of albumin, found concentrations of citrate as high as 3380 μmoles/mmole of albumin; lactate, 746 μmoles/

TABLE II. Effects of Fatty Acids on ^{45}Ca Release from Fetal Rat Bone *in Vitro*.[a]

Expt. no.	Conditions	*N*	^{45}Ca released (%)	*p*
147	0.1% ethanol	4	21.4 ± 0.6	
	0.1% ethanol + 0.1 m*M* oleate	4	21.1 ± 1.8	ns
	0.1 m*M* palmitate	4	20.7 ± 0.6	ns
	0.1 m*M* stearate	4	20.1 ± 1.7	ns
	0.3% ethanol	4	19.3 ± 0.7	
	0.3% ethanol + 0.3 m*M* oleate	4	25.9 ± 1.6	<.05
	0.3 m*M* palmitate	4	22.0 ± 1.8	ns
	0.3 m*M* stearate	4	27.3 ± 2.0	<.05
151	0.1% ethanol	4	15.6 ± 0.5	
	0.1% ethanol + 0.1 m*M* stearate	4	18.2 ± 0.9	ns
	0.01 m*M* citrate	4	16.2 ± 0.7	ns
	0.1 m*M* stearate + 0.01 m*M* citrate	4	19.4 ± 0.9	<.05[b]

[a] Prelabeled bones, incubated 72 hr; values given as means ± standard errors; *p* values based upon comparison with % ^{45}Ca released in the absence of fatty acid.

[b] ^{45}Ca released (%) not significantly greater than with 0.1 m*M* stearate alone.

TABLE III. Comparison of Bone Resorbing Activity and Fatty Acid Content of Several Albumins.

Albumin	Fatty acid conc (m*M*) in a 1 mg/ml albumin solution[a]	Expt. no.	^{45}Ca released (%) from: Control bones[b]	^{45}Ca released (%) from: Albumin-treated bones[c]
		150	20.6 ± 1.5 (5)	
Fraction V bovine serum albumin, lot 72	0.0026			44.6 ± 5.7 (5)
Fraction V, lot 10 (lot 72, after activated charcoal treatment)	0.0012			23.1 ± 3.1 (5)
lot 10 + 0.14 m*M* oleate	0.16			
		139	14.6 ± 0.7 (4)	
Fraction V, lot 80, before activated charcoal treatment	0.0026			36.5 ± 8.9 (4)
after activated charcoal treatment	0.0014			33.0 ± 6.9 (4)
		139	14.6 ± 0.7 (4)	
Crystalline bovine serum albumin, lot 16	0.0134			17.8 ± 1.5 (4)
		137	20.8 ± 4.0 (5)	
Fraction V, lot 66	0.0019			32.0 ± 5.0 (5)
		155	18.2 ± 2.7 (5)	
Fraction V, lot 12 (acetic acid–isooctane extracted)	0.0012			33.5 ± 2.8 (5)

[a] Fatty acids were assayed in a 50 mg/ml albumin solution.
[b] Control bones were incubated without albumin.
[c] An albumin concentration of 4 mg/ml of medium was used in these studies.

mmole; and pyruvate, 43 μmoles/mmole. Fatty acid concentrations of 1–3 mmoles/mmole of albumin have been reported by the above authors and others (3, 7). Conceivably, these substances could be involved in the bone resorption produced by albumin. Citrate and lactate were intriguing possibilities since they had been implicated in bone resorption previously (8–10).

Active albumins produce significant bone resorption at a concentration of 1 mg/ml (1). Assuming a molecular weight of 69,000, the albumin concentration in the culture medium would be 0.014 m*M*. Our results indicate that lactate, pyruvate, and citrate could not have caused the albumin-induced bone resorption even if the albumins had contained the amounts of these substances found by Hanson and Ballard. At higher concentrations, citrate did increase ^{45}Ca loss, but probably by a chelating effect rather than by stimulating resorption. Although the fatty acids stimulated ^{45}Ca release and bone resorption, the concentrations of added fatty acid required for the effect were greater than would be expected in albumin. This factor and the poor correlation between fatty acid content and bone resorbing activity makes it unlikely that the fatty acids are the sole factors responsible for the bone-resorbing activity of the albumins.

The bone resorbing activity of the fatty acids is interesting in view of the demonstrated effects of prostaglandins on bone *in vitro* (11). However, oleic and stearic acids are much less potent than the prostaglandins, some of which were effective at 10^{-6}–10^{-8} *M*. Fatty acids complexed with albumin produced bone resorption at lower concentrations than the added fatty acids, but, even under these conditions, the minimal effective concentration was 0.08 m*M* (12).

Summary. Several substances known to be bound to serum albumin were tested for bone resorbing activity. Citrate, lactate, and pyruvate were ineffective in the concentration

range present in albumin. Fatty acids stimulated bone resorption. However, the fatty acid content of several albumins did not correlate well with the bone resorbing activity of these same proteins.

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1. Stern, P. H., Calcif. Tissue Res. **7**, 67 (1971).

2. Bennhold, H., *in* "Protides of the Biological Fluids" (H. Peeters, ed.), p. 58. Elsevier, New York (1961).

3. Chen, R. F., J. Biol. Chem. **242**, 173 (1967).

4. Hanson, R. W., and Ballard, F. J., J. Lipid Res. **9**, 667 (1968).

5. Stern, P. H., J. Pharmacol. Exp. Ther. **168**, 211 (1969).

6. Dole, V. P., and Meinertz, H., J. Biol. Chem. **235**, 2595 (1960).

7. Goodman, D. S., Science **125**, 1296 (1957).

8. Borle, A. B., Nichols, N., and Nichols, G., Jr., J. Biol. Chem. **235**, 1211 (1960).

9. Neuman, W. F., Firschein, H., Chen, P. S., Jr., Mulryan, B. J., and DiStefano, V., J. Amer. Chem. Soc. **78**, 3863 (1958).

10. Martin, G. R., Mecca, C. E., Schiffman, E., and Goldhaber, P., *in* "The Parathyroid Glands" (P. J. Gaillard, R. V. Talmage, and A. M. Budy, eds.), p. 261. Univ. of Chicago Press, Chicago (1965).

11. Klevin, D. C., and Raisz, L. G., Endocrinology **86**, 1436 (1970).

12. Stern, P. H., Experientia **27**, 1061 (1971).

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