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Effect of Phosphate Loading on Leukocyte Labelling with Inorganic P^{32}.* (26975)

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Deoxyribonucleic acid (DNA) labelling with inorganic radiophosphorus (P^{32}) has been used to study leukocyte kinetics (1-11); the usefulness and reliability of this leukocyte label for appraising cell kinetics has been considered previously(12, 13). It has been shown that isotopic labelling of DNA occurs only during mitotic interphase when new DNA is synthesized(1,2,14-16) and that DNA retains label introduced during synthesis until cell death ensues(1,3,4,16-18).

Previous studies in rabbits(13) demonstrated that significant levels of plasma phosphate activity persist for at least 7 days after a single intravenous injection of only 2 μc of inorganic P^{32}/kg. Ultimate distribution of P^{32} to reach a common level of specific activity of the phosphate in the crude acid soluble, phospholipid, RNA, and DNA fractions of marrow cells was observed to occur by 72 hours. At this time the curve of regression of plasma phosphate activity, comparable in level to that of cell fraction phosphate, was noted to be quite flat. It was concluded that significant DNA labelling must continue for several days following a single intravenous dose of P^{32}. In an effort to shorten the exposure time of proliferating cells to high levels of activity in the extracellular phosphate pool, a load of non-isotopic inorganic phosphate was given intravenously and its effect on plasma clearance of P^{32} and on cell labelling evaluated.

Materials and methods. These studies were done in 3-5 kg rabbits each of which received intravenously 2.0 μc of P^{32}/kg of body weight. Technical and analytical procedures have been described(12,13). Serial blood samples to evaluate plasma clearance following intravenous P^{32} were obtained from the superior vena caval system via a size PE-50 polyethylene catheter inserted through a marginal ear vein. Samples showing visible hemolysis were discarded. Plasma was treated with an equal volume of 10% trichloroacetic acid and the supernate obtained after centrifugation was wet ashed in concentrated sulfuric acid prior to phosphorus determination.

Leukocytes were obtained from heparinized blood by the method of Athens, *et al.*(19). Red cells were sedimented in dextran and those remaining in the leukocyte containing supernate were hemolyzed with gramicidin-lysolecithin. Leukocytes were washed in saline and platelets removed by differential centrifugation. The remaining leukocyte suspension was then fractionated by a modification of the Schmidt-Thannhauser procedure, quantitative phosphorus analysis performed by an adaptation of the method of Berenblum and Chain, and radioactivity determined by scintillation counting(12).

Non-isotopic inorganic phosphate was given as a sterile, pyrogen-free solution containing approximately 100 millimols of phosphate/liter. This was prepared by dissolving 128.7

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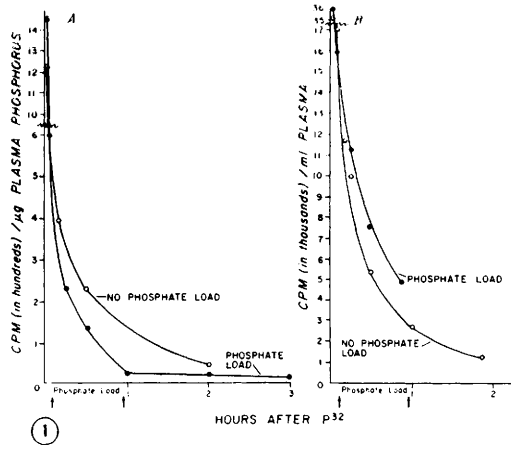

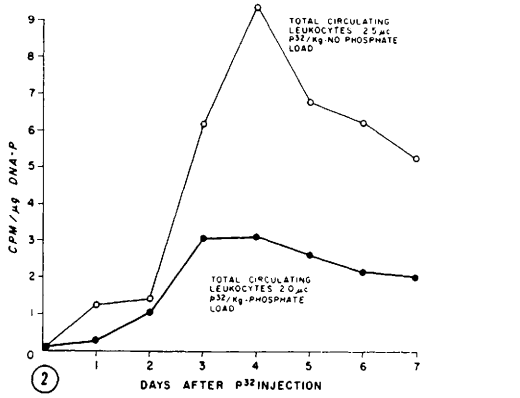

FIGS. 1, A and B. The effect of a phosphate load on plasma clearance of inorganic P^{32}.
Each curve represents determinations in a single animal.

FIG. 2. The effect of a phosphate load on leukocyte labelling with inorganic P^{32}.
Each point represents a mean value from 4–12 animals.

g of $Na_2HPO_4 \cdot 7H_2O$ and 16 g of $NaH_2PO_4 \cdot H_2O$ in enough distilled water to make 6 liters of solution. The phosphate load, 20 ml of the above solution/kg of body weight in every case, was given intravenously either by drip through a polyethylene catheter or by separate injections of 10 ml aliquots. Loading was initiated between 5 and 10 minutes following P^{32} administration and was completed within 1 hour after beginning. Volumes in excess of 20 ml/kg given within 1 hour frequently produced severe pulmonary edema and could not be used.

Results. The effect of a phosphate load on plasma clearance of inorganic P^{32} following an intravenous dose of 2 µc/kg is shown in Fig. 1-A and 1-B. It is apparent from Fig. 1-A that the phosphate load produced immediate dilution of the specific activity of plasma phosphate. Fig. 1-B shows, however, that the actual rate of clearance of radioactivity from the plasma was decreased by the load. Table 1 compares the specific activities of plasma and leukocyte acid soluble phosphorus from phosphate loaded animals with those from controls. The values from phosphate loaded animals were lower in every case.

DNA-P specific activity of circulating leukocytes from animals loaded with inorganic phosphate after 2.0 µc of P^{32}/kg is plotted in Fig. 2 which presents also for comparison the specific activity curve of DNA-P of circulating leukocytes from animals that received no phosphate load after 2.5 µc of P^{32}/kg. The absolute level of DNA-P specific activity obtained in the loaded animals was less than that produced by the same dose of isotope in animals not given a phosphate load(13) and as Fig. 2 shows no sharp peak in DNA-P specific activity occurred in the loaded animals.

Discussion. This study has shown that a load of non-isotopic phosphate given promptly after injection of P^{32} produces immediate dilution of the activity of the plasma phosphate, decreases the rate of loss of activity from the plasma during the period of loading, diminishes the level of tagging of circulating leukocyte acid soluble and DNA phosphorus, and prevents the sharp peak in DNA-P activity observed in unloaded animals.

It has been shown(13) that with a dose of 0.1 µc of P^{32}/kg no significant activity was present in plasma phosphorus 4 hours after injection and maximum specific activity at 1 minute was only 35.4 cpm/µg of plasma phosphorus. This small dose produced very low labelling of DNA-P (0.8 cpm/µg) in leukocytes from circulating blood on the fourth day after P^{32} administration. It was anticipated that a load of non-isotopic phosphate might quickly dilute the high level of plasma phosphate activity produced by 2.0 µc of P^{32}/kg to the low level induced by 0.1 µc/kg and thereby decrease DNA tagging time. It is apparent that an acutely administered phosphate load does effect a dilution of this magnitude within an hour; however, after cessation

TABLE I. Specific Activity* of Plasma and Leukocyte Acid Soluble Phosphorus after 2.0 μc P^{32}/kg.

Phosphate load		Controls		
Plasma P	Leukocyte acid Soluble P	Plasma P	Leukocyte acid Soluble P	Days after P^{32}
22	—	42	—	1/12
3.6	1.5	5.4	2.4	1
2.5	2.8	—	4.2	2
—	4.0	—	4.5	3
—	4.8	2.6	5.0	4

* Counts/min./μg of phosphorus. Each value represents a mean from at least 4 animals.

of loading the fall in plasma phosphate activity is quite gradual. The ratio of plasma phosphate activity 24 hours post-injection to that 2 hours post-injection is 0.13 in unloaded animals and 0.16 in those receiving a phosphate load, indicating that rate of plasma clearance after 2 hours is roughly equivalent in both groups. A significant level of plasma phosphate activity persists for at least 2 days (Table 1) in loaded animals. Cellular uptake of phosphate of significant activity can therefore continue and significant labelling of DNA can occur for at least 2 days in animals receiving a phosphate load. As plasma clearance at this time is dilatory, 2 days probably represents a lower limit.

The lower level of tagging of leukocyte acid soluble and DNA phosphorus found in the phosphate loaded animals is not surprising since the label in these fractions comes ultimately from the plasma phosphate. The specific activity of the phosphate of these fractions will be in all cases the resultant of rate of cellular uptake or exchange of phosphate and specific activity of the precursor. As the phosphate load rapidly dilutes the activity of the plasma phosphate a specific activity of cellular acid soluble and DNA phosphorus lower than that found in controls would be expected in the absence of an accelerated rate of uptake in loaded animals during the brief interval when activity of the plasma phosphorus is still relatively high. This seems to be the case.

Fig. 2 shows that administration of a phosphate load immediately after P^{32} injection prevents development of the peak in DNA-P specific activity seen in control animals. The events producing a peak in circulating leukocyte DNA-P specific activity 4 days after injection of label are not clear. In a chemical sense the peak is merely the time when the quantity of labelled DNA-P relative to total DNA-P is maximal. Further, this should be the time when there is circulating the maximum number of cells bearing the highest average level of DNA label per cell. The precise mechanics producing this situation depend upon the availability of labelled precursor phosphate, the kinetics of DNA synthesis, and the rate and manner cells enter into and exit from the circulating blood. There is no evidence that the phosphate load alters either of the latter two, but there is direct evidence that it does affect the availability of labelled precursor phosphate. Therefore, that after 2 hours, rate of plasma clearance in loaded and unloaded animals is roughly the same, suggests that the initial effect of the phosphate load during the first 2 hours after P^{32} injection prevents development of a peak in DNA-P specific activity of circulating leukocytes. This in turn implies that in control animals, the labelling of marrow leukocyte progenitors during the first 2 hours post-injection conditions the occurrence of the peak in circulating leukocyte DNA-P specific activity.

Summary. The effect of an intravenous load of non-isotopic inorganic phosphate following P^{32} on plasma clearance and on leukocyte labelling has been studied. Such a load produces immediate dilution of plasma phosphate activity, decreases rate of clearance of plasma radioactivity during the period of loading, diminishes the level of labelling of circulating leukocyte acid soluble and DNA phosphorus, and prevents development of a peak in DNA-P specific activity. The findings suggest that marrow labelling during the

first 2 hours after P^{32} injection is responsible for development of the peak in DNA-P specific activity found in peripheral blood leukocytes.

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Pyelonephritis. III. Observations on the Association between Chronic Pyelonephritis and Hypertension in the Rat.* (26976)

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The relationship between hypertension and pyelonephritis has not been clearly defined. Various clinical studies have suggested: 1) pyelonephritis can cause hypertension; 2) pyelonephritis can aggravate a pre-existing hypertension and result in "malignant hypertension"; and 3) there is no relationship between renal infection and elevated blood pressure. Experimental consideration of this problem has been somewhat limited by the lack of suitable laboratory model of pyelonephritis. Studies performed in animals with "obstructive" pyelonephritis have with one exception(1) failed to reveal sustained hypertension(3). Similar findings have been recorded in experiments when renal infection was produced by various other manipulative procedures(2,4).

With the availability of a chronic, progressive pyelonephritis in the rat, produced without ureteral obstruction, experiments were designed to study the possible etiologic relationship between kidney infection and hypertension.

Methods. White, male Wistar rats were used. Previous studies have shown that following intravenous injection of a standard inoculum of *Str. faecalis* pyelonephritis uniformly resulted(5). This infection was characterized by the persistence of bacteria in relatively constant numbers in the kidney during the period when the disease progressed from the acute to the chronic stage histologically. In other experiments, microorganisms were found in the majority of rat kidneys

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