minidase (0.25 unit/ml) for 15 minutes caused the release of 49.3×10^{-9} mole/mg into the supernatant or 61 percent of the total cellular sialic acid content as compared to the insignificant amounts of free sialic acid released from cells exposed to the perfusate solution alone.

Thus, removal of sialic acid from heart cells increased their Ca^{2+} exchangeability. Previous work (*4, 15*) showed that in intact cells La^{3+} does not penetrate intracellularly and displaced only surface-bound Ca^{2+}. The effect of La^{3+} on ^{45}Ca exchange in control and neuraminidase-treated cells is shown in Fig. 2B. Again, the ^{45}Ca exchange from two cultures is shown, normalized for cell mass and ^{45}Ca specific activity. The control achieved asymptotic ^{45}Ca activity at 40 to 45 minutes representing 21.1 mmole of Ca^{2+} per kilogram (dry cells). At 50 minutes 0.5 m*M* $LaCl_3$ was added to the perfusate. This produced a displacement of 11.5 percent (2.4 mmole) of the labeled Ca^{2+} within 10 minutes. In ten control experiments, 0.5 m*M* La^{3+} displaced 1.84 ± 0.13 mmole of Ca^{2+} per kilogram or 11.4 percent of labeled Ca^{2+}. A culture treated with neuraminidase (0.67 unit/ml) for 5 minutes before ^{45}Ca labeling (Fig. 2B) showed asymptotic labeling at 7 minutes, representing 15.8 mmole of Ca per kilogram (dry weight). The addition of 0.5 m*M* $LaCl_3$ at 23 minutes produced a displacement of 74.1 percent (11.7 mmole) of the labeled Ca^{2+} over the course of 10 minutes. In a series of 11 cultures first treated with neuraminidase, 0.5 m*M* La^{3+} caused the displacement of 83 percent of the labeled Ca or 15.6 ± 1.27 mmole/kg (dry weight). This displacement compared to control (1.84 mmole or 11.4 percent) is significant ($P <<< .001$).

The increased ability of La^{3+} to displace Ca^{2+} indicated that removal of sialic acid from the cellular surface permitted La^{3+} to freely enter the cell and displace intracellular Ca^{2+}. A heart cell culture was exposed for 15 minutes to neuraminidase (0.25 unit/ml) and then to a solution containing 0.5 m*M* $LaCl_3$ for 10 minutes (Fig. 1C). The cell surface shows only sparse La deposits, but the interior of the cell, especially the mitochondria, shows heavy La deposits.

Our results indicate an important role for the complex of surface coat and external lamina in the regulation of Ca exchange in cultured heart cells. More specifically, the presence of the C_9 amino sugar sialic acid is required to control Ca^{2+} flux and to inhibit the entry of normally excluded cations such as La^{3+}. Sialic acid removal, on the other hand, does not increase the exchangeability of the major intracellular cation, K^+. This suggests a specific role for cell surface components in the control of ionic exchange in the heart.

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Cortisol Stimulation of Parathyroid Hormone Secretion by Rat Parathyroid Glands in Organ Culture

Abstract. *Addition of cortisol (10^{-6} to 10^{-8}M) and related glucocorticoid congeners to cultures of rat parathyroid glands stimulated dose-related increases in parathyroid hormone secretion; the addition of deoxycorticosterone or cortexolone was without effect. Cortexolone, however, inhibited the stimulatory activity of cortisol when both were added to the culture medium. This direct stimulatory effect of cortisol on parathyroid gland secretion may account in part for the increased concentration of parathyroid hormone in the serum of cortisol-treated animals.*

Cortisol and other active glucocorticoid congeners can produce hypocalcemia in parathyroidectomized animals but not in intact animals. This suggests that an interaction occurs between adrenocorticosteroid and parathyroid hormone (PTH) in calcium homeostasis (*1*). In recent studies, the concentration of immunoreactive PTH in the serum increased significantly when cortisol was administered to intact animals, including man, while the amount of calcium remained unchanged (*2*). The increase in PTH secretion has been attributed to an

Table 1. Effect of different adrenocorticosteroid congeners on PTH secretion by rat parathyroid gland (PTG) cultured in thyroparathyroidectomized rat serum (5 percent) in Eagle's basal medium containing 1.25 m*M* calcium and 1.0 m*M* magnesium. The ratio *T/C* is for the rate of PTH secretion in a 24- or 48-hour treatment period (*T*) over the rate of secretion in the 24-hour control period prior to the steroid treatment (*C*) for each culture dish. Values are means ± S.E. for the number of culture dishes (*N*) in each steroid-treated group. Control cultures received no steroids throughout the 72 hours; each experiment included three or four such control cultures.

Steroid and concentration (*M*)	Time period (hours)			
	24		48	
	N	*T/C*	*N*	*T/C*
Control cultures	15	1.46 ± 0.17	13	1.29 ± 0.18
Cortisol (10^{-8})	4	2.69 ± 0.95	4	2.20 ± 0.81
Cortisol (10^{-7})	11	4.83 ± 0.69*	9	4.68 ± 0.90*
Cortisol (10^{-6})	7	5.80 ± 0.84*	7	8.05 ± 1.35*
Dexamethasone (10^{-8})	4	5.20 ± 0.67*	4	5.16 ± 1.13*
Corticosterone (10^{-6})	4	4.68 ± 0.75*	4	4.80 ± 0.92*
Deoxycorticosterone (10^{-6})	4	1.62 ± 0.53	4	1.06 ± 0.21
Cortexolone (10^{-6})	7	2.06 ± 0.24	7	1.99 ± 0.31
Cortisol (10^{-7}) plus cortexolone (10^{-6})	4	1.80 ± 0.48†	4	1.14 ± 0.57†

*Significantly different from control group for the same period, $P < .01$. † Significantly different from cortisol (10^{-7}*M*) group, $P < .05$.

Table 2. Effect of cortisol on the uptake (T/M) and incorporation into protein as measured by trichloroacetic acid–precipitated radioactivity (^{3}H-labeled precipitate) of ^{3}H-labeled amino acid, and on the PTH content (immunoreactive PTH and P-10-rPTH) of rat parathyroid glands cultured in a medium containing normal concentrations of calcium (1.25 m*M*) and magnesium (1.0 m*M*) in thyroparathyroidectomized rat serum (5 percent) in Eagle's basal medium. Values are means ± S.E. for four culture dishes (four glands per dish); dpm, disintegrations per minute; micrograms are for dry weight.

Treatment	Dry weight (μg)	T/M	^{3}H-labeled precipitate (dpm/μg)	Immunoreactive PTH (nl-eq/μg)	P-10–rPTH (dpm/μg)
Control	118 ± 5.2	4.6 ± 0.2	2375 ± 197	15.3 ± 0.44	39
Cortisol ($10^{-7}M$)	132 ± 6.1*	5.8 ± 0.2*	3037 ± 223*	23.7 ± 4.0	52

*Significantly different from control group, $P < .05$.

indirect, compensatory response to a postulated transient cortisol-induced hypocalcemia. The experiments reported here present evidence for a direct effect of cortisol in stimulating PTH secretion by rat parathyroid glands maintained in organ culture.

The methods used for parathyroid gland culture have been described (*3*). The glands were obtained from male albino rats, cleaned by microdissection, and cultured in 5 percent serum (obtained from thyroparathyroidectomized rat and heat-inactivated at 56°C for 30 minutes) in Eagle's basal medium containing a mixture of tritiated amino acids (New England Nuclear, 10 μc/ml of medium) (*4*). The calcium in the medium was adjusted for low (0.75 m*M*), normal (1.25 m*M*), or high (1.75 m*M*) concentration; the magnesium (1.0 m*M*, normal concentration) was adjusted as required for different experiments. The glands (three glands per 0.5 ml of medium per dish) were kept in an atmosphere of 95 percent O_2 and 5 percent CO_2 at 37°C for a total of 72 hours, divided into six 12-hour periods. At the end of each 12-hour period the medium was collected and the glands transferred into fresh medium and culture dishes. In each experiment groups of glands were cultured for two 12-hour periods in control medium (24-hour control groups); one group of these glands was cultured throughout the remaining 12-hour periods in control medium (72-hour control groups); the other groups of glands were transferred to medium with steroids added (steroid-treated groups) for four more 12-hour periods. Results were obtained by comparing the rate of secretion of rat PTH in steroid-treated glands with the rate of secretion in the 24-hour control groups. For each 12-hour period the mean PTH secretion values for each steroid-treated group (three to five culture dishes per group) were also compared with the mean value for the 72-hour control group.

The secretion of rat PTH into the medium was estimated by a radioimmunoassay method based on that of Arnaud *et al.* for human PTH (*5*). Dilutions of a guinea pig antiserum to bovine PTH, which cross-reacted with rat PTH, highly purified bovine PTH labeled with ^{125}I, and a standard medium containing reference amounts of rat PTH (*6*) were used to determine the standard curves for each assay. The amount of rat PTH in the culture medium was expressed as a volume equivalence of the standard rat PTH medium. Within each assay, there was a coefficient of variation of 6.2 ± 3.1 percent (mean ± standard deviation); between assays the coefficient of variation was 12.9 ± 7.1 percent. At the end of each experiment glands were removed, lyophilized, and weighed. After extraction of ^{3}H-labeled amino acids with cold 10 percent trichloroacetic acid (TCA), the gland residue insoluble in TCA was further extracted with 90 percent phenol. The radioactivity in a portion of the phenol extract was counted by a liquid scintillation method for determining the incorporation of ^{3}H-labeled amino acids into peptides. In one experiment, the phenol extracts of glands from each steroid-treated group were pooled and examined by chromatography (Biogel P-10 column, 1.5 by 70 cm) with 3*M* guanidine hydrochloride in 2.3*M* formic acid buffer (*7*). The uptake of ^{3}H-labeled amino acids by the parathyroid glands is expressed as the ratio of tissue to medium (T/M), and is determined as previously described by Raisz (*8*).

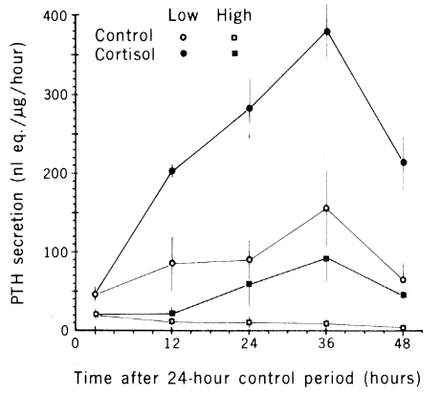

Fig. 1. Rat parathyroid glands were cultured (three glands per 0.5 ml of medium per dish) in a medium containing low (0.75 m*M*) or high (1.75 m*M*) concentrations of calcium for two 12-hour control periods. One-half of the glands for each calcium concentration were transferred to medium containing cortisol ($10^{-7}M$), the other half to the same medium as for the control period; all were incubated for four more 12-hour treatment periods. Values are means ± S.E. for three culture dishes. Differences between cortisol-treated and control glands were significant at $P < .02$ for all treatment periods when the medium contained low concentrations of calcium; at $P < .05$ for the 36-hour and 48-hour periods when the medium contained high concentrations of calcium. The PTH secretion is measured as nanoliter equivalents of rat PTH standard per microgram of dry weight of gland per hour.

The effect of cortisol at low (0.75 m*M*) and high (1.75 m*M*) concentrations of calcium in the medium on the secretion rate of rat PTH is shown in Fig. 1. Low concentrations of calcium stimulated and sustained high rates of PTH secretion, while high calcium concentrations suppressed PTH secretion throughout all the observation periods. Cortisol ($10^{-7}M$) stimulated a sustained increase in the rate of rat PTH secretion not only in glands exposed to high concentrations of calcium, but also in glands already stimulated by exposure to low calcium concentrations. In other experiments cortisol also stimulated PTH secretion in rat glands cultured in media containing normal concentrations of calcium (1.25 m*M*) and magnesium (1.0 m*M*) (Table 1).

Table 1 shows the effect of various glucocorticoid congeners on rat parathyroid gland secretion. A dose-related response for PTH secretion was obtained for cortisol at 10^{-8} to $10^{-6}M$ concentrations. Corticosterone ($10^{-6}M$) and dexamethasone ($10^{-8}M$) also stimulated 3.5- to 7-fold increases in the rates of secretion compared with the values for the 24-hour control groups, and six- to ten-fold increases over the values for the 72-hour control groups for the same time period. Deoxycorticosterone or cortexolone treatment did not significantly alter the rates of PTH secretion when these were compared with the rates for the 72-hour controls. Though cortexolone ($10^{-6}M$) alone did not affect PTH secretion, it significantly inhibited the stimulatory effect produced by cortisol ($10^{-7}M$).

Cortisol increased the uptake of ^{3}H-labeled amino acids; mean T/M ratios for cortisol-treated glands were significantly different from those for control glands, $P < .05$ (Table 2). More ^{3}H-labeled amino acids

were incorporated into glandular proteins in cortisol-treated than in control glands. Cortisol-treated glands also had higher immunoreactive PTH content than control glands, though the differences were not statistically significant. Phenol extracts of glands chromatographed on Biogel P-10 in 3*M* guanidine hydrochloride and 2.3*M* formic acid yielded a substance with a consistent small peak of radioactivity for which the partition coefficient, K_d, was 0.18 to 0.2, the same as that for highly purified bovine PTH labeled with ^{125}I. If one assumes that this ^{3}H-labeled peptide is ^{3}H-labeled rat PTH (P-10–rPTH), the cortisol-treated glands also contained 30 percent more ^{3}H-labeled P-10–rPTH per microgram of dry tissue than the control glands.

These results suggest that cortisol stimulates the synthesis of proteins and PTH. Although the changes in the glandular content of ^{3}H-labeled proteins and PTH were relatively small, they were sufficient to prevent decreases in glandular hormone content during 3 days of culture when PTH secretion was stimulated. This reflects a rapid turnover rate for PTH synthesis and secretion which has been previously observed in ultrastructural studies of rat parathyroid glands (*9*) as well as in short-term studies in vitro with bovine parathyroid gland slices (*10*). It is possible that cortisol acts indirectly to increase net PTH synthesis either by increasing active membrane transport of amino acids or by decreasing the rate of intracellular degradation of PTH. Habener and co-workers have proposed that the control mechanism for the secretion of PTH in bovine parathyroid gland slices is mediated through a calcium control of intracellular degradation of newly synthesized PTH; a high concentration of calcium in the medium decreases the secretion rate by increasing the rate of PTH degradation (*11*). Cortisol can thus affect secretion through inhibition of calcium uptake by the parathyroid gland, analogous to its ability to inhibit the absorption of calcium in the gut (*12*). It is also possible that cortisol, through its known ability to stabilize lysosomal membranes and decrease protease release (*13*), could decrease intracellular PTH degradation and thus increase secretion.

The specific relative effect of glucocorticoid congeners on parathyroid hormone secretion, and the findings that cortexolone alone has no effect but can inhibit the effects of cortisol, are consistent with the presence of cortisol receptors in the parathyroid cell (*14*). The extremely small size of each rat gland (15 to 30 μg dry weight) makes the isolation and demonstration of specific receptors in these glands technically difficult.

This study provides another explanation for the increase in plasma PTH in cortisol-treated animals and suggests that some of the effects of therapeutic doses of cortisol to decrease bone mass might be mediated through this action. Thus, in cortisol-induced osteopenia the direct stimulation of PTH secretion by cortisol might overcome the inhibitory effect of cortisol on bone resorption (*15*). An additional effect from secondary stimulation of parathyroid hormone secretion could also result from hypocalcemia produced by cortisol inhibition of gut calcium absorption (*12*) and bone resorption (*15*). The net result would be an increase in the concentration of PTH in the plasma, normocalcemia, and increased bone resorption (*16*) in the parathyroid-intact animal treated with cortisol.

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Complete Covalent Structure of a Human IgA1 Immunoglobulin

Abstract. *The complete covalent structure has been determined for a human myeloma IgA1 immunoglobulin. This protein has unique features in the amino acid sequence and disulfide bridge structure of the variable (V) and constant (C) regions of both the α heavy and the λ light chains, and in the number and loci of oligosaccharides. Whereas C region domains of heavy chains have evolved independently over eons, recent isotypic variations have occurred in λ light chains and possibly in α heavy chains.*

Despite the vast amount of partial sequence data for human immunoglobulins (*1, 2*), until recently the complete covalent structure had been reported for only one IgG molecule (*3*) and two IgM molecules (*4*). We here report the complete covalent structure of a human IgA1 myeloma globulin (designated Bur) including the complete amino acid sequence of the α1 heavy chain, the λ light chain, the location and kind of the oligosaccharides, and the probable disulfide bridge structure (Fig. 1). In the constant (C) region of the α1 chain our sequence differs in only 8 positions out of 353 from another α1 chain (Tro) recently reported (*5*) in which the location of the carbohydrates and disulfide bridges was not given. However, there are 42 differences in the variable (V) regions of the two α1 chains, excluding differences in amide assignments, gaps, and the presence of carbohydrate in the V region of the Bur α1 chain. These studies verify the schematic structure we have previously proposed for human IgA1 immunoglobulin (*2, 6*); they validate, extend, and correct many partial sequences of portions of the human α1 chain proposed by other workers (*7*) and the complete Fcα sequence reported by us (*8*). The λ light chain of IgA1 Bur has three unusual positions of variation in the C region, independently described by us (*9*) and by Fett and Deutsch (*10*). In both the light and the heavy chains of IgA1 Bur, the switch point (the division between the V and C regions of chains of the same class) extends into what was previously regarded as the C region, thereby casting doubt on the existence of a nucleotide sequence that signals the point of union for the postulated V and C genes (*2–9*).

The order of the 472 amino acid residues of the Bur α1 chain was determined by automatic or manual sequence analysis of more than 200 different peptides from the whole α1 chain, its CNBr frag-