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Renal Arteriovenous Reduction in Hemoglobin Concentration in the Anesthetized Dog.* (30962)

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A renal arteriovenous reduction in hemoglobin concentration has been observed in the dog in terms of oxygen capacity(1,2) and in the rat in terms of oxyhemoglobin(3). Since this finding would not necessarily have been expected from what was known of the physiology of hemoglobin and the physiology of the kidney, it was considered of interest to investigate the matter further.

Procedure. The hemoglobin concentration in arterial blood was compared with that drawn from a renal vein at the same time. Adult mongrel dogs were anesthetized with 20 mg/kg of sodium pentobarbital given intravenously, supplemented with additional doses of 5 mg/kg as needed. Two groups of experiments were performed. In the first, samples of about 1 ml were taken from the right femoral artery and left renal vein by puncturing the vessels with hypodermic needles just before they were to be drawn. The vessels were exposed by dissection. The renal vein was approached through a ventral laparotomy and the viscera were usually retracted out of the way just before the samples were to be taken. A total of 109 pairs of blood samples were taken from 13 different dogs.

In the second group of experiments samples of about 1 ml were drawn from the aorta opposite the left renal artery and from the left renal vein through polyethylene catheters that had been implanted some time before. The animals were heparinized to avoid clotting in the catheters. Two to 5 ml of blood were drawn through each catheter and discarded just before the samples were taken to clear the catheters of the blood that had remained in them. A total of 89 pairs of blood samples were taken from 15 different dogs.

In both groups of experiments duplicate subsamples of each sample of blood were analyzed for hemoglobin concentration in terms of both carbon monoxide hemoglobin and acid hematin and in the second group also in terms of cyanmethemoglobin. The sample, which was discharged from the syringe or catheter into a small paraffin cup, was agitated at the time the subsamples were taken to avoid any possibility of settling of the red cells. In the first group of experiments subsamples of 0.1 ml were each measured into 25 ml of distilled water. Two tenths ml of concentrated ammonium hydroxide was added to clear this somewhat cloudy solution. Aliquots of the resulting clear solution were then analyzed for carbon monoxide hemoglobin and acid hematin. In the second group of experiments 0.2 ml subsamples were each measured into 20 ml of M/60 phosphate buffer (pH 6.6). This buffered solution was cleared by addition of 0.05 ml of concentrated ammonium hydroxide. Aliquots of it were then analyzed for carbon monoxide hemoglobin, acid hematin, and cyanmethemoglobin.

Results are summarized in Tables I and II. The results of the first group of experiments demonstrate that a renal arteriovenous reduction in hemoglobin, as reported by others, may be observed. Reductions of as much as 3.58 g % were found. In 27 instances, the

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TABLE I. Renal Arteriovenous Reduction in Hemoglobin Concentration Determined by Different Methods of Measuring Hemoglobin.

Method	No. of pairs of samples	Mean and standard error of mean (g %)	P from distribution of t
1st group of exp			
a. Carbon monoxide hemoglobin	109	.32 ± .08	<.001 diff from zero
b. Acid hematin	109	.34 ± .08	*Idem*
2nd group of exp			
c. Carbon monoxide hemoglobin	89	.01 ± .04	.9-.8 diff from zero <.001 diff from a
d. Acid hematin	89	.02 ± .03	.6-.5 diff from zero <.001 diff from b
e. Cyanmethemoglobin	89	.20 ± .07	.01-.001 diff from zero <.001 diff from c <.001 diff from d

reduction exceeded 1 g % for both the methods used to measure hemoglobin. In general, the interval between pairs of samples from the same subject ranged from some minutes to over an hour. In 12 instances, however, a second pair of samples was taken within 30 seconds. In these it was found that both the arterial and venous hemoglobin concentrations were changing rapidly.

TABLE II. Comparison of Variances of Differences Between Simultaneous Pairs of Arterial and Venous Samples and Between Subsamples of Each of These Samples in Hemoglobin Concentration Determined by Different Methods.

Difference between	Degrees of freedom	Variance $(g\ \%)^2$	P from distribution of F
1st group of exp			
Carbon monoxide hemoglobin			
A-V difference	109	.623	<.001
Subsamples	212	.049	
Acid hematin			
A-V difference	109	.736	<.001
Subsamples	210	.055	
2nd group of exp			
Carbon monoxide hemoglobin			
A-V difference	87	.151	>.20
Subsamples	172	.128	
Acid hematin			
A-V difference	88	.063	>.20
Subsamples	174	.064	
Cyanmethemoglobin			
A-V difference	85	.334	.01–.001
Subsamples	168	.176	

The results of the second group of experiments where hemoglobin was measured as carbon monoxide hemoglobin or acid hematin show that the renal arteriovenous reduction in hemoglobin observed in the first group may largely disappear if care is taken to avoid producing changes in the animal by the sampling procedure. The results in which hemoglobin was determined as cyanmethemoglobin, however, shows a renal arteriovenous reduction in hemoglobin where no statistically significant reduction was found when the hemoglobin was determined as carbon monoxide hemoglobin or acid hematin.

Discussion. The finding that the renal arteriovenous reduction in hemoglobin concentration measured as carbon monoxide hemoglobin or acid hematin largely disappeared in the second group of experiments would seem to indicate that the renal arteriovenous reduction in hemoglobin in the first group of experiments was largely a transient phenomenon evoked by the sampling procedure. Such a transient reduction might be explained, for example, in terms of currently accepted concepts of physiology as a lag in

the increase of renal venous hemoglobin concentration behind a rapid rise in arterial concentration due to a compression or contraction of the spleen produced by the sampling procedure(4).

The results of the second group of experiments would seem likely to be less immediately affected by the sampling procedure and therefore reflect less transient processes. The comparison of variances indicates that the renal arteriovenous differences in hemoglobin concentration determined as carbon monoxide hemoglobin or acid hematin can be largely accounted for by the differences between subsamples. The mean renal arteriovenous reduction in hemoglobin concentration measured as cyanmethemoglobin, however, cannot be explained either in terms of the differences between subsamples ($P = .01$-$.001$ from the distribution of F) or in terms of the variation of the size of the reduction itself ($P = 0.01$-0.001 from the distribution of t). Since it is significantly greater than the reduction determined as carbon monoxide hemoglobin ($P<0.001$) or acid hematin ($P<0.001$) it would seem to be at least in part due to processes intrinsic to the kidney, for it would be difficult to explain it as due wholly to an extrarenal process such as might account for the reduction measured in term of these in the first goup of experiments. It would also appear that the method in which hemoglobin is determined as cyanmethemoglobin must measure some substance other than hemoglobin or possibly a modification of the hemoglobin molecule to a different degree than the other methods. Since it is a reduction, it cannot be explained as due to the formation of urine and lymph. It also cannot be explained as due to a continuing irreverisible change in the hemoglobin molecule (5). The 95% confidence limits for this renal arteriovenous reduction in hemoglobin measured as cyanmethemoglobin are 0.06 and 0.34 g %. Even the smaller of these is at least 10-fold too large to be accounted for in this way

The renal arteriovenous reduction in hemoglobin found by others(1-3) can be explained readily in the same way as the reduction in the first group of experiments. In one of these(2) it was also found that at least the larger reductions disappeared when the sampling procedure was changed so that it would be expected to have had less immediate effect on the subject. This would seem to be comparable to the disappearance of much of the reduction in the second group of experiments where hemoglobin was measured as carbon monoxide hemoglobin or acid hematin.

In a number of investigations(2,6,7,8) in which the arterial and venous hemoglobin concentrations were studied incidentally to other things, it was found that any arteriovenous changes in hemoglobin concentration that might have been present were insignificant in relation to whatever was primarily being investigated. However, arteriovenous changes comparable in size to those found in the first group of experiments may well have gone unrecognized, in part because the number of arteriovenous differences studied typically was too small to have revealed much smaller changes and in part because the investigators reported such differences more from the point of view of whether or not they were large enough to be a significant complication in relation to other factors.

Summary. A renal arteriovenous reduction in hemoglobin concentration measured as carbon hemoglobin or acid hematin was observed in the dog. It largely disappeared when care was taken to avoid producing changes in the animal by the sampling procedure. When such care was taken, however, a renal arteriovenous reduction in hemoglobin concentration measured as cyanmethemoglobin was still found. It would seem that this reduction was at least in part due to a process intrinsic to the kidney and that it must have involved the measurement of an unidentified substance or possibly a modification of the hemoglobin molecule by the method for determining hemoglobin as cyanmethemoglobin to a different degree than by the other methods.

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Effect of Heparin and Parathyroid Extract on Acid Phosphatase In Bone.* (30963)

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It is generally agreed that normal remodelling of bone is a cellular process taking place in well defined areas influenced by growth and mechanical stress(1,2). The mechanism of the process is less well understood, but osteoclasts and giant cells are usually to be found in resorptive cavities in the area being remodelled(3,4). Various hormones have been shown to alter the normal process when given in pharmacologic amounts(1). Parathormone is used experimentally to increase resorption presumably by accelerating normal processes (1,5,6). After treatment of an animal with parthyroid extract (PTE) changes can be shown in both the stable highly mineralized diaphyseal region, and in the more cellular metabolically active metaphyseal regions. These changes may reflect different independent parameters of parathyroid action(7-10).

Since the early work of Gutman, Huggins, Kochakian, and Klendshoj, parathyroid induced resorption or pathologic destruction of bone in metastatic cancer has been associated with elevated serum acid phosphatase and elevated bone acid phosphatase(11-15). In more recent studies various workers have associated acid phosphatase with osteoclasts in PTE-treated and normal bone by histochemical means(16-21).

Acid phosphatase as well as collagenase and hydrolases are found in many tissues in subcellular particles, the lysosomal bodies of de Duve(22). De Duve has suggested that release of these enzymes from lysosomal bodies of osteoclasts may help to explain the local action of osteoclasts on bone(23). It has been postulated that in order for this to happen the lysosomal membrane or cell membrane must be altered to permit escape of the enzymes(24). Experimental evidence for this has been presented by Asher and Nichols, studying heparin induced osteoporosis, who found that heparin could cause a marked increase in collagenase activity in bone. These same authors also reported a similar action for saponin and chondroitin sulfate(25). Another lysosomal membrane active substance, vitamin A, has been shown to enhance resorption of bone in tissue culture(26). These considerations have become clinically important with the discovery of osteoporosis in patients on long term heparin therapy(27,28). Increased serum levels of acid phosphatase have been reported in hyperparathyroid states(11-14). It appears that the clinical observations of Griffith *et al*(27) might be explained on the basis of a still further liberation of acid phosphatase resulting from heparin activity. To test this hypothesis we injected heparin into animals made hyperparathyroid with PTE and analyzed the bone for acid phosphatase in areas known to be subject to PTE ac-

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