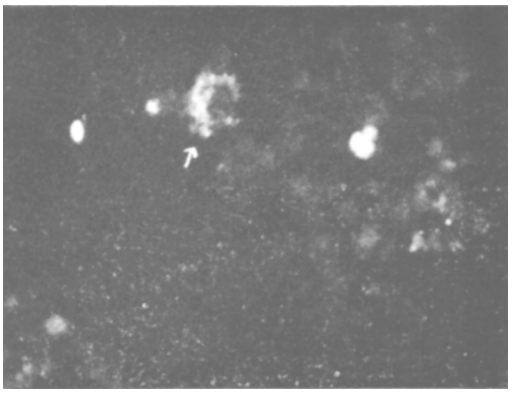

FIG. 2. NDV-infected megakaryocyte in guinea pig bone marrow cell culture after 15 hr incubation. Fluorescent antibody staining × 128.

or incomplete virus; megakaryocytes comprise only a small fraction of the total number of cells in bone marrow culture, therefore it is very difficult to determine by infectivity counts whether the virus multiplied in the megakaryocytes. It is supposed that the antigen found in megakaryocytes in cell cultures is newly formed since the infection with NDV is followed by an eclipse period of about 4 hours when no antigen is demonstrable inside the cells in infected cultures. In the study of Prince and Ginsberg(11), infection of Ehrlich ascites tumor cells with NDV also resulted in formation of new antigen following an eclipse period. They showed the viral antigen to be associated with incomplete NDV.

Whether the suppression of thrombopoiesis by NDV might have a bearing on the mechanism of thrombocytopenia occurring in virus infections is not known.

Summary. Incubation of bone marrow cells with NDV results in morphological changes in megakaryocytes and suppression of thrombopoiesis. Fluorescent antibody staining of infected megakaryocytes in cell culture shows adsorption of NDV, an eclipse period, and formation of new viral antigen starting at 4 hours after infection.

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The Blood Calcium Lowering Effect of Hydrocortisone in Parathyroidectomized Rats. (28772)

HERBERT C. STOERK,* ARLENE C. PETERSON AND VIOLA C. JELINEK
Merck Institute for Therapeutic Research, Rahway, N. J.

Previously it has been observed that hydrocortisone (Cpd. F) lowers the blood Ca in hypoparathyroid patients(1) and in parathyroidextomized (PTX) animals of several species(2,3). The same treatment has no effect on the blood Ca of patients or animals with functioning parathyroids. Since the parathyroids are known to maintain Ca-homeostasis by secreting more parathormone (PTH) in response to lowered Ca concentrations, we have suggested that compensatory

* Present address: Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.

TABLE I. Effect of Cpd. F (3 mg/kg) upon Serum Ca of Parathyroidectomized Rats.

Trials	PTX		PTX + F		Difference	Significance P
	No. of rats	Ca	No. of rats	Ca		
1	10	8.16 ±.21	9	6.84 ±.17	1.32	<.001
2	7	8.61 ±.11	7	7.29 ±.28	1.32	<.01
3	5	7.96 ±.20	5	7.22 ±.14	.74	<.05
4	7	7.84 ±.28	7	6.80 ±.22	1.04	<.05
5	10	7.99 ±.23	10	6.61 ±.26	1.38	<.001
6	5	7.50 ±.29	5	6.42 ±.07	1.08	<.01
7	11	8.32 ±.25	11	7.89 ±.26	.43	N.S.
		Weighted mean difference			1.04	<.001
		95% confidence limits			.78, 1.30	

Trials	ADX-PTX		ADX-PTX + F		Difference	Significance P
	No. of rats	Ca	No. of rats	Ca		
1	4	9.00 ±.15	5	7.56 ±.21	1.44	<.01
2	10	9.00 ±.28	10	7.03 ±.26	1.97	<.001
3	6	9.35 ±.29	5	7.08 ±.07	2.27	<.001
4	12	10.65 ±.36	12	8.83 ±.56	1.82	<.05
5	7	9.34 ±.29	8	8.18 ±.37	1.16	<.05
		Weighted mean difference			1.74	<.001
		95% confidence limits			1.33, 2.15	

hyperactivity of the parathyroids prevents the blood Ca-lowering effect of excess Cpd. F in presence of functioning parathyroids(3). Further evidence compatible with parathyroid hyperactivity in steroid treated animals consisted of the following observations: It was found that Cpd. F caused striking inhibition of tubular reabsorption in intact but not in PTX rats and that the steroid suppressed the appearance of orally fed labeled Ca in the blood of parathyroidectomized but not in unoperated rats(3).

The present report deals with further studies of the blood Ca in Cpd. F-treated PTX rats. Attempts were also made to determine the extent of the compensatory hyperactivity of the parathyroids in Cpd. F-treated rats. Finally, the possibility was examined that changes in blood citrate may have mediated the steroid effect upon the blood Ca.

Materials and methods. Male rats of the Sprague-Dawley strain, Holtzman, were used throughout the experiments. Observations on serum calcium and citrate were made on rats ranging in weight from 115-300 g. Parathyroidectomies, by electrocautery, adrenalectomies and nephrectomies were performed under ether anesthesia. Eli Lilly bovine parathyroid extract (PTE) and Merck Hydrocortisone were injected subcutaneously. PTE injections were controlled by injections of saline acidified with HCl to a pH of 3.4. Dilutions of PTE were made in the same vehicle. Hydrocortisone was injected in 30% EtOH while controls received 30% EtOH alone. In some experiments the rats were fed a semi-synthetic diet (Ca, .04%; P, .07%) composed of lactalbumin 24%, sucrose 63%, corn oil 5%, KH_2PO_4 4.4%, Ca and P free salt mixture 2.6%, and a mixture of all known vitamins, except Vit. D, in the usual quantities. Ca and P were added to this diet in amounts indicated in the text. In all other experiments the animals were maintained on a stock diet (Purina Chow). Serum calcium was determined in 1 ml of serum by the method of Berger(4), serum citrate was determined in 2 ml serum by the method of Beutler and Yeh(5).

Results. Table I illustrates the effect of Cpd. F (30 mg/kg body weight upon the serum Ca of 94 PTX rats compared to that of the same number of controls. The animals in Group A were PTX only, while in Group B adrenalectomies were performed in addition to the parathyroidectomies. The operations were immediately followed by the injections and Ca determined from serum obtained 6 hours after the start of the experiments.

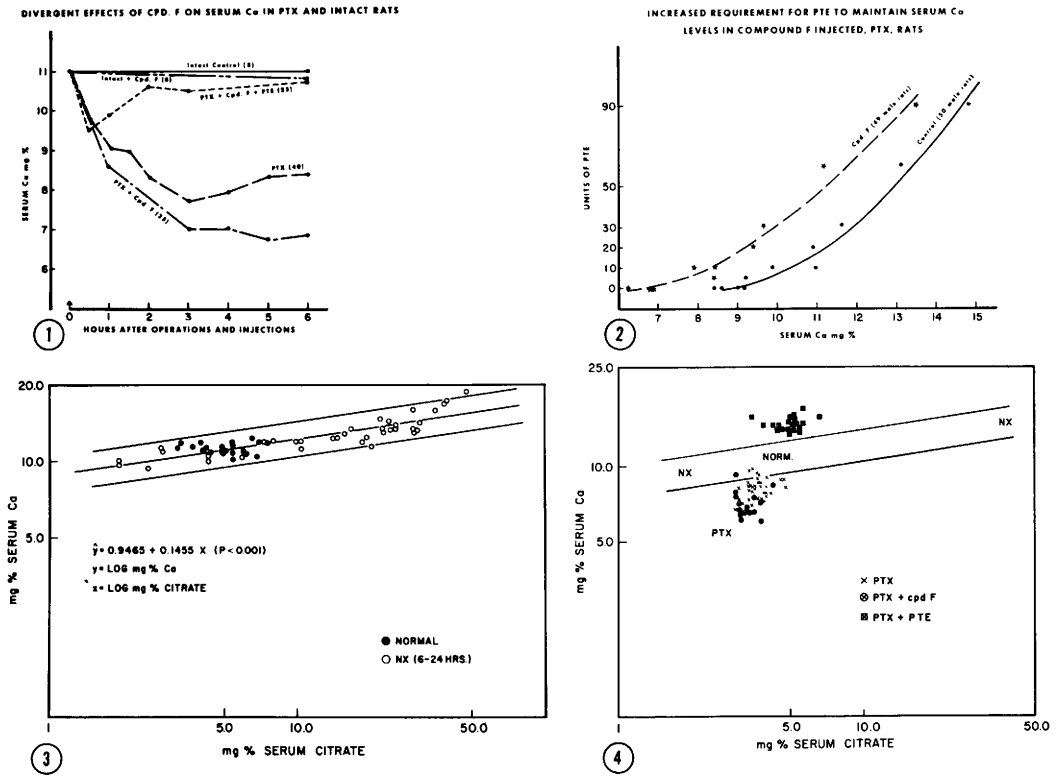

FIG. 1. Comparison of serum Ca levels in groups of rats taken at various intervals up to 6 hours.

FIG. 2. Comparison of 2 main groups of PTX, ADX rats—one injected with hydrocortisone (Cpd. F) and with increasing amounts of PTE, the other with PTE alone.

FIG. 3. Relation of serum Ca to serum citrate concentrations in nephrectomized and in normal rats.

FIG. 4. Serum Ca and serum citrate levels in rats 6 hr after parathyroidectomy and simultaneous injection of Cpd. F (30 mg/kg) and 24 hr after 2 injections of bovine PTE (50 units).

In 7 different trials it was observed that 6 hours after parathyroidectomy, the blood Ca of control rats decreased from the usual levels to values which varied from 7.5 to 8.6 mg%. Cpd. F-treated, PTX rats exhibited a greater drop in serum Ca (6.4-7.9 mg%). In Group B adrenals were removed to eliminate the source of endogenous adrenal steroids. Accordingly, following parathyroidectomy, serum Ca declined much less than in the non-ADX animals in Group A. Since the steroid in these animals produced hypocalcemia of about the same order as in Group A, the difference between Cpd. F-treated rats and their controls became even more striking.

The data summarized in Fig. 1 serve to illustrate the time sequence of the changes in serum calcium. Pooled sera from groups of at least 5 animals each were taken from rats killed at the time intervals indicated on the horizontal axis. The average serum Ca (11.1 mg%) of 10 non-PTX, ADX rats at start of the experiment is compared to that of 2 groups of 8 animals 6 hours later. At 0 hour, one of these groups was injected with hydrocortisone in 30% ethanol (30 mg/kg), the other with ethanol alone. Ca determinations in these rats were done individually. In the presence of functioning parathyroids no change in serum Ca was caused by the steroid. The serum Ca of PTX rats fell to about 8.5 mg%, that of the PTX steroid-treated groups to about 7 mg%. PTE (50 units) injected together with the steroid prevented the fall in blood Ca and during the most part of the 6 hours maintained nearly normal blood Ca levels. These findings indi-

TABLE II. Effect of 21 Daily Injections of Cpd. F to Parathyroidectomized Rats on a Low P Diet.

Exp group	% in diet Ca	% in diet P	mg % serum Ca	No. of rats
Control	.3	.07	10.6 ±.19	12
Cpd. F	.3	.07	8.8 ±.33	14
Control	.1	.07	9.3 ±.15	9
Cpd. F	.1	.07	6.1 ±.49	10

cate that the effect of Cpd. F upon blood Ca levels escapes detection unless the regulating activity of hyperfunctioning parathyroids is eliminated. Attempts to determine the order of magnitude of this compensatory hyperactivity of the glands are summarized in Fig. 2. The procedure in this experiment is essentially the same as in the preceding one. Ninety-nine rats were parathyroidectomized, divided in groups of usually 5 animals each, then immediately injected, subcutaneously, with Cpd. F (20 mg/kg in EtOH). The controls were given a subcutaneous injection of 30% EtOH alone. Subgroups in both categories were injected subcutaneously at a separate site but simultaneously with graded doses of bovine PTE (5-90 U.S.P. units). In the graph the average serum Ca in each group determined individually, is plotted against the number of units of PTE. PTX rats injected with PTE showed a rise in serum Ca which over a wide range of calcium concentrations was proportional to the number of units injected. The quantity of PTE required to restore the serum Ca in PTX controls to normal levels represents adequate replacement therapy and was found to be 10-20 U.S.P. units. PTX rats injected with hydrocortisone required about 60 units of PTE to maintain normal blood Ca levels. This increased requirement for PTE to maintain normal serum Ca levels represents a measure of the extent of the hyperactivity of the parathyroids in hydrocortisone-injected rats and over the entire range of observations, was calculated to be 3.5 (95% confidence limit 2.2-5.9) times greater in the steroid-treated groups than in controls.†

The findings in Table II show that the blood Ca-lowering effect of Cpd. F is not a transient one and that PTX rats treated with the steroid daily for a prolonged period persistently retain much lower blood Ca levels than untreated PTX controls. In these experiments PTX rats were kept on a low P intake in order to permit the majority of them to survive. On these diets, controls maintained nearly normal blood Ca levels.

The sum of these findings demonstrates that the blood Ca of PTX rats is reproducibly lowered by the action of Cpd. F. Because of the prompt appearance of this effect, hours after injection of the steroid, the possibility that the reduction of total calcium was due to a decrease of the protein-bound calcium appeared most unlikely. However, serum citrate is known to affect the solubility of calcium and has been shown by Harrison and Harrison(6) and Agrell(7) to be lowered during hydrocortisone treatment. It appeared, therefore, possible that the hypocalcemia was due to a depression of citrate. A regular relationship between calcium and citrate levels in the blood of nephrectomized rats has been observed by several investigators and was defined by Freeman for 3 levels of serum citrate(8). We have repeated these experiments and extended them to explore the effect of Cpd. F and of PTH upon the relation of calcium to citrate in the serum of PTX rats.

Fig. 3 illustrates the relation of calcium to citrate in the serum of normal and of nephrectomized rats. In the graph, calcium has been plotted against citrate on a log, log scale. Each point represents an individual observation. A highly significant ($P<0.001$) straight line relationship was obtained ($Y = 0.9465 + 0.1455\ X$) when the log mg% Ca (Y) was related to the log mg% citrate (X). About 1/3 of the rats nephrectomized for 24 hours, or starved nephrectomized rats (which are not included in the graph) exhibited serum Ca and citrates lower than those observed in normal rats. The highest values for both calcium and citrate were usually obtained in rats nephrectomized 18 hours prior to taking the blood samples.

In Fig. 4 the range of the data from the previous graph is outlined by the solid lines.

† We are indebted to Mr. J. Ciminera for the statistical evaluation.

Compared to this is the serum calcium in relation to citrate of PTX rats and similar rats injected with Cpd. F or bovine PTE. Blood calcium and citrate levels of PTX or PTX-Cpd. F-injected rats do not follow the linear relationship observed in Fig. 3 but in 6 hours show a sharp drop in calcium without any appreciable concomitant fall in citrate. Conversely, injection of bovine PTE caused a marked increase in serum calcium but no significant rise in serum citrate. It appears, therefore, that neither the effect of parathormone nor that of Cpd. F upon blood calcium is mediated by circulating citrate.

Discussion. The blood Ca-lowering effect of hydrocortisone in PTX rats could be due to a number of possible actions of the steroid. Increased uptake of Ca by bone or its greatly enhanced excretion could account for the diminished quantities in blood. However, experimental evidence does not lend support to either of these possibilities. The uptake of labeled Ca into tibia of Cpd. F treated rats was found essentially unchanged(9). While striking calciuria follows administration of the steroid, the quantities of Ca excreted appear much too small to account for the rapid drop in blood Ca. Other possibilities include inhibition of Ca-absorption from the intestine or of Ca-mobilization from bone. Evidence for both of these possibilities has been obtained from experiments in rats. Earlier, we have reported that the appearance in the peripheral circulation of Ca^{45} fed orally was strikingly suppressed by Cpd. F in PTX but not in intact rats(3). In PTX rats, the rate of disappearance of intravenously injected Ca^{45} was not altered by the steroid. These findings suggested that intestinal absorption was suppressed by the steroid but enhanced by PTH.

Other findings compatible with the possibility of intestinal malabsorption of Ca in steroid-treated animals are the following:

In isolated intestinal loops, Cpd. F inhibited the transfer of Ca(10). Furthermore, we have observed that in rats on a Ca-deficient diet for 10 days, then PTX and injected with Cpd. F 6 hours before sacrifice, blood Ca was not lowered by the steroid (10 Cpd. F-treated rats, 5.6 $\pm$ 0.29; 10 controls, 5.5 $\pm$ 0.14). This finding might be interpreted to mean that in the absence of dietary Ca the blood Ca-lowering action of Cpd. F cannot be obtained although it also seems possible that such extreme hypocalcemia cannot be further exaggerated. Findings affirming the possibility that Cpd. F may counteract the Ca-mobilizing effect of PTH have recently been presented(11). It was observed that an excess of both PTH and of Cpd. F caused rarefication of metaphyseal spongiosa in rats but it was also seen that the development of "osteitis fibrosa," characteristic of excess PTH was largely suppressed by the steroid treatment. Quantitative studies are required to clarify which action of the steroid predominates in lowering the blood Ca of PTX animals.

A number of investigators have come to believe with Nordin(12) that "idiopathic" osteoporosis may be due to prolonged Ca-deficiency. Ca-deprivation is known to lead to secondary hyperparathyroidism and it seems likely that if osteoporosis is due to lack of Ca it should be associated with increased parathyroid activity. Spontaneous and iatrogenic Cushing's disease are invariably associated with osteoporosis. The circumstantial evidence presented here strongly suggests that excess glucocorticoid in some way leads to Ca-deprivation and parathyroid stimulation. While the morphological changes typical of hyperparathyroidism and of osteoporosis have one feature in common—the rarefication of bone—they are otherwise unmistakably different. Is it then conceivable that hyperparathyroidism of a low order of magnitude may lead to osteoporosis without exhibiting any of the classic features of "osteitis fibrosa"? Also in view of the findings of suppressed osteitis in steroid treated rats, is Ca-deficiency perhaps associated with adrenal cortical stimulation and are the bone changes of "idiopathic" osteoporosis explained by an excess of both PTH and Cpd. F? Sensitive methods for the measurements of PTH and of Cpd. F in biological fluids have recently been developed. Their use in patients with idiopathic, and with steroid osteoporosis should resolve these questions.

Summary and conclusions. PTX rats, 6

hours after injection of hydrocortisone, exhibited more pronounced hypocalcemia than PTX controls. In non-PTX rats the serum Ca was not lowered after injection of the steroid. Comparing the effect of graded doses of bovine PTE (5-90 U.S.P. units) upon the serum Ca of PTX, hydrocortisone-injected rats and of PTX controls 3.5 times more PTE had to be administered to the hydrocortisone-injected group to sustain normal serum Ca concentrations in the 2 groups of PTX animals. Thus it appears that the parathyroids of hydrocortisone-injected rats secrete about 3 times more hormone than those of non-injected controls. The serum Ca-lowering effect of hydrocortisone was not a transient one and was demonstrable after 3 weeks of daily treatment. The effect of hydrocortisone upon the blood Ca of PTX rats was not mediated by circulating citrate.

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Effect of Endotoxin-Tolerance, Cortisone, and Thorotrast on Release of Enzymes from Subcellular Particles of Mouse Liver.* (28773)

AARON JANOFF AND B. W. ZWEIFACH

Department of Pathology, New York University School of Medicine, New York City

In vitro studies by Weissmann(1,2) on a large-granule fraction of rabbit liver homogenate have shown that release of acid-hydrolases from the lysosomes of this fraction can be partially inhibited by pretreatment of the rabbits with cortisone or by prior adaptation of the animals to bacterial endotoxin. In our laboratory, adaptation of rats to traumatic shock (drum-tolerance) was also found to inhibit the release of acid-hydrolases from hepatic lysosomes when the corresponding granule fraction was exposed to ultraviolet irradiation *in vitro*(3). In view of deDuve's suggestion that the acid-hydrolases contained within these particles may contribute to the destruction of cells during pathological states(4), we and others have postulated that stabilization of lysosomal membranes in tolerant animals might constitute a mechanism of adaptation to trauma and toxic substances(1,3). As a further extension of these studies in adapted animals, experiments have been undertaken to determine whether stabilization of lysosomal membranes reflects a general alteration in permeability of subcellular particles resulting from tolerance, or represents instead a special response of lysosomes to repeated stress. In addition, since tolerance to endotoxin or trauma can be overcome by injection of a reticuloendothelial-blocking agent such as Thorotrast(5), we also investigated the effect of administration of this agent upon the *in vitro* release of acid-hydrolases from lysosomes of normal and endotoxin-tolerant mice.

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