

THE HYPERCALCEMIA OF ADRENAL INSUFFICIENCY *

BY MACKENZIE WALSER, BRIAN H. B. ROBINSON,† AND JOHN W. DUCKETT, JR.‡

(From the Department of Pharmacology and Experimental Therapeutics and the Department of Medicine, The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital, Baltimore, Md.)

(Submitted for publication October 3, 1962; accepted December 6, 1962)

An interrelationship between the adrenal glands and calcium metabolism was demonstrated in 1911 by Guleke (1), who showed that adrenalectomy ameliorates tetany in parathyroidectomized animals. This observation has been repeatedly confirmed (2).

Hypercalcemia after adrenalectomy was first demonstrated in 1924 by Kisch (3) in rabbits, and soon thereafter by other workers in dogs (4-8), cats (5, 9), and in patients with Addison's disease (10-12). An early report (13) of hypercalcemia in adrenalectomized rats was based upon dubious methods of analysis; later reports indicate that plasma calcium remains constant or falls in this species after adrenalectomy (14-16).

Administration of adrenocortical steroids often ameliorates spontaneous hypercalcemia (17), except when it is caused by hyperparathyroidism (18). Nevertheless, in rats cortisone modifies or prevents the hypercalcemic response to parathyroid extract (16, 2). This discrepancy may be a matter of dosage, since large amounts of steroids may sometimes reduce plasma calcium even in hyperparathyroidism (19, 20). In normal subjects, administration of adrenocortical steroids has little effect on plasma calcium concentration, and Cushing's disease is not associated with hypocalcemia. A report of hypercalcemia after cortisone administration to nephrectomized dogs (21) has not been confirmed (22).

Several hypotheses have been advanced to explain these observations. The possibility that hemoconcentration might explain the hypercalcemia of adrenal insufficiency was suggested by the earliest workers; however, no measurements of plasma protein concentration or ultrafiltrable cal-

cium in this condition appear to have been reported. Increased parathyroid gland activity during adrenal insufficiency was suggested by the reported finding of parathyroid hyperplasia in adrenalectomized animals (23). Against this view is the occurrence of hypercalcemia in adrenalectomized parathyroidectomized dogs (22), as well as the antagonistic effects of cortisone and parathyroid extract mentioned above.

Adrenal steroids block the excessive intestinal absorption of calcium in sarcoidosis (24) and tend to inhibit the active transport of calcium by the isolated gut (25). Calcium absorption might therefore be excessive in adrenal insufficiency. Balance studies in an Addisonian subject (26) and in adrenalectomized rats (27) do not support this possibility. The same objections apply to the hypothesis that cortisone acts by antagonizing the intestinal action of vitamin D. The possibility that the skeletal actions of vitamin D are antagonized by adrenal steroids has not been excluded, although the morphologic effects of vitamin D deficiency and steroid excess on bone are quite different (28).

Steroid administration and hypercorticism are usually associated with increased urinary calcium excretion (29, 30). After withdrawal of hormone therapy in Addison's disease (26), urinary excretion of calcium may fall. Contrary results have also been reported (31). In hypercalcemic states treated with steroids, urinary calcium excretion falls concomitantly with the reduction in plasma calcium (22); clearly this effect on plasma calcium cannot be ascribed to a renal effect of the hormone. Nevertheless, a renal role in the hypercalcemia of adrenal insufficiency remains a possibility.

Finally, evidence for a direct action of adrenal steroids on the equilibrium between bone and extracellular fluid has been presented (32). Apparently it has not been determined whether changes in ionic calcium concentration or some

* Supported by U. S. Public Health Service grant A-2306.

† Present address: Guy's Hospital, London, England.

‡ Present address: Peter Bent Brigham Hospital, Boston, Mass.

relevant ion product occur in hyper- and hypo-adrenal states as plasma calcium concentration changes.

In the present study, the incidence of hypercalcemia in adrenocortical insufficiency has been examined in three species, man, dog, and rat, and the mechanism of this change has been investigated in the dog.

In studying the incidence of hypercalcemia, it is necessary to establish some criterion for adrenal insufficiency. The absence of adrenal function is clearly an inadequate criterion for this purpose, since the syndrome of adrenal insufficiency may fail to occur in adrenalectomized animals maintained on salt alone, whereas adrenal crisis may develop despite hormone therapy under conditions of stress. One of the most characteristic features of the syndrome of adrenal insufficiency is hyponatremia. Therefore the incidence of hypercalcemia has been compared with the incidence of hyponatremia in patients with Addison's disease and in adrenalectomized dogs. In rats, hypercalcemia failed to occur after adrenalectomy, so no further study of this species was made.

In examining the mechanism of this form of hypercalcemia, observations in human subjects are not readily available because patients with the syndrome of adrenal insufficiency are uncommon and are treated as soon as the diagnosis is made. Therefore the mechanism of this disturbance has been studied in dogs.

MATERIALS AND METHODS

Subjects

Patients. Records of 31 patients with Addison's disease have been collected in which hyponatremia, or hypercalcemia, or both have been observed in a single blood sample or in a single day. These include both treated and untreated subjects. We have arbitrarily selected 135 mEq per L as the lower limit of normal sodium concentration and 11 mg per 100 ml (2.75 mmoles per L) as the upper limit of normal calcium concentration. Sixty cases of proven Addison's disease in the records of the Johns Hopkins Hospital have been examined, and 22 reports have been found of analyses of blood samples from sixteen cases that meet these criteria. Apart from scattered reports of one or two cases, the only published accounts that include appreciable numbers of calcium determinations in Addisonian patients appear to be those of Loeb (10), Helve (11) and Leeksa, De Graeff, and De Cock (12). The results of these authors are included in Figure 1.

Rats. Adrenalectomized, Sprague-Dawley, female rats

and litter-mate controls weighing 150 to 200 g were obtained the day after operation¹ and placed on diets of either milk or sodium-free milk (Lonalac) containing 20% dextrose. Each day for 5 days, groups of animals were anesthetized with ether and exsanguinated via the abdominal aorta. The adrenal regions were inspected to confirm completeness of adrenalectomy.

Dogs. Male and female mongrel dogs weighing from 7 to 16 kg were used. Twenty-five successful bilateral adrenalectomies were performed in one or two stages through flank incisions under pentobarbital anesthesia. Cortisone,² desoxycorticosterone,³ and antibiotics were provided postoperatively and when required. From time to time, hormone therapy was withdrawn and daily blood samples were obtained until frank adrenal insufficiency developed. This required from 2 to 10 days (usually 4 to 6) and was heralded by loss of appetite and decline in weight.

Three feeding regimens were employed: canned dog meat supplemented with sodium chloride and sodium bicarbonate, sweetened milk freed of calcium and magnesium, and ordinary dog chow. The milk diet was prepared by passing homogenized pasteurized cow's milk through a well-washed 10 × 50 cm column of Dowex A1 resin in the sodium form, acidifying to pH 6 to 7 with HCl, adding 200 g dextrose per L, and freezing 400-ml samples in plastic containers. Neither calcium nor magnesium could be detected in this material. Sodium concentrations varied from 100 to 120 mEq per L. Activated charcoal was mixed with the resin in order to remove a bitter-tasting material which this resin releases. The rate of onset of symptoms of adrenal insufficiency was not appreciably different with any of these three regimens.

Venous blood samples, usually from the jugular vein, were obtained in syringes containing heparin.

Analytical methods

The methods for determination of plasma pH, protein concentration, calcium, magnesium, sodium, potassium, phosphate, and citrate were the same as those cited in previous reports from this laboratory (33, 34). Ultrafiltrates were prepared from a number of samples, and the concentration of free calcium ions and free magnesium ions was determined spectrophotometrically (33). It was not possible to perform all of the determinations on every sample. Consequently, in the calculation of individual calcium complexes in plasma ultrafiltrate (34), values for magnesium ion concentration have been assumed when not available.

RESULTS

Incidence of hypercalcemia in adrenal insufficiency

In man. In Figure 1, plasma calcium concentrations have been plotted against plasma sodium

¹ Charles River Breeding Laboratories, Boston, Mass.

² 17 α ,21-dihydroxy-4-pregnene-3,11,20-trione.

³ 21-hydroxy-4-pregnene-3,20-dione.

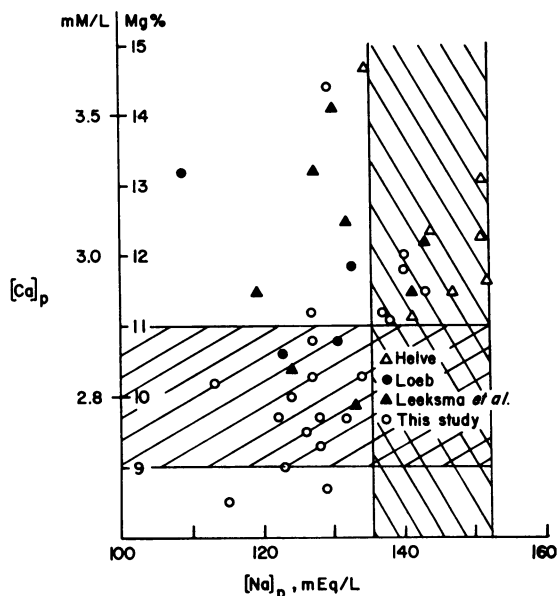


FIG. 1. PLASMA CALCIUM IN RELATION TO PLASMA SODIUM IN 31 PATIENTS WITH ADDISON'S DISEASE, EXCLUDING THOSE IN WHICH BOTH VALUES WERE NORMAL. The normal limits were arbitrarily defined. Values published by Loeb (10), Helve (11), and Leeksma, De Graeff, and De Cock (12) are included.

concentrations in thirty-one patients with Addison's disease, including cases from the Johns Hopkins Hospital records and three published reports (10-12). In each case, the calcium and sodium determinations were performed on the same blood samples, or at least on the same day. In the Johns Hopkins Hospital patients, hyponatremia appears to be about twice as frequent as hypercalcemia. Helve (11), however, found several patients with hypercalcemia without hyponatremia. These results obviously suffer from the lack of controls, differing methods, and sampling bias. Considering all the data, however, it appears that hypercalcemia approaches hyponatremia both in frequency and in severity in Addison's disease. There is no correlation, positive or negative, between hypercalcemia and hyponatremia.

In rats. Table I summarizes the results in 37 adrenalectomized and 33 control rats. Day 0 refers to the first day after adrenalectomy, when the diet was started. Plasma calcium concentrations did not change in controls or adrenalectomized animals, even when fed on sodium-free milk, despite a high mortality in the adrenalectomized rats on this diet.

In dogs. Figure 2 represents the incidence of hypercalcemia as compared with hyponatremia in adrenalectomized dogs. As in the human subjects, any sample that revealed hypercalcemia, hyponatremia, or both has been included, whether the animals were receiving therapy or not. Twenty observations from one dog are included, and from one to six observations from 24 others. The distribution of points from the one dog is similar to that from the others. The frequency of hypercalcemia is seen to be nearly identical with that of hyponatremia in these animals, even in those on a calcium-free, magnesium-free diet. The severity of hypercalcemia, however, is much greater than that of hyponatremia. One-sixth of the 58 samples exhibited calcium concentrations more than 40% above the mean normal value and nearly half were increased 20% or more. In only one of the samples was the sodium concentration reduced as much as 20%; half were reduced 10% or more. There is no correlation, either positive or negative, between hypercalcemia and hyponatremia in this group of observations. Twelve samples exhibited hyponatremia without hypercalcemia, 12 hypercalcemia without hyponatremia, and 34 showed both. The highest calcium concentration observed was 4.18 mM, or 16.7 mg per 100 ml.

There was no apparent difference between the clinical significance of hypercalcemia and that of hyponatremia in these dogs. In most cases of either disturbance, the animals were symptomatic, but a few appeared well and remained so for sev-

TABLE I

Mean plasma calcium concentration in adrenalectomized and control rats fed milk and dextrose, and sodium-free milk and dextrose*

Days on diet	Mean plasma calcium concentration			
	Milk and dextrose diet		Sodium-free milk and dextrose diet	
	Controls	Adrenalectomized rats	Controls	Adrenalectomized rats
	<i>mmoles/L</i>		<i>mmoles/L</i>	
0	2.70 [4]	2.57 [4]	2.70 [4]	2.57 [4]
1		2.50 [1]	2.86 [4]	2.62 [4]
2	2.52 [1]	2.50 [1]	2.68 [4]	2.58 [4]
3	2.62 [1]	2.48 [1]	2.74 [4]	2.74 [4]
4	2.64 [3]	2.63 [3]	2.66 [4]	2.59 [4]
5		2.52 [3]	2.67 [4]	2.66 [4]

* Numbers in brackets refer to the number of observations in each group.

TABLE II
Plasma composition in normal dogs and in hypercalcemic adrenalectomized dogs

Sample	Observed data										Derived data									
	Plasma					Ultrafiltrate					Protein-bound					Complexed Ca				
	pH (37°C)	Ca	Mg	P	Na	Ca	Ca ⁺⁺	Mg	Mg ⁺⁺	P	Cit	Ca	Mg	P	K _{CaProt} *	K' _{CaProt} *	Total	CaCit ⁻	CaHPO ₄	
	g/100 ml	mmoles/L	mmoles/L	mmoles/L	mmoles/L	mmoles/L	mmoles/L	mmoles/L	%	%	%	%	%	%	%	mmoles/L	mmoles/L	mmoles/L		
Normal dogs																				
2Z0	7.41	5.51	2.68	0.98	1.79	1.69	1.56	0.53	0.43	0.20	37	46	11	0.0092	0.13	0.08	0.07			
3A0	7.48	5.59	2.63	1.23	1.44	1.58	1.53	0.61	0.47	0.20	40	51	11	0.0084	0.05	0.08	0.06			
3B0	7.52	5.42	2.61	1.09	0.73	1.68	1.50	0.66	0.45	0.33	36	39	11	0.0092	0.18	0.13	0.03			
3C0	7.45	5.70	2.58	0.96	1.39	1.51	1.20	0.72	0.48	0.17	42	25	13	0.0066	0.31	0.06	0.04			
3D0	7.42	5.85	2.46	0.94	1.46	1.50	1.23	0.73	0.55	0.19	39	23	10	0.0079	0.27	0.06	0.05			
3E0	7.32	6.04	2.58	1.02	2.19	1.66	1.37	0.77	0.51	0.14	36	24	8	0.0096	0.29	0.05	0.08			
3F0	7.37	5.47	2.62	0.87	1.68	1.72	1.37	0.58	0.49	0.18	34	33	14	0.0084	0.35	0.07	0.06			
4I1	7.32	5.42	2.64	0.87	1.74	1.74	1.21	0.62	0.51	0.22	34	29	15	0.0076	0.53	0.07	0.05			
5K1D	7.36	5.73	2.61	0.87	1.74	1.62	1.24	0.58	0.58	0.17	38	33	15	0.0075	0.38	0.06	0.06			
Mean		5.64	2.61	0.96	1.55	1.62	1.36	0.64	0.49	1.58	38	36	11	0.0083	0.27	0.07	0.06			
SD		±.21	±.06	±.12	±.39	±.08	±.13	±.08	±.04	±.40	±.3	±.10	±.2	±.0009	±.13	±.02	±.01			
Adrenalectomized dogs																				
4B13	7.32	7.05†	3.06†	0.64†	1.74	1.69	1.45	0.49†	0.42†	1.79	45†	23	12	0.0076	0.0086	0.24	0.09	0.07		
4C5	7.41	6.08†	3.69†	0.93	3.11†	1.60	1.21	0.55	0.47	3.32†	57†	41	9	0.0031†	0.0040†	0.39	0.15†	0.10†		
4H11	7.37	8.00†	3.02†	1.86	1.39	1.68	1.29	0.37†		1.21	44†	41	45†	0.0081	0.0086	0.39	0.11	0.04		
4J2	7.55†	4.96†	2.88†	1.04	2.86†	1.97†	1.55	0.51†	0.48	2.87†	32	49	15	0.0087	0.0095	0.42	0.30†	0.11†		
4O2	7.27†	8.70†	3.69†	0.51†	3.20†	1.68	1.23	0.37†		1.22	54†	49	68†	0.0053†	0.0069	0.35	0.13	0.04		
5B34	7.34	7.08†	4.11†	0.60†	3.23†	2.66†	1.33	0.30†		3.08†	34	49	19†	0.0066	0.0069	1.33†	0.37†	0.10†		
5D15	7.30†	6.62†	3.75†	0.51†	2.12	2.30†	1.67†	0.34†		2.06	39	39	17†	0.0075	0.0086	0.63†	0.15†	0.09†		
5I13	7.35	6.75†	3.39†	0.77	2.72†	1.68	1.37	0.42†		2.65†	60†	39	17†	0.0062†	0.0067	0.31	0.16†	0.09†		
5I18	7.31†	7.08†	3.70†	0.77	3.17†	1.94†	1.36	0.82†		1.23	48†	48†	67†	0.0042†	0.0048†	0.57†	0.31†	0.04		
5N3	7.34	6.48†	3.30†	1.90	1.50	1.73	1.49	1.22	0.17	1.22	42	45†	45†	0.0060†	0.0058†	0.24	0.07	0.05		
Mean		6.88†	3.46†	0.71	2.59†	1.89	1.40	0.48	0.46	2.06	46†	38	31†	0.0063†	0.0070†	0.49	0.18†	0.07		
SD		±.96	±.38	±.19	±.59	±.33	±.14	±.81	±.26	±.81	±.3	±.10	±.21	±.0017	±.0017	±.30	±.10	±.03		

* K_{CaProt} = calculated dissociation constant of calcium proteinate complex and K'_{CaProt} = calculated dissociation constant of calcium proteinate complex, corrected to μ = 0.16.

† Values outside the normal range.

‡ Values differ significantly from normal means (p < 0.02).

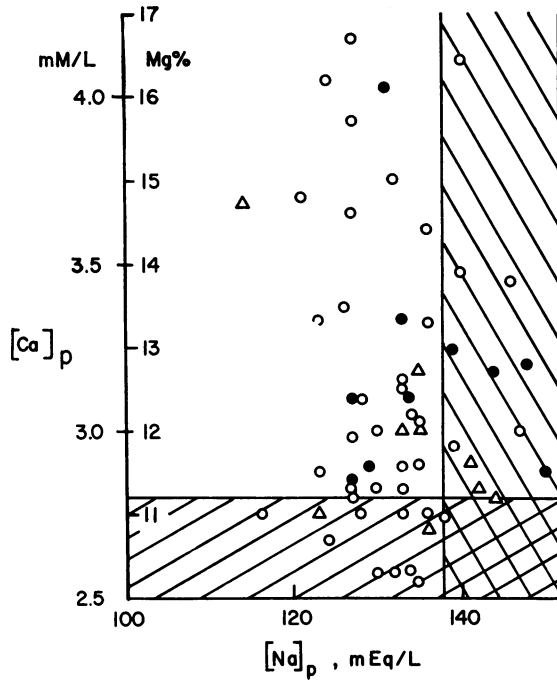


FIG. 2. PLASMA CALCIUM IN RELATION TO PLASMA SODIUM IN ADRENALECTOMIZED DOGS, EXCLUDING SAMPLES IN WHICH BOTH VALUES WERE NORMAL. Mean values ± 2 SD for 20 normal dogs are shown. Triangles: dogs on a calcium-free, magnesium-free diet. Open circles: dogs on a regular diet. Closed circles: dogs on a milk diet.

eral days. No dogs with both disturbances remained well for more than a day.

Magnesium concentrations were subnormal (less than 0.81 mM) in half of the 22 adrenalectomized dogs on magnesium-containing diets. The lowest value was 0.33 mM. Low magnesium values were not correlated with hypercalcemia, hyponatremia, or hyperkalemia. No high values were observed, contrary to an early report by Harrop, Soffer, Ellsworth, and Trescher (8).

Ionic composition of the plasma in the hypercalcemia of adrenal insufficiency

In Table II are summarized the results of analysis of plasma in nine normal dogs and ten samples from nine adrenalectomized dogs that were hypercalcemic. The normal dogs were not the same animals as the operated dogs, except for dog 4J. The values in normal dogs are similar to those previously reported in normal human subjects (34), with the following statistically significant

differences: total, ultrafiltrable, free ionic calcium, phosphate, and citrate were slightly higher; protein concentration was lower. In the adrenalectomized animals, the major finding is that free calcium ion concentration is normal in all but one dog, in which it is slightly elevated.⁴ Total plasma calcium is increased 33%, on the average, whereas ultrafiltrable calcium is increased 17% and free ionic calcium only 3%.

Complexed calcium, calculated as the difference between ultrafiltrable calcium and free ionic calcium, was not increased significantly in the group as a whole, but was beyond the normal range in three dogs, being markedly increased in one. Part of this increase is attributable to the calcium citrate complex, CaCit^- , which was abnormally increased in six out of ten dogs; a smaller part is attributable to increased amounts of the complex CaHPO_4 ; a portion is unaccounted for, particularly in one sample. Figure 3 illustrates the difference between normal dog plasma and these hypercalcemic samples.

The cause of these changes in the plasma calcium fractions is revealed in part by the data in Table II. Hyperproteinemia was seen in all but one sample. In order to determine whether the increase in protein concentration was adequate to

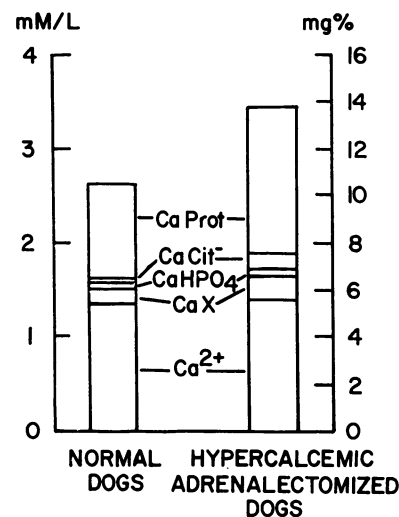


FIG. 3. MEAN VALUES FOR FRACTIONS OF PLASMA CALCIUM IN 9 NORMAL DOGS AND IN SAMPLES FROM 10 HYPERCALCEMIC ADRENALECTOMIZED DOGS. CaX = unidentified calcium complexes.

⁴ This value lies 2.4 SD from the normal mean, a deviation to be expected by chance alone 1 out of 60 times.

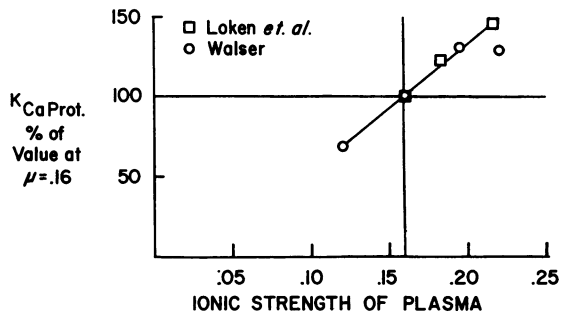


FIG. 4. EFFECT OF VARYING IONIC STRENGTH OF PLASMA ON THE ABILITY OF PLASMA PROTEIN TO BIND CALCIUM. Results of Loken, Havel, Gordan, and Whittington (37) are from normal human plasma, and results of Walser (36), from normal dog serum.

account for the increase in nonultrafiltrable calcium, the dissociation constant of calcium proteinate was calculated by the following formula: $K_{CaProt} = [Ca^{++}] (1.22 [Prot] - [CaProt]) / [CaProt]$, where $[CaProt] = [Ca]_P - [Ca]_{UF}$ (plasma and ultrafiltrate concentrations). This formula differs from that used in a previous report (35) in that free ionic calcium concentration replaces ultrafiltrable calcium. As stated previously, the choice between these two alternatives depends upon the extent of protein-binding of the filtrable calcium complexes. Since $CaCit^-$ accounts for a portion of the filtrable complexes in these samples and is not appreciably bound to protein (36), the free ionic calcium concentration was employed. As shown in Table II, five samples from four dogs exhibit abnormally low values for K_{CaProt} by this calculation; that is, the elevation of protein concentration is not sufficient to account for the increase in protein-bound calcium, and an abnormal affinity of plasma protein for calcium apparently exists.

One possible cause for this abnormal affinity is hyponatremia. When the ionic strength of plasma is varied, marked changes in the affinity of plasma protein for calcium occur (36, 37). Figure 4 represents K_{CaProt} as the percentage of normal in two reported studies in which a single plasma sample was diluted with water or salt solutions and then subjected to ultrafiltration. K_{CaProt} , as percentage of normal, can be approximated as $[Ca]_{UF} / ([Ca]_P - [Ca]_{UF})$, expressed as the percentage of the value obtained in plasma diluted with isotonic saline. The results in Figure 4 can be represented approximately by a straight line. The results of Loken, Havel, Gordan, and Whit-

tington (37) are the mean of several determinations and have therefore been given greater weight. From this line, it is possible to calculate the change in plasma calcium concentration required to maintain constant ultrafiltrable calcium when ionic strength varies. If normal ionic strength is taken as $\mu = 0.16$ and normal sodium concentration as 145 mEq per L, these results can be expressed in terms of plasma sodium, as in Figure 5. This graph shows that hyponatremia to the extent of 110 mEq per L can be expected to increase plasma calcium 2 mg per 100 ml if ultrafiltrable calcium is to remain constant.

When the values for K_{CaProt} shown in Table II are corrected for hyponatremia according to the line in Figure 4, three remain abnormally low. Thus an abnormally high affinity of plasma protein for calcium evidently exists in these three dogs.

Citrate concentrations were abnormally elevated in seven out of ten samples. In three, very high values were seen. Obviously, free citrate ion concentration was increased, as well as the amounts of $NaCit^-$, $MgCit^-$, and $CaCit^-$. The hypercitremia did not account, however, for more than 20% of the increase in plasma calcium in any dog. In patients with Addison's disease, slight elevations of plasma citrate have been reported (38).

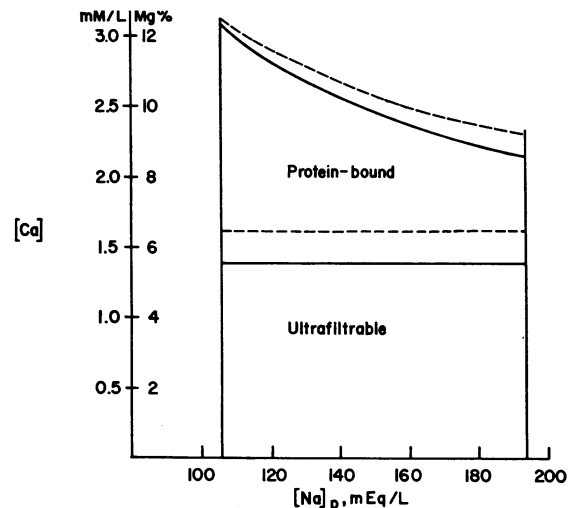


FIG. 5. TOTAL PLASMA CALCIUM VALUE REQUIRED TO MAINTAIN CONSTANT ULTRAFILTRABLE CALCIUM VALUE IN MAN (SOLID LINE) AND IN DOGS (DOTTED LINE) WHEN IONIC STRENGTH OF PLASMA CHANGES, CALCULATED FROM DATA IN FIGURE 4.

Plasma phosphate was elevated in six of ten samples and in the group as a whole. In seven samples, the fraction of filtrable phosphate was reduced, in four cases to a marked degree. When these plasma samples were subjected to ultrafiltration after the addition of 4 mmoles per L of the trisodium salt of EDTA, the filtrability of phosphate became normal. This same finding was reported in the other hypercalcemic states (35), and was attributed to the formation of so-called "colloidal calcium phosphate," either *in vivo* or *in vitro*. In the present samples, this explanation may not be tenable because of the large amounts of nonultrafiltrable phosphate involved. In sample 5I18, for example, nearly 2 mmoles per L of plasma phosphate were nonultrafiltrable until EDTA was added. Yet the total amount of nonultrafiltrable calcium and magnesium was only 2 mmoles per L. If the nonultrafiltrable phosphate was associated with calcium and magnesium, as the results with EDTA indicate, the entire amounts of nonultrafiltrable calcium and magnesium must have been in this form. These considerations lead us to suspect that this form of nonultrafiltrable phosphate is at least in part an artefact of some kind, as against the nonultrafiltrable phosphate in normal plasma, which is unaffected by EDTA (39). Conceivably, there may be labile, nondialyzable, organic phosphate compounds in plasma that are converted to inorganic phosphate either by trichloroacetic acid or by EDTA, but this seems improbable. This phenomenon may account for some of the hypercalcemia of adrenal insufficiency, since two of the animals with large amounts of nonultrafiltrable phosphate were those with abnormal values for K'_{CaProt} ⁵ (5I18 and 5N3). In other words, the apparently excessive affinity of protein for calcium in these dogs may be due to the presence of a nonfiltrable compound of calcium and phosphate. This compound must be present *in vivo* to contribute to hypercalcemia, but in all probability the amount of this compound increased *in vitro* after the blood was drawn.

Magnesium concentrations were reduced in one-third to one-half of the samples in which measurements were made. None of these dogs was receiving calcium-free, magnesium-free milk. Pro-

⁵ Calculated dissociation constant of calcium proteinate complex, corrected to $\mu = 0.16$.

tein-binding of magnesium was normal in three samples.

DISCUSSION

Hypercalcemia is evidently a common feature of adrenocortical insufficiency both in adrenalectomized dogs and in patients with Addison's disease. For unknown reasons, it apparently does not occur in rats.

In contrast to the findings in other types of hypercalcemia (35), the free ionic calcium concentration is not increased. Several other factors, singly or in combination, account for this hypercalcemia: hemoconcentration, increased plasma concentration of citrate and other complexing anions, and an abnormal affinity of plasma protein for calcium, attributable in part to hyponatremia itself.

Considering the dogs as a group, the average increase in plasma calcium was 0.85 mmole per L. Hyperproteinemia alone accounted for only a fourth of this increment. A like amount is attributable to increased complexed calcium. The other half of the increase is the result of an apparently increased affinity of plasma protein for calcium. Part of this change in affinity is attributable to hyponatremia itself; the remainder is unexplained. Only three samples, however, exhibited this change and in two of the three, a nonfiltrable form of calcium phosphate appears to be responsible.

There is no reason to implicate excessive intestinal absorption of calcium as a contributory cause of this type of hypercalcemia. The total amount of calcium in the extracellular fluid is probably close to normal. Although total calcium concentration in plasma was increased an average of 33%, plasma volume was probably considerably reduced. Likewise, interstitial fluid volume was probably reduced, so that the 17% increase in ultrafiltrable calcium would not be inconsistent with a normal amount of calcium in the interstitial fluid. Furthermore, hypercalcemia occurred with comparable frequency in adrenalectomized dogs fed a calcium-free, magnesium-free diet.

A renal role in this hypercalcemia has not been excluded. As shown elsewhere (40), renal tubular reabsorption of calcium is excessive in adrenally insufficient dogs. Daily urinary calcium excretion was determined in a number of animals

and was usually less than 0.3 mmole (12 mg), despite hypercalcemia. An increase in calcium excretion could presumably restore a normal plasma calcium concentration, but only at the cost of a subnormal ionic calcium concentration. If more calcium were released from bone, hypercalcemia would recur.

Since the concentration of free calcium ions was normal, there is no need to postulate an increase in parathyroid hormone secretion, an increased sensitivity to vitamin D, or a direct effect of deficiency of adrenal hormones on the equilibrium between bone mineral and extracellular fluid.

There is no evidence that the hypercalcemia of adrenal insufficiency is deleterious to the organism. Since the effective calcium concentration is normal, the only possible effects are those of increased protein-bound or complexed calcium. These fractions are not known to exert any physiological actions, with the possible exception of the complex CaHPO_4 (35).

In some respects, this form of hypercalcemia resembles that produced locally by prolonged venous obstruction, so-called "*in vivo* filtration" (41). In the venous blood from an arm whose venous outflow has been occluded, protein concentration and total calcium are increased, plasma volume is reduced, and ultrafiltrable calcium concentration is normal. It is uncertain whether hypercalcemia is a common feature of the hemoconcentration seen in salt and water depletion.

The peculiar nonultrafiltrability of plasma phosphate in half of these samples makes somewhat uncertain any conclusions about the free ion product $[\text{Ca}^{++}][\text{HPO}_4^{--}]$. The observed values for this product are proportional to the concentrations of the complex CaHPO_4 given in Table II, since the quotient of these quantities, $[\text{Ca}^{++}][\text{HPO}_4^{--}]/[\text{CaHPO}_4]$, is the dissociation constant of the complex. As indicated by the data in the table, increased products were observed in half of the samples. Even higher products would have been obtained if the filtrability of phosphate had been normal. These high products may be a reflection of renal failure, with a consequent accumulation of inhibitors of calcification in the plasma (35). There may also be some connection between nonultrafiltrable phosphate and renal failure, since the four samples in which this phenomenon was most pronounced were also among the most hy-

perphosphatemic and acidotic ones. An alternative possibility is that inhibitors of calcification fail to occur in adrenal insufficiency, despite azotemia, and that calcium phosphate microcrystals consequently form in plasma on cooling and standing. The calcification of the external ears sometimes found in Addison's disease might conceivably have something to do with this phenomenon.

Just as slight hypercalcemia may be considered as a normal response to hyponatremia (see Figure 5), the hypocalcemia seen in hypernatremic states (42) may be explained in part on this basis. It would be of interest to determine ultrafiltrable calcium in this disorder.

The hypomagnesemia seen in adrenal insufficiency in these dogs is apparently a new observation, for which we have no explanation. It is interesting to note that hypomagnesemia is also seen occasionally in hyperaldosteronism (43). Reports of plasma magnesium in patients with adrenal insufficiency are apparently lacking.

SUMMARY

Hypercalcemia occurs as frequently as hyponatremia in adrenalectomized dogs, and is considerably more pronounced. In patients with Addison's disease, it appears to be somewhat less frequent, but adequate data are lacking. In adrenalectomized rats, it does not occur at all, even when they are fed a high-calcium, low-sodium diet.

In dogs, the hypercalcemia of adrenal insufficiency may be severe (up to 4.18 mmoles per L. or 16.7 mg per 100 ml), but the concentration of free calcium ions was nevertheless found to be normal. Plasma magnesium was usually reduced. Three alterations in the plasma combined to produce hypercalcemia: first, the elevated plasma protein concentration associated with hemoconcentration; second, an increase in filtrable calcium complexes, especially calcium citrate; and third, an increase in the affinity of plasma protein for calcium, which could be explained in part as a consequence of hyponatremia and the resulting reduction in ionic strength of plasma, and in part as a consequence of excessive amounts of a nonfiltrable compound of calcium and phosphate, formed either *in vivo*, or *in vitro*, or both.

The increased calcium concentration of the

plasma is not dependent upon increased intestinal absorption of calcium, since it also occurred on a calcium-free diet.

ACKNOWLEDGMENTS

We are greatly indebted to medical students J. Brown, P. Briscoe, J. Fries, M. Robson, and C. Van Way for permission to include the observations on rats that were made during a student research project as part of the course in pharmacology, and to Sylvia Butler, Mary Jane Ford, and Edna Richardson for technical assistance.

REFERENCES

- Guleke, P. Experimentelle Untersuchungen über Tetanie. *Langenbecks Arch. klin. Chir.* 1911, **94**, 496.
- Laron, Z., J. P. Muhlethaler, and R. Klein. The interrelationship between cortisone and parathyroid extract in rats. *Arch. Path.* 1958, **65**, 125.
- Kisch, B. Experimentelle Untersuchungen über die Funktion der Nebennieren. *Klin. Wschr.* 1924, **3**, 1661.
- Lucas, G. H. W. Blood and urine findings in desuprarenalized dogs. *Amer. J. Physiol.* 1926, **77**, 114.
- Taylor, N. B., and W. R. Caven. Observations upon the serum calcium after adrenalectomy (abstract). *Amer. J. Physiol.* 1927, **81**, 511.
- Rogoff, J. M., and G. N. Stewart. Studies on adrenal insufficiency. VII. Further blood studies (cholesterol and calcium) in control adrenalectomized dogs. *Amer. J. Physiol.* 1928, **86**, 25.
- Hastings, A. B., and E. L. Compere. Effect of bilateral suprarenalectomy on certain constituents of the blood of dogs. *Proc. Soc. exp. Biol. (N. Y.)* 1931, **28**, 376.
- Harrop, G. A., L. J. Soffer, R. Ellsworth, and J. H. Trescher. Studies on the suprarenal cortex. III. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. *J. exp. Med.* 1933, **58**, 17.
- Baumann, E. J., and S. Kurland. Changes in the inorganic constituents of blood in suprarenalectomized cats and rabbits. *J. biol. Chem.* 1926, **71**, 281.
- Loeb, R. F. Chemical changes in the blood in Addison's disease. *Science* 1932, **76**, 420.
- Helve, O. A study of the metabolism in Addison's disease. II. On the metabolism of lipids, nitrogen, and minerals, and on the vitamin C household. *Acta med. scand.* 1947, **128**, 1.
- Leeksa, C. H. W., J. De Graeff, and J. De Cock. Hypercalcaemia in adrenal insufficiency. *Acta med. scand.* 1957, **156**, 455.
- Rohdenburg, G. L., and O. F. Krehbiel. On the relation of certain endocrines to the salt content of rat blood. *J. Cancer Res.* 1925, **9**, 422.
- Helve, O. E. Studien über den Einfluss der Nebennieren-exstirpation auf den tierischen Stoffwechsel. *Biochem. Z.* 1940, **306**, 343.
- Conway, E. J., and D. Hingerty. The influence of adrenalectomy on muscle constituents. *Biochem. J.* 1946, **40**, 561.
- Kleeman, C. R., D. Bernstein, J. T. Dowling, J. M. Koplowitz, and M. H. Maxwell. The combined influence of the adrenal and parathyroid glands in the regulation of serum calcium (abstract). *J. clin. Invest.* 1959, **38**, 1018.
- Connor, T. B., T. R. Hopkins, W. C. Thomas, Jr., R. A. Carey, and J. E. Howard. The use of cortisone and ACTH in hypercalcemic states (abstract). *J. clin. Endocr.* 1956, **16**, 945.
- Dent, C. E. Cortisone test for hyperparathyroidism. *Brit. med. J.* 1956, **1**, 230.
- Jackson, W. P. U. Lowering by prednisone of serum-calcium in hyperparathyroidism. *Lancet* 1961, **1**, 893.
- Gordan, G. S. The Yearbook of Endocrinology, 1960-61. Chicago, Year Book Medical Publishers, 1961, p. 75.
- Grollman, A. Effect of cortisone on serum calcium, magnesium and phosphate levels in nephrectomized dogs. *Proc. Soc. exp. Biol. (N. Y.)* 1954, **85**, 582.
- Myers, W. P. L. Studies of serum calcium regulation in *Advances in Internal Medicine*, I. Snapper, Ed. Chicago, Year Book Publishers, 1962.
- Rogoff, J. M. Observations on functional interrelationship between the adrenal and parathyroid glands. *Science* 1934, **80**, 319.
- Anderson, J., C. E. Dent, C. Harper, and G. R. Philpot. Effect of cortisone metabolism in sarcoidosis with hypercalcaemia. Possibly antagonistic actions of cortisone and Vitamin D. *Lancet* 1954, **2**, 720.
- Kimberg, D. V., D. Schachter, and H. Schenker. Active transport of calcium by intestine: effects of dietary calcium. *Amer. J. Physiol.* 1961, **200**, 1256.
- Tibbetts, D. M., and J. C. Aub. Magnesium metabolism in health and disease. III. In exophthalmic goiter, basophilic adenoma, Addison's disease and steatorrhea. *J. clin. Invest.* 1937, **16**, 511.
- Rubin, M. I., and E. T. Krick. The salt and water metabolism of adrenal insufficiency and partial starvation in rats. *J. clin. Invest.* 1936, **15**, 685.
- Laron, Z., B. D. Canlas, Jr., and J. D. Crawford. The interaction of cortisone and vitamin D on bone of rachitic rats. *Arch. Path.* 1958, **65**, 403.
- Molinatti, G. M., F. Camanni, and M. Olivetti. A study on the metabolism of calcium in the hyperadrenocortical syndrome. *Acta Endocrinol.* 1960, **34**, 323.
- Laake, H. The action of corticosteroids on the renal reabsorption of calcium. *Acta Endocr. (Kbh.)* 1960, **34**, 60.

31. Tuttle, S. G., and W. G. Figueroa. Hypercalciuria associated with reduction in corticoid therapy after prolonged administration of prednisone and after bilateral adrenalectomy for Cushing's syndrome (abstract). *J. clin. Invest.* 1958, **37**, 937.
32. Schartum, S., and G. Nichols, Jr. Influence of adrenal glucocorticoids on distribution of calcium and phosphorus between bone and its surrounding fluids. *Proc. Soc. exp. Biol. (N. Y.)* 1961, **108**, 228.
33. Walser, M. Determination of free magnesium ions in body fluids. Improved methods for free calcium ions, total calcium, and total magnesium. *Analyt. Chem.* 1960, **32**, 711.
34. Walser, M. Ion Association. VI. Interactions between calcium, magnesium, inorganic phosphate, citrate and protein in normal human plasma. *J. clin. Invest.* 1961, **40**, 723.
35. Walser, M. The separate effects of hyperparathyroidism, hypercalcemia of malignancy, renal failure, and acidosis on the state of calcium, phosphate, and other ions in plasma. *J. clin. Invest.* 1962, **41**, 1454.
36. Walser, M. Ion Association. I. The effect of multivalent anions on the protein-bound and complexed calcium in serum. *J. cell. comp. Physiol.* 1960, **55**, 245.
37. Loken, H. F., R. J. Havel, G. S. Gordan, and S. L. Whittington. Ultracentrifugal analysis of protein-bound and free calcium in human serum. *J. biol. Chem.* 1960, **235**, 3654.
38. Mårtensson, J. Hypercitricemia in Addison's disease. *Acta med. scand.* 1949, **134**, 61.
39. Walser, M. Protein-binding of inorganic phosphate in plasma of normal subjects and patients with renal disease. *J. clin. Invest.* 1960, **39**, 501.
40. Robinson, B. H. B., M. Walser, and E. B. Marsh, Jr. Unpublished observations.
41. Gerbrandy, J., A. M. van Leeuwen, H. B. A. Hellen-doorn, and L. A. de Vries. The binding between electrolytes and serum proteins calculated from an *in vivo* filtration method. *Clin. Sci.* 1960, **19**, 181.
42. Finberg, L. Experimental studies of the mechanisms producing hypocalcemia in hypernatremic states. *J. clin. Invest.* 1957, **36**, 434.
43. Milne, M. O., R. C. Muehrcke, and I. Aird. Primary aldosteronism. *Quart. J. Med.* 1957, **26**, 317.

ANNOUNCEMENT OF MEETINGS

THE AMERICAN FEDERATION FOR CLINICAL RESEARCH will hold its Twentieth Annual Meeting in Atlantic City, N. J., at the Casino Theatre on the Steel Pier on Sunday, April 28, 1963, at 9:00 a.m. Joint sectional meetings with The American Society for Clinical Investigation will be held on Sunday afternoon at Chalfonte-Haddon Hall, and additional meetings sponsored by The American Federation for Clinical Research will be held there on Sunday evening.

THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, INC., will hold its Fifty-fifth Annual Meeting in Atlantic City, N. J., on Monday, April 29, at 9:00 a.m. at the Casino Theatre on the Steel Pier and in simultaneous programs sponsored with The American Federation for Clinical Research on Sunday afternoon, April 28, in Chalfonte-Haddon Hall.

THE ASSOCIATION OF AMERICAN PHYSICIANS will hold its Seventy-sixth Annual Meeting in Atlantic City, N. J., at the Casino Theatre on the Steel Pier on Tuesday, April 30, at 9:30 a.m., and in the Vernon Room, Chalfonte-Haddon Hall, on Wednesday, May 1, at 9:30 a.m.

THE AMERICAN SOCIETY FOR CLINICAL NUTRITION will hold its Third Annual Meeting in Atlantic City, N. J., at the Colton Manor Hotel on Saturday, April 27, from 1:00 to 5:00 p.m.