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Blood Levels of 17-Hydroxycorticosteroids in Hyperthermic Dogs.* (22732)

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(Introduced by Richard R. Overman)

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That stress produces an elevation in the level of 17-hydroxycorticosteroids has been indicated by a number of publications(1-5). These observations have ranged from noting the decreased quantity of cholesterol present in the adrenal glands of animals subjected to hemorrhage, to direct measurements of plasma 17-hydroxycorticosteroids in animals and man subjected to surgical, bacterial pyrogen, E.C.T., and whole body radiation procedures. Recently Eik-Nes has reported by abstract, that dogs given an intravenous pyrogenic dose of bacterial mucopolysaccharides exhibited prolonged elevations in plasma 17-hydroxycorticosteroids. The adrenal cortical hormone(s) are known to exert considerable influence not only on the bodily economy of fluids and electrolytes, but also on the partition of these substances. Overman *et al.*(6) have shown that in man and the monkey, marked alterations in Na and K distribution result from prolonged exposure to a febrile condition having its origin in the malarious state. It was suggested that this is indicative of an increased level of certain adrenal cortical hormones in the body.

Since little of a correlative nature is known concerning the stressful hyperpyrexic state, efforts have been made in this laboratory to understand the aberrant physiology of fever. The report of one facet of that investigation —concerning alterations in plasma concentrations of 17-hydroxycorticosteroids, of Na and of K—is the principal concern of this communication.

Materials and methods. Twenty-four healthy mongrel dogs of both sexes and weighing between 10 and 22 kg were subjected to exogenous hyperthermia. Before the animals were placed in the hypertherm cabinet but *after* the administration of intraperitoneal pentobarbital sodium (1 ml of 7% solution/5 lbs. body weight) and after placing of the arterial cannulae, control determinations were made of plasma and R.B.C. electrolytes, rectal temperature and plasma steroid concentrations. Na and K were determined by flame photometry. Rectal temperatures were taken 4 inches proximal to the anus using a thermistor probe. Plasma 17-hydroxycorticosteroids were determined by the method of Nelson and Samuels(7). The dogs were exposed to an ambient temperature of 50°C, generated by shielded infrared heat lamps, for periods sufficient to provoke a rise in rectal temperature to 42.5°C. This temperature was maintained for one hour. All blood samples were obtained from a cannulated carotid artery and were heparinized. Ten dogs, treated in a manner similar to that of the fever experiments with regard to anesthesia, minor surgery and elapsed experimental time, were utilized as controls. The survival of experimental animals was found to be less than 10% 24 hours after heating.

Results. The values obtained from the analysis of plasma for 17-hydroxycorticosteroids are presented in Table I. It will be noted

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TABLE I. Control and Experimental Plasma 17-Hydroxycorticosteroid Concentrations (μg %) in Dogs Subjected to Hyperpyrexia.

Control, 37.5°C	39.4°C	At 42.5°C	42.5°C + 0.5 hr	42.5°C + 1.0 hr	1.5 hr after cabinet removal
3		2		5	
2		5		5	
3		6		28	
3	11	8		18	
3	4	3	11	9	
4	9	13	9	11	
7	12	12	14	14	
5	0	7	10	4	
3*	5	8	22	21	
0*	3	5	11	8	
2*	5	5	11	11	
2*	3	3	9	10	
0*	1	3	8	18	
0*		1			
0*		6			
6*				16	
6*				17	
8*				25	
7*				28	36
6*				17	15
4*				16	28
4*				9	12
8*				27	32
2*				17	28
		Avg			
4	5	6	12	15	25
		% change			
	+25	+50	+200	+275	+525

* Blood removed from these dogs was replaced from donor dogs.

that after the rectal temperatures had been increased from the average control of 37.5°C to 42.5°C and maintained at that level for one hour, plasma cortical steroid level had risen from 4 to 15 μg/100 ml plasma. Observations were made at the following times during the experimental period: at a point half way between the control rectal temperature and 42.5°C (39.4°C in Table I), after the temperature had reached 42.5°C, after one half hour at 42.5°C, and after one hour at 42.5°C. In addition, plasma steroid concentrations were measured in 6 dogs about 1.5 hours after removal from the cabinet (average rectal temperature, 39.4°C).

Fig. 1 shows that during the first 120 minutes, both experimental and control animals presented increments in circulating plasma 17-hydroxycorticosteroids of 2 and 3 μg % respectively. During this interval the control animals' rectal temperatures decreased an average of 1.5°C from their control value (38.8°C). It is conceivable that the hypothermic tendency produced this increment in the control animals; no comparable decrease in rectal temperature occurred in the experimental dogs. After 120 minutes, however, the course of the 2 groups of dogs became quite divergent, with those exposed to elevated rectal temperatures exhibiting marked increments in circulating plasma 17-hydroxycorticosteroid levels (Fig. 1). It was feared that the bleeding necessary to obtain adequate plasma for electrolyte and 17-hydroxycorticosteroid determinations (approximately 35 ml of whole blood), in combination with marked diminution in circulating plasma volume as reported by Agersborg *et al.*† might augment the stress produced by the hyperthermic state. Consequently, the volume of blood removed for analysis in the last 16 animals presented in Table I was replaced from healthy, normal, donor dogs. It will be seen that similar increments in plasma 17-hydroxycorticosteroids occurred despite the replacement.

The average steroid level of the 6 dogs after removal from the hypertherm cabinet was 25 μg/100 ml plasma in contrast to only 4 μg % in the controls (Table I, Fig. 1). Removal of the external stimulus apparently does not reverse the tendency for increasingly augmented 17-hydroxycorticosteroid levels.

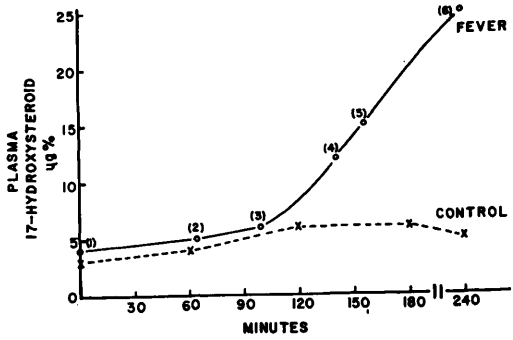

FIG. 1. Each point represents the avg of 6 or more determinations: (1) Avg control rectal temp., 37.5°C. (2) Avg rectal temp., 39.4°C; p = 0.5. (3) Rectal temp., 42.5°C; p = 0.05. (4) Rectal temp., 42.5°C; p = <0.001. (5) Rectal temp., 42.5°C; p = <0.001. (6) Avg rectal temp., 39.5° C; p = <0.001.

† Abstract

Plasma K concentrations were increased from control values of 3.9 to 5.3 mEq/l at the end of the experimental period ($p<0.001$). In general, plasma Na levels were not altered. However, the erythrocyte Na concentration was increased from a control value of 111 mEq/l to an experimental average of 115 mEq/l which is significant at the 1% level. In addition, red blood cell K concentration increased.

Discussion. The data show that severe hyperthermia produces a marked rise in the circulating level of 17-hydroxycorticosteroids in anesthetized dogs. It cannot be ascertained from these data what mechanisms are responsible for such pronounced increments; however, judging from the control determinations, anesthesia, minor surgical trauma and duration of experiment cannot be indicted. Several other possibilities might be suggested.

1) It is possible that thermal stimulation of adrenal cortical function occurs either directly or via ACTH.

2) The rate of hormone destruction in the liver might be impaired. It has been shown by Tyler *et al.*(8) and Sandberg and his colleagues(1) that during surgical stress the adrenal cortex is only submaximally stimulated, and the resulting elevation of corticosteroids results from a decreased ability of the liver to remove 17-hydroxycorticosteroids from the plasma. In this regard two modes of action are distinct possibilities in animals subjected to hyperpyrexia: a) the elevated body temperature might reduce the capacity of liver cells to conjugate 17-hydroxycorticosteroids, and b) the altered circulation of these dogs might contribute to the inability of the liver to handle adrenal steroids in a normal manner. Numerous observations of hemodynamics in hyperpyrexic dogs in this laboratory have shown marked skin dilation associated with measured rises in peripheral resistance. This suggests visceral vasoconstriction which might well decrease liver blood flow. Thus, while liver cells may still be functional or even hyperactive in the face of thermal stimulation, a serious reduction in liver blood flow could result in diminished removal of 17-hydroxycorticosteroids from plasma.

3) There is a possibility that a decreased rate of utilization or destruction could occur in the peripheral tissues of the body, but no means has yet been devised to determine either rate of utilization or destruction of adrenal cortical hormone(s) by tissue cells.

4) The increased plasma steroid levels might result from a concentration effect due to diminished plasma volume. Consideration of a typical animal in which the plasma 17-hydroxycorticosteroid level rose from 6 to 17 μg % during the experimental period reveals that such is not the case. With a control plasma volume of 1015 cc, this dog had 61 μg of circulating 17-hydroxycorticosteroids. At the end of the febrile period, while the plasma volume had been reduced to 791 cc, the total amount of circulating 17-hydroxycorticosteroids equalled 134 μg. If concentration (due to plasma volume reduction) were alone operative, one would expect very little, if any, increment in total circulating plasma 17-hydroxycorticosteroids. The observed increase of 120% in total circulating steroid level cannot be reconciled on the basis of a concentrating mechanism. As a result of these findings, it is suggested that the observed hyper-17-hydroxycorticosteroidemia is due to (1) the possibility of thermal stimulation of the adrenal cortex, (2) diminished rate of hormone destruction in the liver, (3) decreased peripheral tissue utilization, or (4) some subtle combination of these mechanisms.

The electrolyte alterations encountered were all statistically significant at the 2.5% level or lower with the exception of plasma Na concentrations. The marked increase in plasma K concentration ($p<0.001$) is deserving of commentary. Either a concentration mechanism is operative in this instance which is not readily apparent from plasma Na values, or perhaps the plasma K concentration is elevated through the release of adrenaline. Numerous investigators(9-11) have reported that the injection of adrenaline results in the elevation of plasma K concentration, and although the site of release of this K remains unknown, it has been generally attributed to the glycogenolytic influence of

TABLE II. Plasma and Erythrocyte Na and K Concentrations Obtained before and after Dogs Were Subjected to Rectal Temperature of 42.5°C for One Hour.

	Plasma electrolytes, mEq/l				Erythrocyte electrolytes, mEq/l			
	Control		Exp.		Control		Exp.	
	Na	K	Na	K	Na	K	Na	K
	154	3.9	151	6.3				
	154	3.9	151	6.7				
	146	3.9	147	4.0	99	4.2	115	5.0
	141	3.7	144	5.3	106	6.1	118	6.8
	144	4.0	145	5.4	111	6.0	113	6.4
	141	4.2	144	6.2	118	5.4	119	5.7
	146	3.9	144	7.6	115	5.3	116	5.6
	139	3.4	139	4.7	107	6.6	114	5.8
	134	4.0	138	6.0				
	140	3.5	140	4.7	113	4.0	118	4.5
	143	3.7	143	6.8	108	3.9	111	5.0
	148	3.3	145	4.6	115	5.4	113	5.1
	140	4.0	142	5.0	106	5.0	109	5.4
	149	3.8	148	4.7	110	6.1	120	6.2
	150	4.2	151	4.4	118	5.9	107	5.4
	149	4.4	150	4.8	109	6.3	119	6.9
	149	4.2	149	5.1	115	5.7	119	5.5
	150	4.0	149	5.8	112	5.8	115	6.7
	154	3.5	152	3.6				
	143	3.9	145	4.7	112	4.8	118	6.1
	145	4.1	147	4.9	112	5.3	116	5.4
Avg	146	3.9	146	5.3	111	5.4	115	5.8

adrenaline in the liver.

A consideration of 6 dogs in which plasma volume measurements were made shows that no change in total circulating plasma K occurs after the hyperthermic experience. This would appear to offer concrete evidence of a concentrating mechanism. However, in these experiments, transfer of K occurred into erythrocytes (Table II) which may have contributed to the absence of elevated total circulating K. The magnitude of the K uptake by the red blood cells is not too clear from these studies and a more complete treatment must await thorough investigations concerning alterations in the amount of cell ion. Preliminary determinations indicate that the magnitude of increase in the *amount* of erythrocyte K is quite sizeable (c. 190%).

With regard to plasma volume reductions it should be pointed out that the plasma represents the most labile fraction of the body fluid. Since the electrolyte concentrations are essentially similar on either side of the endothelial barrier, the increment in plasma K concentration would be reflected in the interstitial fluid. It seems unlikely, however, that the reduction of the plasma volume would be accompanied by an equivalent decrement in the larger and more stable interstitial fluid compartment. Therefore, the distinct possibility remains that an actual increment existed in the amount of extracellular K in these animals. The point to be emphasized here is that in many instances it is difficult to evaluate the real meaning of electrolyte alterations on the basis of altered plasma concentration alone. Such considerations apply equally to all plasma constituents. Thus, these experiments give us no real measure of the absolute amount of extracellular K, and one can only surmise that it has increased.

With these considerations in mind it would be well to regard the respiratory musculature as a possible source of some of the K. It has been repeatedly shown that muscular activity promotes loss of much cell K, and that such loss is proportional to the strength and duration of contraction(10). All animals used in these studies had very rapid breathing rates (>250/min) many of which exceeded the ability of the investigators to count by palpation, and all of which involved not only the thoracic musculature, but also the accessory respiratory muscles of the abdomen. It is therefore suggested that marked muscular effort might materially add to the extracellular K levels.

Likewise, in the face of dehydration it was noted that no change occurred in plasma Na concentration (Table II). However, the total amount of circulating Na diminished from an average of 114 meq to 91 meq in the 6 animals in which plasma volumes were measured. Clearly Na is being lost from the vascular system and the data presented in Table II would indicate that some of it is making an appearance in the erythrocytes. It is also conceivable that Na is moving into respiratory muscle cells in exchange for K, but such measurements have not been made in this experiment. The transfer of Na into muscle and red blood cells could account for the lack of rise in plasma Na concentration seen at the end of the experimental period instead of an increased concentration as might be expected

to result from the loss of water. That increments in erythrocyte Na occurred is clear, and while it is very difficult to assess the importance of alterations in canine red blood cell electrolytes, because their chemical anatomy is quite different from other body cells, such increases could be mediated by the influence of excessive amounts of 17-hydroxycorticosteroids on membrane permeability.

Summary. Twenty-four dogs were subjected to an ambient temperature of sufficient magnitude to elevate rectal temperatures to 42.5°C for one hour. These animals were studied with regard to plasma 17-hydroxycorticosteroid concentration and plasma and erythrocyte electrolyte levels. Significant changes were observed in all measured variables with the exception of plasma Na concentration, which was not altered from the control value. The physiological implications of these data are discussed.

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Increased Activity of Renal Glutaminases in Guinea Pig Following Prolonged Administration of Acid or Alkali.* (22733)

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The relationship between ammonia excretion and renal glutaminase activity has attracted much attention in recent years. Davies(1) discovered a marked increase in the ability of kidney slices from chronically acidotic rats to deaminate glutamine and other amino acids. On the other hand, Handler(2) found no increased hydrolysis of glutamine in slices from rats chronically treated with acid or alkali. White(3) observed no increase in renal glutaminase activity in homogenates from acidotic rats, while Rector(4) demonstrated a sharp day to day rise in the glutaminase activity of kidney homogenates of rats treated with ammonium chloride. Contrary to observations in man, dog and rat, we observed that, in the guinea pig, not only does ammonia excretion increase in acute acidosis but also in acute alkalosis(5). It was thought that investigation of the activity of renal glutaminases in the two conditions might aid in elucidating the biochemical mechanisms responsible for the increased urinary ammonia excretion. As a basis for work on the enzymatic mechanism of ammonia production, it was mandatory to characterize the enzymes involved. This had not been done previously and might explain the controversial observations. We have been able to distinguish three enzymes involved in glutamine metabolism in

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