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BLOOD ORGANIC PHOSPHATE IN HYPERTHERMIC DOGS

There is a marked fall in plasma inorganic phosphate (P) concentration and urinary inorganic phosphate excretion in unanesthetized dogs exposed to heat. If the fall in plasma inorganic P is due to an increase in metabolism, then one should expect to find an increase in intracellular organic P. The red blood cells, as the most easily obtainable cellular sample, were selected as the yardstick for measuring cellular changes. Acid soluble organic phosphate was determined by the difference between the total P and inorganic P in aliquots of the same sample. Sixteen experiments were conducted on 8 unanesthetized dogs. In a group of 8 dogs exposed to 120° F., the whole blood inorganic P fell from a control value of 3.2 to 0.7 mg. percent at the end of 4 hours in a hot room, while the whole blood organic P increased from a control value of 23.1 to 27.2 mg. percent at the end of 4 hours' exposure to heat. Six control dogs exposed to 76° F. air temperature for 4 hours showed no significant changes in whole blood inorganic or organic P. The increase in metabolism which is responsible for the inorganic-organic shift is mainly due to vigorous panting; dogs tested at 105° F., requiring mild panting, showed no fall in inorganic P nor increase in organic P. In 1 anesthetized dog all skeletal muscle activity was inhibited by curarization. Upon exposure to heat no fall in inorganic P or increase in organic P occurred. The fall in inorganic P is, therefore, directly related to the large increase in metabolism which occurs as a result of the exercise involved in panting.

In a previous paper (1) we described the marked fall in plasma inorganic phosphate (P) concentration and urinary inorganic phosphate excretion which occur in unanesthetized dogs exposed to heat. During 4 hours' exposure to air temperature of 120° F. the plasma levels fell from 5.2 to 2.6 mg. percent P, while the urine concentration fell from 184 to 5 mg. percent P. The fall in urinary phosphate is believed to be due to renal dynamics involving almost complete reabsorption because of low plasma phosphate concentration and low loads delivered to the tubules (2,3,4). The cause of the fall in plasma inorganic phosphate was not ascertained. As the fall in P was not due to excretion it probably was metabolic in origin. It was decided, therefore, to investigate organic phosphate regulation in hyperthermia to see if the fall in inorganic P could be ascribed to metabolic alteration.

METHODS

Sixteen experiments were conducted on 8 trained, unanesthetized dogs. One additional experiment was run on an untrained, anesthetized dog. The animals were loosely confined in a standing position in a dog stall with collapsible legs which rested on a movable table. A Fairbanks balance was placed under the stall so that the animals could be weighed without being disturbed. If the fall in plasma inorganic P was due to an increase in metabolism, then one should expect to find an increase in intracellular organic phosphate. The red blood cells, being the most easily obtainable cellular sample, were selected as the yardstick for measuring cellular changes. Venous blood samples were drawn periodically and analyzed for whole blood total acid soluble phosphate and inorganic phosphate, as well as plasma total

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acid soluble phosphate and plasma inorganic phosphate. Organic phosphate was determined by the difference between the total phosphate and inorganic phosphate in aliquots of the same sample. Inorganic P determinations were done by the Gomori method (6) while total acid soluble phosphate was done by wet-ashing whole blood with perchloric acid and heat and, after digestion, using the Gomori inorganic method to determine total P present. Four series of experiments were run. In the first group, 8 dogs were deprived of drinking water and exposed to a temperature of 120° F. and low humidity for 4 hours. In the second group, which formed the control series, 6 dogs were tested for 4 hours, under the same conditions as the preceding group, but at an air temperature of 76° F. In another group, 2 dogs were run at an intermediate air temperature of 105°

F. so that heat stress would be minimized but some rapid dehydration would occur. Lastly, one anesthetized dog was exposed to high environmental temperatures; this dog's respiratory muscles were paralyzed with flaxedil" so as to prevent panting, and artificial respiration was used. In this way, the effect of the previously accelerated respiratory movements (panting) on phosphate regulation was ascertained.

RESULTS

Exposure of 8 unanesthetized dogs to air temperatures of 120° F. and low humidity caused a precipitous fall in whole blood inorganic phosphate as well as a marked increase in whole blood organic phosphate (figure 1). The whole blood inorganic phosphate fell from
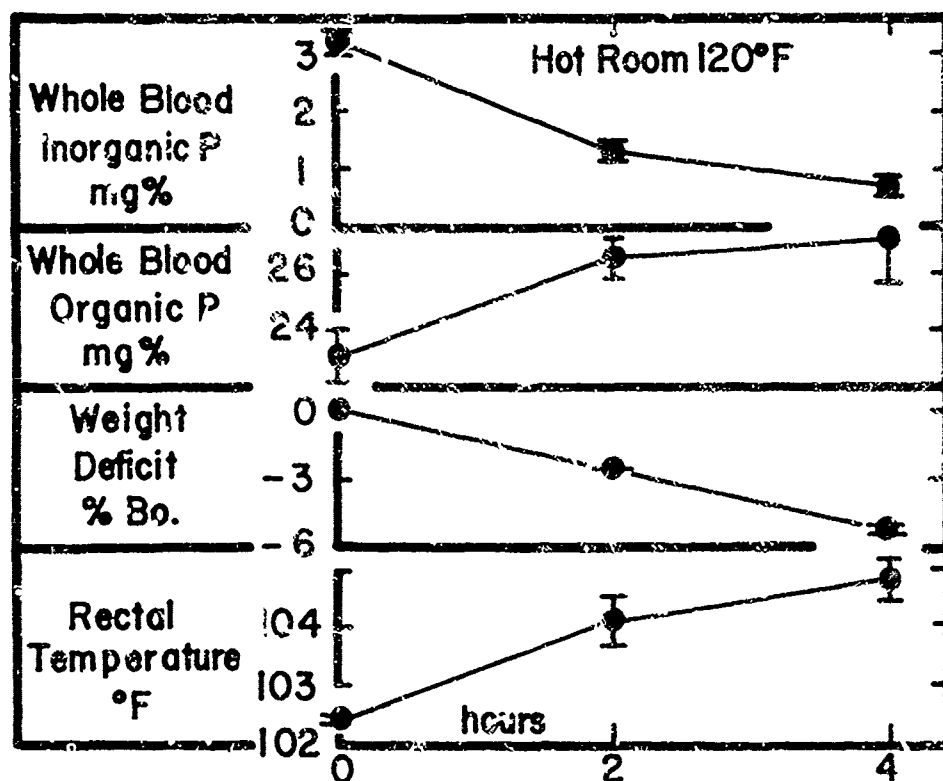

FIGURE 1

Eight dogs, deprived of drinking water, tested at an air temperature of 120° F. for 4 hours. Averages and standard errors of the mean are included.

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a control value of 3.2 to 0.7 mg. percent at the end of 4 hours in a hot room while the whole blood organic phosphate increased from a control value of 23.1 to 27.2 mg. percent at the end of 4 hours of exposure to heat. This increase in whole blood organic phosphate concentration was greater than could be accounted for on the basis of dehydration (weight decrease) so that an actual increase in organic phosphate did occur. The weight decrease in this group at the end of 4 hours was 5.2 percent body weight while the increase in whole blood organic phosphate was 17.7 percent.

There is practically no organic phosphate in plasma. In six experiments of this series in which the plasma organic phosphate was measured, the average control plasma organic phosphate was 0.1 mg. percent and after 4 hours' exposure to heat and dehydration it was still 0.1 mg. percent.

If a large share of the fluid loss came from the plasma compartment, then the increase in whole blood organic phosphate would not be so significant. With the loss of plasma, which contains little organic phosphate, a relative increase in red blood cells per unit volume would occur and, therefore, an increase in organic phosphate in whole blood on the basis of increased hematocrit, even without considering any shift from the inorganic form in plasma to the organic form intracellularly. Our data show, however, that this did not occur, for the small increase in the hematocrit (+ 5.9 percent) indicates that the fluid required for evaporative cooling from the respiratory surfaces was drawn from the intracellular as well as extracellular spaces. Even with rapid panting and the loss of 5.2 percent body weight, mainly as water, the rectal temperature increased from a control value of 102.4° F. to
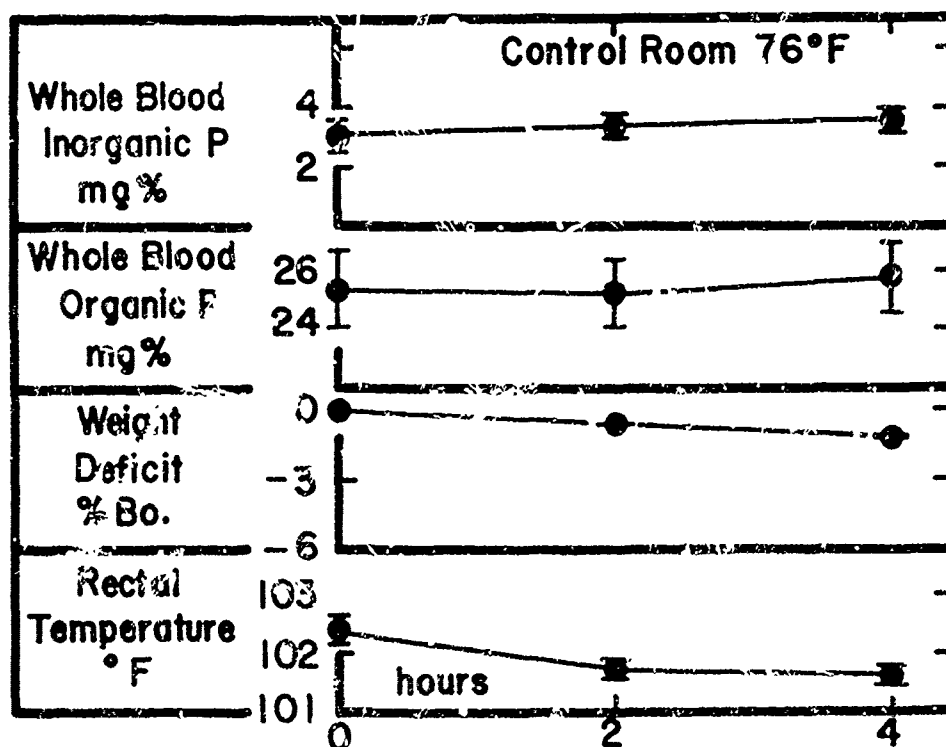

FIGURE 2

Six dogs, deprived of drinking water, tested at an air temperature of 76° F. for 4 hours. Averages and standard errors of the mean are included.

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TABLE I

Phosphate regulation in a curarized dog exposed to heat for 2 hours

Time (min.)	Rectal temperature (°F.)	Hematocrit (percent)	Inorganic phosphate (mg. percent P)	Organic phosphate (mg. percent P)	Body weight (kg.)
Control — dog anesthetized at —75 min.					
0	101.7	42.5	5.2	19.2	23.46
+30	101.7	42.4	5.3	18.8	23.42
Flaxedil® injected 0.2 mg./kg. at +31 min.					
+60	101.7	43.0	5.3	18.8	
+75	101.7	43.0	5.2	18.6	23.39
Animal exposed to heat at +76 min.					
+105	102.6	46.2	4.5	20.3	23.34
+135	104.2	45.7	4.2	20.1	23.32
+165	105.1	50.8	5.1	25.6	23.29
+195	106.3	54.1	5.4	24.8	23.25

104.8° F. at the end of 4 hours of exposure. Therefore, both dehydration and hyperthermia were present.

When 6 dogs were tested at an air temperature of 76° F. no panting occurred and the weight deficit was about 0.3 percent body weight per hour (figure 2). The rectal temperature decreased slightly from a control of 102.4° to 101.6° F. at the end of 4 hours in the control room. There was no significant change in either whole blood inorganic or whole blood organic phosphate concentration. The whole blood inorganic P went from 3.1 ± 0.6 to 3.6 ± 0.5 in 4 hours while the whole blood organic P went from 25.4 ± 1.5 to 25.8 ± 1.4 in 4 hours.

As no change in organic phosphate occurred under control conditions, yet did occur under hyperthermic stress, it was decided to test an intermediate temperature (105° F.) to see whether with dehydration but a minimum of panting the typical heat-caused inorganic phosphate decline and organic phosphate increase would occur. Two animals were tested and,

again, no significant change occurred in either the whole blood inorganic or organic phosphate (figure 3). Although panting was very mild, 3.2 percent body weight was lost in 4 hours. The evaporation of this quantity of water allowed maintenance of rectal temperature, and hyperthermia did not develop. In fact, rectal temperature fell slightly from a control value of 102.4° to 101.8° F. at the end of 4 hours' exposure to 105° F. air temperature. By decreasing the heat stress so that hyperthermia and, in turn, severe panting did not occur, the usual pattern of decrease in inorganic phosphate and increase in organic phosphate also failed to occur.

Lastly, an anesthetized dog was curarized with flaxedil* (2.0 mg./kg.) and exposed to heat. All skeletal muscle activity was eliminated and no panting occurred. Under such conditions temperature regulation was impaired and a rapid hyperthermia developed. This allowed us to isolate the effect of hyperthermia from accelerated respiratory muscle activity. It was found (table I) that the inorganic phosphate in whole blood fell slightly as the
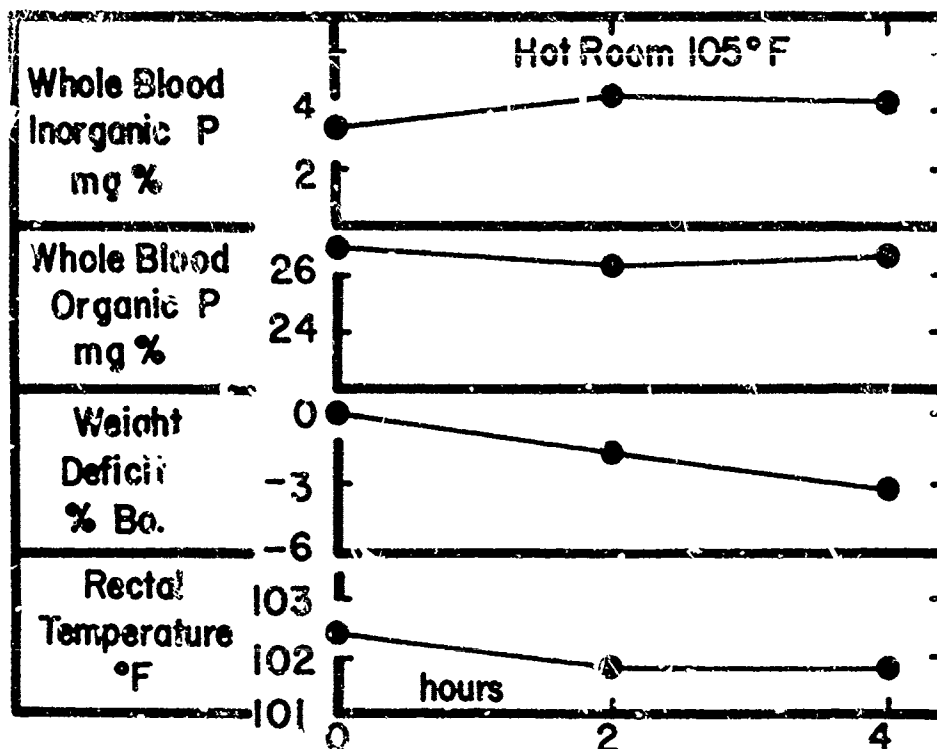
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FIGURE 3

Two dogs, deprived of drinking water, tested at an air temperature of 105° F. for 4 hours.

av ~al was initially exposed to heat. This initial dec ase was probably due to the speed up of met lism which drew additional inorganic phos, ite for incorporation into the expanding metabolic pool. The amount required is far below that amount of phosphate in sported when panting occurs. With continued exposure to heat, the inorganic phosphate levels increase gradually, reflecting the progressive dehydration. The previous found drastic and rapid fall in inorganic phosphate does not occur in the curarized animal. While the increase in organic phosphate from control to the end of the exposure to heat was 27 percent, the increase in hematocrit from control was also 27 percent. With panting eliminated, therefore, the effect of heat on inc anic-organic phosphate levels is largely the physical effect of dehydration and increased hematocrit.

DISCUSSION

Unanesthetized dogs exposed to heat show a marked decrease in plasma and whole blood inorganic phosphate levels (1). Under similar environmental conditions hypoglycemia also occurs (5). It appears probable, therefore, that since the fall in phosphate was not due to excretion, the decrease was metabolic in origin. With the increased activity of the respiratory muscles to maintain temperature by evaporative cooling from oral passages, glucose is utilized at a more rapid rate. This acceleration of glucose metabolism causes added phosphate to be incorporated into the expanded metabolic cycle. The phosphate is largely supplied from the extracellular inorganic source and is transformed to the organic state intracellularly.

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It was Harden and Young (7, 8) who found that inorganic phosphate added to fermenting yeast caused an increase in fermentation and a disappearance of the inorganic phosphate and an increase of organic-bound phosphate. Halpern (9), in 1936, showed that glycolysis itself favored the synthesis of organic phosphate. In our study we have a comparable in vivo situation.

A reflection of the increase in intracellular organic phosphate can be seen in the whole blood analyses which include the red blood cell content. If one compares the plasma organic phosphate to the whole blood organic phosphate it is apparent that practically all but a trace of the organic phosphate is found intracellularly. Any increase, therefore, in whole blood organic phosphate is really an increase in red blood cell organic phosphate.

That the inorganic phosphate is incorporated intracellularly was shown in our first series of experiments (figure 1). This intracellular uptake is then responsible for the previously observed fall in plasma inorganic phosphate in heat-exposed unanesthetized dogs. That the increase in metabolism, mainly due to vigorous panting, is responsible for the inorganic-organic shift is seen from the results of experiments conducted at 105° F. At this temperature panting decreased and no fall in phosphate was observed. Final proof was the curarization experiment (table I) in which all skeletal muscle activity was inhibited and, in spite of hyperthermia, no fall in inorganic phosphate occurred. The fall in inorganic phosphate is, therefore, related directly to the large increase in metabolism due to the exercise involved in panting. Hyperthermia alone does not increase metabolism sufficiently to show clearly the alteration in inorganic-organic phosphate balance.

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