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Incorporation *in vivo* of P^{32} from Condensed Phosphates. (24520)

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The reported symptomatic benefit obtained in treatment of tumor metastasis to bone with P^{32} phosphate(1,2,3) has suggested the use of other forms of phosphate. In particular, condensed phosphates such as trimetaphosphate and polymetaphosphate (previously misnamed hexametaphosphate) appeared worthy of investigation since they have been hydrolyzed by mammalian tissues(4,5). Additional favorable features of these substances are their strong binding to proteins(6,7), and relatively high molecular weights(8). Both characteristics would tend to increase body retention, thus prolonging their action. This, in addition to ease with which these condensed phosphates can be prepared from other phosphates, has prompted the present study of localization of P^{32} from these higher forms of phosphates.

Methods. Radioactive trimetaphosphate was prepared in platinum crucibles from $NaH_2P^{32}O_4 \cdot H_2O$ by the method of Jones(9). Larger quantities of a non-radioactive preparation were obtained simultaneously for analytical purposes. The final preparation was an amber-colored clear glass, not precipitable in solution with $AgNO_3$ indicating an absence of phosphate. The pH of 1% solutions was 5.5. Phosphorus content was 28%. Polymetaphosphate also was prepared from $NaH_2P^{32}O_4 \cdot H_2O$ by the method of Jones(9). This material was a colorless transparent glass which dissolved slowly in water. In solution, it did not precipitate with $AgNO_3$. One % solutions had a pH of 5.8. Phosphorus content was 26%. Both substances probably do not represent pure compounds but contain small amounts of other condensation products. According to Jones(9), 92-95% of pure metaphosphates may be prepared by these procedures. Injections of $NaH_2P^{32}O_4$, radioactive trimetaphosphate or polymetaphosphate, were given intraperitoneally to 25 g male mice for 4 successive days, and the mice were sacrificed 24 hours after last injection. When larger bone specimens were required, 5 kg male rabbits were used. No toxic reactions or discomfort were observable. Preliminary experiments established intravenous and intraperitoneal routes as being equivalent and markedly superior to oral administration. That condensed phosphates are poorly absorbed by the intestine has been noted by other workers(10). Amount of radioactivity given was approximately 3×10^5 counts/minute for the 3 substances. Depending upon the experiment, representative samples of pooled organs of 2-5 animals were obtained, wet-ashed with HNO_3 and H_2O_2 and aliquots plated on stainless steel planchets. Radioactivity was counted with an end window counter coupled with an automatic sample changer attachment. All counts were corrected for decay. No correction for self absorption was necessary for the mass of sample taken. Standard deviation for the counts measured is $\pm$ 2 cts/min. To evaluate biological retention of these substances, radioactivity of approximately 4×10^5

counts/minute was given in single intravenous injection. Specific activities of both metaphosphates were about 40 μc/mg. $NaH_2P^{32}O_4$ was essentially carrier free. These may be considered equivalent in view of the tracer dose actually given. Two animals from each group were sacrificed at 24, 72 and 120 hours. Representative organs were pooled, ashed, and radioactivity assays performed as above. Localization of P^{32} in soft tissue such as liver was evaluated on the subcellular level by using ultracentrifugal fractionation of Schneider and Hogeboom(11).

Results. Table I shows results of a 4-day injection experiment using radioactive sodium trimetaphosphate, sodium polymetaphosphate and $NaH_2P^{32}O_4$. It demonstrates active concentration of P^{32} from the 3 substances in bone. The *in vitro* experiments(4,5) would suggest that the metaphosphates are rapidly hydrolyzed by tissues with high phosphatase activity, and that actual localization is the same in all cases, mainly as phosphate. For concentration in bone, both metaphosphates appear superior to $NaH_2P^{32}O_4$ when multiple injections are given, the factors being 1.8 times $NaH_2P^{32}O_4$ for trimetaphosphate and 1.4 times for polymetaphosphate. In evaluating the ratio of P^{32} concentration in bone to average concentration in soft tissue, again the

TABLE I. Tissue Localization of P^{32}.

Organ	Counts/min./g	% total counts/min./g
	A. $NaH_2P^{32}O_4$	
Liver	8,221	4.1
Intestine	8,211	4.1
Kidney	7,255	3.6
Lung	5,849	2.9
Femur	25,697	12.8
Muscle	7,620	3.8
	B. Trimetaphosphate	
Liver	10,013	3.9
Intestine	10,773	4.2
Kidney	9,463	3.7
Lung	7,445	2.9
Femur	47,480	18.6
Muscle	9,844	3.9
	C. Polymetaphosphate	
Liver	4,665	2.3
Intestine	5,319	2.7
Kidney	4,309	2.2
Lung	3,431	1.7
Femur	37,043	18.6
Muscle	4,002	2.0

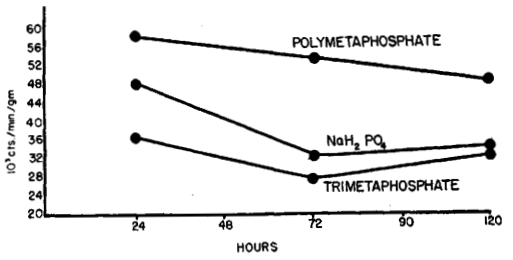

FIG. 1. Localization of P^{32} from different phosphates in bone.

metaphosphates are superior. $NaH_2P^{32}O_4$ averages 3.5, trimetaphosphate averages 5 and polymetaphosphate 8.2 times soft tissue concentration. Duplicate experiment yielded essentially the same result.

In a single injection, differential concentration of P^{32} by bone becomes more marked. After 120 hours, for $NaH_2P^{32}O_4$ it is 12, for trimetaphosphate 9.6 and polymetaphosphate 15. From rate of disappearance curve (Fig. 1), it appears that polymetaphosphate is retained longer than either metaphosphate or $NaH_2P^{32}O_4$. This is compatible with the high molecular weight of polymetaphosphate(8).

Evidence that both metaphosphates are enzymatically hydrolyzed to phosphate is obtained from radioautographs of mouse and rabbit femur and tibia. In these instances, incorporation of P^{32} into actively growing area of bone is qualitatively identical to that of phosphate.* Similarly, incorporation of P^{32} into the subcellular fractions of liver cells follows an identical pattern for all 3 phosphates (Table II). The extensive localization in nuclei and mitochondria is consistent with the high nucleic acid content of these fractions.

In view of the high alkaline phosphatase activity of certain bone tumors(12), it would appear from the present data that the metaphosphates, in particular polymetaphosphate, possess considerable therapeutic potential. These substances are being studied further.

Summary. P^{32} from trimetaphosphate and polymetaphosphate localizes preferentially in bone, particularly in actively growing areas. The metaphosphates are superior to NaH_2-

* Retention of P^{32} from the 3 phosphates in bone matrix of demineralized bone also has been found to be equivalent.

TABLE II. Subcellular Localization of P^{32} in Liver.

Fraction	Counts/min./Mg N	% total counts
A. $NaH_2P^{32}O_4$		
Cell debris I	753	12.8
II	1,102	18.7
Nuclei	1,465	24.9
Mitochondria	1,554	26.4
Supernatant	1,011	17.2
B. Trimetaphosphate		
Cell debris I	811	13.9
II	652	11.2
Nuclei	1,519	26.1
Mitochondria	1,618	27.7
Supernatant	1,229	21.1
C. Polymetaphosphate		
Cell debris I	409	14.4
II	365	12.8
Nuclei	750	26.4
Mitochondria	765	26.9
Supernatant	551	19.4

$P^{32}O_4$ as a P^{32} donor to bone, the extent depending upon experimental conditions. In particular, polymetaphosphate has a longer body retention and a higher bone-soft tissue ratio. The equivalent localization of P^{32} from $NaH_2P^{32}O_4$, trimetaphosphate and polymetaphosphate into the nuclei and mitochondria of liver cells is offered as evidence that the metaphosphates are enzymatically broken down to phosphates before incorporation.

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Electrophoretic Studies of Bovine Serum. II. Concurrent Hypoglobulinemia and Natural Infections of Eperythrozoonosis.* (24521)

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Although data have been accumulated on pathogenesis of eperythrozoonosis together with descriptions of the causative agent (1-4), no work, thus far, has been directed toward physicochemical characterization of alterations manifested in the host. During the present investigations, in which bovine anaplasmosis was studied primarily, it was occasionally observed that stained blood smears showed microscopic evidence of eperythrozoonosis prior to experimental infection with anaplasmosis. This report presents results of serum protein changes concurrent with natural infections of eperythrozoonosis in calves.

Materials and methods. Cattle: In our investigation a total of 30 dairy calves of mixed breeds, ranging in age from 3 weeks to 6 months, were studied. They were housed and fed as previously described(5). Of this num-

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