The dynamics of adrenocortical secretion

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The experiments reported here were undertaken to describe the dynamic relations between the concentration of adrenocorticotropic hormone (ACTH) in systemic blood and the secretory function of the adrenal cortex. We have studied the temporal course of adrenocortical secretion rate, while experimentally varying ACTH concentration in adrenal arterial blood according to different temporal patterns. These different views of time-dependent adrenocortical secretory function provide many constraints to guide its dynamic modeling.

The term "dynamic modeling" designates either of two activities, both aimed at generalizing about adrenocortical dynamics from the experimental data. The first is the formulation of arbitrary mathematical expressions which can be forced with functions representing time variations in ACTH concentration and not only simulate the experimental results but also predict adrenocortical secretory responses to temporal variations in ACTH concentration other than those studied experimentally. Such models can serve to describe the dynamic secretory function of the intact adrenal, but bear no relation to the underlying biochemical details of ACTH action and steroidogenesis. The alternate approach is to formulate dynamic models which derive from current knowledge or hypothesis about the mechanisms of action of ACTH. Physically appropriate bases for such models are differential equations which express the conservation of mass of known or postulated biochemical compounds involved in the coupling between ACTH and the biosynthesis of adrenocortical hormones. Such models not only serve to describe the dynamic function of the intact adrenal, but also reveal the functional implications of the biochemical ideas from which the models were formulated.

The availability of computers makes it practical to undertake dynamic modeling, which in turn provides the basis for the study and description of simultaneous interactions among many dynamic physiological processes. Systems theory provides the conceptual basis for this approach, which seems especially appropriate in endocrinology because of the communication and control aspects of hormone action. We have focused on these

adrenal; analog computer; adrenal cortex; systems analysis; cortisol; ACTH; perfusion; mathematical modeling
aspects of ACTH action on adrenocortical secretion. Although it is common to describe this endocrine phenomenon with statements such as "the pituitary hormone, ACTH, stimulates the adrenocortical secretion of steroid hormones," the emphasis in the present study is conveyed more clearly by the following, more limited description: "the adrenal cortex converts an endocrine signal of one chemical form and biological activity into an endocrine signal of quite different chemical form and biological activity." This statement emphasizes the flow of signals and prompts questions, which we have sought to answer, about the fidelity of the signal conversion process. The answers relate to function at levels of physiological organization both higher and lower than that of the intact adrenal gland. The higher level functions are communication and control in the pituitary adrenal system, and the lower level functions are the biochemical mechanisms of ACTH action on steroidogenesis, which one might regard in this context as the "logic" of the signal conversion process.

A biological systems analysis poses considerable methodologic difficulties, but the present study was made feasible by the existence of sensitive and specific chemical methods for quantifying various adrenocortical steroids, and good surgical techniques for gaining access to the adrenal vascular supply. Also, ACTH acts sufficiently rapidly so that the studies could be done in acute experiments of several hours' duration.

Previous studies (1, 10-13, 15, 16) did not provide answers to the questions we have raised, nor could the data support dynamic modeling, for one or more of the following reasons. Either the level of adrenocortical stimulation by ACTH was maximal or supramaximal, the time course of ACTH concentration in arterial blood was unknown, or the period of stimulation was too short to permit the distinction between transient and steady state events. In retrospect, these same reasons appear to explain why the earlier work failed to reveal some of the striking qualitative features of adrenocortical secretory dynamics which are described in this paper.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Blood Flow, mI/min</th>
<th>Gland Wt, g</th>
<th>Perfusion Pressure, mm Hg</th>
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<tr>
<td>112</td>
<td>7.0-8.0</td>
<td>1.20</td>
<td>110-120</td>
</tr>
<tr>
<td>114</td>
<td>4.4-5.0</td>
<td>1.09</td>
<td>100-110</td>
</tr>
<tr>
<td>115</td>
<td>7.4-8.9</td>
<td>1.60</td>
<td>70-90</td>
</tr>
<tr>
<td>137</td>
<td>4.4-5.0</td>
<td>0.83</td>
<td>85-100</td>
</tr>
<tr>
<td>138</td>
<td>4.6-5.0</td>
<td>1.01</td>
<td>115-125</td>
</tr>
<tr>
<td>142</td>
<td>4.0-5.6</td>
<td>0.81</td>
<td>85-90</td>
</tr>
<tr>
<td>145</td>
<td>3.0-4.0</td>
<td>1.02</td>
<td>95-115</td>
</tr>
<tr>
<td>148</td>
<td>6.0-6.4</td>
<td>1.47</td>
<td>100-105</td>
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<tr>
<td>164</td>
<td>2.4-3.0</td>
<td>0.77</td>
<td>95-110</td>
</tr>
<tr>
<td>168</td>
<td>3.0-4.4</td>
<td>1.10</td>
<td>115-130</td>
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<td>184</td>
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<td>90-100</td>
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<td>185</td>
<td>5.7-6.9</td>
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<tr>
<td>186</td>
<td>3.6-4.0</td>
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<td>191</td>
<td>3.6-4.3</td>
<td>1.05</td>
<td>90-95</td>
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METHODS

Perfusion technique. The experimental studies were performed on the left adrenal gland of acutely hypophysectomized dogs (14-20 kg body wt) of both sexes, anesthetized with pentobarbital, 30 mg/kg. The adrenal was perfused in situ with autogenous arterial blood, using a procedure previously reported (18). Briefly, the perfusion procedure involved leading blood from a carotid artery through a Harvard peristaltic pump and heater (39°C) to a cannula in the left renal artery. All visible branches of the abdominal aorta, between the celiac axis and the left renal artery, were ligated, with the exception of the small vessels which pass directly from the aorta to the left adrenal. The vessels of the right adrenal were ligated. Then the dogs were given 20,000 units heparin intravenously, blood flow was established from carotid to aorta by the pump, and the aorta was ligated immediately caudal to the left renal artery and immediately cephalad to the superior mesenteric artery. A cannula in the left lumboadrenal vein conveyed the left adrenal effluent. When the effluent was not being sampled, it was allowed to flow back to the animal through a cannula in the inferior vena cava. The resistance to adrenal venous blood flow offered by the adrenal venous and caval cannulas, along with their connecting device, was 30-40 peripheral resistance units (PRU, 1 PRU defined as a Poiseuille resistance of 1,333 dynes-sec/cm²) so that adrenal venous blood pressure was increased by only 3-6 mm Hg with the adrenal blood flows encountered in this study. The adrenal venous blood flow in these experiments averaged 61% of the pump-controlled arterial input into the isolated segment of abdominal aorta, the difference being attributable to flow through extra adrenal branches of the perfused segment of abdominal aorta. The perfusion pump was set to hold pressure in the aortic segment in excess of the dog's systemic arterial pressure. The preparation was

**FIG. 1.** Relative time courses of cortisol secretion rate following the injection of 250 microunits (solid line) or 2,500 microunits (dashed line) into the perfusion at zero time. The two experiments were performed on different perfused glands. Our estimates of the strengths of the stimuli to the two glands are 42 μU/ml for 22 sec and 227 μU/ml for 28 sec.
judged technically unsatisfactory if adrenal vascular resistance exceeded 200 PRU.

**ACTH.** The ACTH utilized was a partially purified preparation (Parke, Davis), with a potency of 16–17 U/mg. Before each experiment, a quantity of the preparation, rated to be 25 units by the manufacturer, was diluted in isotonic saline containing 0.1% bovine albumin and kept at 0°C in polypropylene beakers. The albumin-saline solution was infused into the perfusion line throughout the experiment to obtain control values of cortisol secretion rate before and after ACTH addition. The exogenous ACTH was administered to the perfused gland by mixing two flows, the one being carotid arterial blood, pumped at a known rate (Q), and the other being an ACTH solution of known concentration (C), pumped at an independently controlled rate (S). The increment in ACTH concentration in the perfusate, ΔA, over the concentration in systemic arterial blood, is given by

$$\Delta A = \frac{C \cdot S}{Q + S} \quad (1)$$

The value S was kept at approximately 10% of Q. Under conditions where cortisol secretion rate after acute hypophysectomy was below 0.85 μg/min, it was assumed that the concentration of endogenous ACTH was zero, and by using equation 1, it becomes possible to calculate the absolute concentration of ACTH in the perfusate, on the basis that none of the administered ACTH passed through the gland or leaks in the perfusion circuit and reappeared in systemic arterial blood. In fact, however, the experimental design does permit exogenous ACTH to recirculate in this manner. The maximal contribution of this type of recirculation of ACTH may be gauged by comparing the rates of ACTH infusion required to achieve a calculated ACTH concentration in the perfusate of 2 μU/ml (0.5–1.5 μU/min per kg body wt) with the systemic intravenous infusion rate required to stimulate cortisol secretion rate to a comparable extent (6–12 μU/min per kg body wt) (19). Thus, at worst, the systemic ACTH concentration appears to have been an order of magnitude less than that in the perfusate. In the ACTH concentration ranges used in these experiments, this may be considered to have had negligible effect.

In these experiments, the concentration of ACTH in the perfusate was made to follow four different temporal patterns. The first was a brief pulse, which was approximated by the rapid injection of a small volume of ACTH solution into the perfusion line. The second and third were a stepwise increase or decrease in concentration, which were approximated by switching the tubing, conveying an infusate at fixed rate, from a solution of one ACTH concentration to one of another. The fourth was a sinusoidal fluctuation in ACTH concentration about a mean level. This was approximated by modulating in the manner of a sine wave the flow (S) of ACTH solution whose concentration (C) was held constant. The sinusoidally modulated pump was built by the Harvard Apparatus Co. The frequency, mean flow, and amplitude of the flow changes were independently controllable. The performance of this pump was monitored throughout each experiment by having its second channel pump water into the dome of a pressure transducer and then through a high-resistance needle, recording the resulting sinusoidal pressure changes. Fourier analysis of the pressure changes showed no component up through the fifth harmonic of the desired frequency whose amplitude exceeded 1% of the amplitude of the desired frequency. The operation of the pump was pulsatile in the range 0.5–2/sec, but this distortion was at least two decades higher than the highest frequency employed in the experiments. Accordingly, the pump was judged reliable for delivering a sinusoidal input. However, equation 1 reveals that ΔA will not be truly sinusoidal, because the sinusoidally modulated term, S, appears in both numerator and denominator. Since the mean of S was kept below 10% of Q, the deviation from a true sinusoidal variation in ΔA was small. We corrected for this distortion by simulating equation 1 with representative experimental values for S and Q on an analog computer, and subjecting the computed time course of ΔA to Fourier series analysis. With unit variation in S, the amplitude of the sinusoidal component of ΔA at the frequency of S was 1.14, and the amplitude of higher harmonics, up through the fifth, was below 0.04. Calculations based on experimental data have been corrected for this distortion. This and other simulation studies were carried out on an EAI analog computer, model TR-48.

All four of the experimental modes of varying ACTH concentration were subject to temporal distortion to the extent that dispersion and mixing occurred in the aortic segment and in the perfusion tubing which lay beyond the point at which the ACTH solution entered the perfusion line. This distortion effect was estimated by infusing Evans blue dye and measuring the time course of dispersion.
dye concentration in blood drawn from the aortic segment using a recording densitometer (Gillford Instrument Co.). The dispersion and mixing of dye in the perfusion line and aortic segment considered together could be represented by a single time constant of 15–25 sec, depending on the size of the dog and on the perfusion rate. No correction was made for this dynamic lag, which was small in relation to adrenocortical secretory dynamics.

The time course of dye concentration in adrenal venous blood was also examined in four experiments under representative flow conditions. This technique permitted measurement of the over all delay from the time of switching between ACTH solutions until the ACTH concentration would have changed in blood at the end of the adrenal venous catheter, which was the sampling point. This delay was 60–120 sec, and was corrected for, so that the reported delays in the adrenocortical response to ACTH are attributable to glandular mechanisms.

We also attempted to estimate the statistical distribution of transient times in the circulation of the perfused left adrenal under representative flow conditions. The estimate of transit time distribution was in the form of a probability density function computed from the simultaneous time courses of dye concentrations in aortic segment blood and in adrenal venous blood upon infusion of dye into the perfusion line. The simplex method of linear programming was adapted (4) for this purpose. Blood from each of these two sites was conveyed, via catheters of equal dimensions, to each of two identical recording densitometers. The flow of blood in the aortic catheter was adjusted to equal the flow of blood from the adrenal venous catheter. The resulting time courses of dye concentration at the two sites were digitized at 0.5- or 1-sec intervals. In four experiments, the results of the computations showed a mean transit time between 15–25 sec, with over 90% of the area under the probability density function lying between 0 and 10 sec. In consequence of these very short estimates for transit time, microcirculatory dispersion was not further considered as a significant factor in adrenocortical secretory dynamics.

Measurement of adrenocortical secretion. The rate of cortisol secretion was measured, as this steroid is quantitatively the principal glucocorticoid secreted by the canine adrenal cortex (cf. 23). Previous studies have shown that the secretion of corticosterone, which is produced by the canine adrenal at rates approximately 25% of that of cortisol, does not appear to vary independently of cortisol secretion rate (cf. 23). Cortisol concentration in samples of adrenal venous blood was measured with a modification (18) of the Kliman-Peterson double-isotope derivative method (9). Cortisol secretion rate was calculated as the product of cortisol concentration in adrenal venous blood and adrenal venous blood flow.

Timing and duration of experiments; choice of levels of adrenocortical stimulation. In almost all of the experiments, cortisol secretion rate was measured at least 2 hr after hypophysectomy and at least 30 min after the completion of surgery, but prior to the administration of exogenous ACTH. These measurements served to indicate the adequacy of hypophysectomy. With several exceptions, noted below, if these initial cortisol secretions rates were, in excess of 0.85 μg/min, the experiment was deemed technically unsatisfactory and not further analyzed, since the interpretation of the results depended on our maintaining control over the degree of adrenal stimulation by ACTH. We attempted, in most of the experiments, to eliciting an adrenal response whose steady-state value

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Coefficient of Variation of Mean Values, t = 0-60, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>112</td>
<td>9.6</td>
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<td>114</td>
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![Fig. 3. Time course of cortisol secretion rate in response to stepwise changes in ACTH concentration from 0-1, 1-2, and 21-μU/ml.](http://ajplegacy.physiology.org/Downloaded from http://ajplegacy.physiology.org)
would fall between 20 and 50% of maximal. This range of stimulation corresponds to cortisol secretion rates observed in intact undisturbed dogs (6; personal observations), and also minimizes or avoids altogether saturation effects in the transient period of the response. In most of the experiments, ACTH concentrations of 2 μU/ml, as calculated from equation 1, achieved the desired level of adrenal stimulation. The perfusion rate was held constant in each experiment, with the result that adrenal blood flow varied only within a narrow range (Table 1). The experimental studies did not exceed 3 hr duration. The maximal rate of cortisol secretion was estimated at the end of most experiments from measurements made 15–20 min after increasing ACTH concentration to 150–220 μU/ml. Previous studies with the same preparation have shown that such concentrations of ACTH elicit near-maximal rates of cortisol secretion (18).

RESULTS

The experimental results may be conveniently treated in relation to the mode, or temporal pattern, of ACTH input. Table 1 presents the data on adrenal blood flow, gland size, and perfusion pressure in the experiments reported in Tables 2–4, organized according to the mode of ACTH input.

A. Short pulse input. The initial experimental studies were done using a single injection of ACTH into the perfusion line (short pulse input) on the assumption that the results would serve as a guide for both the sampling interval and the duration of sampling in subsequent experiments, with other modes of ACTH input. Figure 1 shows the time courses of cortisol secretion rate in two experiments after a single rapid injection of 250 micro-units (solid line) or 2,500 micro-units (dashed line) of ACTH. The ordinate scales are adjusted so that the

![Graph showing cortisol secretion rate over time](image)

**FIG. 4.** Time course of cortisol secretion rate in response to a 5-min cessation of ACTH stimulation (exp. 184). The reduction in ACTH concentration was made 60 min after initially establishing the ACTH concentration at 2 μU/ml.

### Table 1: Time course of cortisol secretion rate in response to a 5-min reduction in ACTH concentration from 2 to 0 μU/ml.

<table>
<thead>
<tr>
<th>ACTH input</th>
<th>ACTH = 0</th>
<th>ACTH = 2 μU/ml</th>
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<tbody>
<tr>
<td>Time (min)</td>
<td>15–20</td>
<td>15–20</td>
</tr>
<tr>
<td>ACTH = 0</td>
<td>14.8</td>
<td>14.8</td>
</tr>
<tr>
<td>ACTH = 2 μU/ml</td>
<td>14.8</td>
<td>14.8</td>
</tr>
</tbody>
</table>

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relative time courses may be compared easily. The smaller response appeared to be complete by 17 min; the larger one was not followed to completion, but suggested that cortisol secretion rate would have returned to control levels at about 20 min. The results indicate that a 1 min interval between samples is probably adequate for resolving the salient dynamics of adrenocortical secretion. With respect to indicating the duration of sampling after an ACTH concentration change, however, these results proved to be misleading. This was so because of the quite different time courses of the onset and cessation of ACTH action, as the following experiments with stepwise changes in ACTH concentration reveal.

B. Step inputs. Figure 2 shows the adrenal response to a stepwise increase in ACTH concentration, from 0 to 2 µU/ml. The glandular response began in the 3rd min after the increase in ACTH concentration, reached a maximal secretion rate at 11 min, and then quite unexpectedly declined gradually, and did not reach a steady rate of cortisol secretion until about 30 min after the application of the step input. This "overshoot" in the response could be repeated, as shown in Fig. 2. A step increase in ACTH concentration was applied to each of seven glands. Each responded with an appreciable overshoot in cortisol secretion rate. The data from these experiments are presented in Table 2, which includes the first of the two responses shown in Fig. 2 (exp. 142).

Figure 3 shows results from one of two such experiments where overshoot occurred when ACTH concentration was elevated first from 0 to 1 µU/ml, and then once again occurred when the input was subsequently increased from 1 to 2 µU/ml. These findings demonstrate that the overshooting secretory response can be elicited in glands which have already been stimulated by ACTH. Figure 4 shows that after 60 min of constant ACTH stimulation at 2 µU/ml, when ACTH input was reduced to zero for 5 min and then restored to 2 µU/ml, there was no overshoot. Table 3 lists the data from four such experiments. These results are to be contrasted with those shown in Fig. 2, in which overshoot occurred in

| Time courses of cortisol secretion rate in response to a stepwise increase at zero time in ACTH concentration from 0 to 30 µU/ml (X) or to 70 µU/ml (-). Each ACTH concentration was held steady throughout the 60-min interval. The two experiments were performed on different glands. | FIG. 5. |
DYNAMICS OF ADRENOCORTICAL SECRETION

followed a first-order exponential decay, whose mean output began to decline, its time course approximately rapid. Apart from the delay of 2 min before cortisol concentration from 2 to 1 @J/ml, presented in Table reduction in ACTH concentration was surprisingly same gland when ACTH concentration was increased. Thus, the result shown in Fig. 2 where ACTH concentration would show a comparable "undershoot." Obviously, when the control cortisol secretion rate and ACTH concentration was increased.

Cortisol concentration also reveal an absolute time delay before the onset of changes in cortisol secretion rate. The data in Tables 2-4 and Figs. 2-5 show this delay to be of approximately 2 min duration but the accuracy of this estimate is poor because of the 1-min sampling interval.

C. Sinusoidal inputs. ACTH concentration was varied sinusoidally at five different frequencies, in the interval between 0.02 and 0.4 cycles/min. The experiments were performed by modulating ACTH concentration by approximately +20% about a mean of 2 μU/ml. Figure 6, A and B, shows two experiments in which ACTH concentration was varied in this way at 0.1 cycles/min, and serves to illustrate the method of interpretation. On the basis of the data in Table 4 and the well-known logarithmic relationship between ΔCort concentration and cortisol secretion rate over a broad range of ACTH concentrations (provided adrenal blood flow is constant) (10), the relation between cortisol secretion rate and ACTH concentration was considered linear in the concentration range 0-3 μU/ml. The mean rate of cortisol secretion during sinusoidal variation in ACTH concentration was assumed to be independent of frequency, and was in fact evidently so in two experiments in which several different frequencies were tested. On this basis, the two ordinate scales in Fig. 6, A or B, can be related. In the experiment shown in Fig. 6A, the calculated mean ACTH concentration was 2.06 μU/ml, the mean of all 10 measurements of cortisol secretion rate was 1.74 μg/min, and the cortisol secretion rate in the absence of exogenous ACTH was below 0.02 μg/min. Because of the negligible rate of cortisol output at zero ACTH concentration, the two ordinate scales in Fig. 6A were set so that 2.06 μU/ml and 1.74 μg/min would lie on the same horizontal level, signifying a static relationship between ACTH concentration and cortisol output of 0.85 μg/min per milliliter. Thus the plots of cortisol secretion rate and of ACTH concentration vs. time reveal the relative amplitudes of the two functions, as well as their phase relation. Visual inspection of the data in Fig. 6A shows that cortisol secretion rate varied in near-sinusoidal fashion. Its variation about the mean was in approximate proportion to the extent of the variation in ACTH concentration. However, cortisol secretion rate lagged behind ACTH concentration so as to invert the usual relationship between these variables, that is, cortisol output fell as ACTH level rose. In other experiments than the one shown in Fig. 6A there was deviation from a clearly sinusoidal pattern. Figure bD shows one such result. At lower frequencies (0.02 and 0.05 cycles/min) the data from most experiments followed a sinusoidal pattern but at higher frequencies (0.2 and 0.4 cycles/min) there was no clear sinusoidal pattern in the results.

The results at each frequency were subjected to harmonic regression analysis (3). A single harmonic, at
FIG. 7. Polar plot of the frequency dependence of the relationship between ACTH concentration and cortisol secretion rate. The two cortisol secretion rate vectors represent best fit values from a harmonic regression analysis of the pooled data from individual experiments. The regions enclosed by dotted lines show the 95% joint confidence limits on the magnitude and angle of the vectors.

FIG. 8. Two models of the dynamics of adrenocortical secretion. The dashed arrows indicate parametric actions which entail no loss of mass, and the solid arrows represent mass flow. The equations for these models are given in the Appendix. The upper model is treated as model 1 in the Appendix.

(0.314 rad/min) and 0.1 cycles/min (0.63 rad/min) is in accord with the existence of an apparent, dominant time constant of approximately 3 min, as was observed in the experiments with stepwise decreases in ACTH concentration. However, the overshoot observed in the transient response has no counterpart in the limited portion of the gland's frequency response observed in these studies.

**Discussion**

The present results reveal several unexpected features of adrenocortical dynamics. The most striking one was the overshoot in the secretory response to stepwise increases in ACTH concentration. After the secretory response reached its peak, it declined toward the final steady-state value quite slowly in relation to the surprisingly fast decline in cortisol output which followed a stepwise decrease in ACTH concentration. The adrenocortical response to ACTH can be described as dynamically asymmetrical, since the responses to increasing ACTH concentrations follow a quite different temporal pattern than do the responses to decreasing concentrations. These features are obscured at high concentrations of ACTH, and are not suggested by the experiments with short-pulse inputs. The results with sinusoidal variations in ACTH concentration, although quite limited in extent and complicated by wide confidence limits, appear to show a dominant time constant of about 3 min, as was seen in the experimental results with step decreases in input.

Previous work in both the rat (10) and the sheep (1) demonstrated that the onset of action of ACTH was relatively rapid, and presented results which do not differ qualitatively from those shown in Fig. 1. Porter and Klaiber's paper on the adrenal blood flow dependence of ACTH action includes data on the detailed time course of adrenal corticosterone secretion in the
hypophysectomized rat during constant systemic intravenous infusion of ACTH (16). In their studies, the rise time of ACTH concentration in adrenal arterial blood was uncertain because the kinetics of ACTH metabolism and distribution in body fluids are uncertain. The extent of any overshoot in secretion rate would be expected to depend on the rapidity with which ACTH concentration rose in arterial blood. (Both models presented below show this feature, for example.) Also, Porter and Klaiber infused ACTH at rates sufficient to elicit near-maximal rates of corticosterone secretion (15), so that saturational effects might have obscured the overshooting response observed in the present studies. This is illustrated by the contrasting responses to large (Fig. 5) and small (Fig. 2, Table 2) stepwise increases in ACTH concentration.

The most extensive previous work on the response of perfused adrenals to ACTH was that of Macchi and Hechter (11-13). They established that the effective range of blood ACTH concentration lay between 1 and 100 µU/ml (12), which is in agreement with this and a previous study (18). Two of their three glands studied with a 1 µU/ml input showed responses which suggest the overshoot reported here, but their data on secretion rates were determined from blood samples collected over an 18-min interval, which would have blurred the transients shown by the canine gland. One striking difference between their data and ours is the much longer half-time (about 25 min) with which corticosteroid output declined after they had switched from an ACTH-rich to an ACTH-free perfusate (13). In those experiments, the ACTH concentration before switching was 1000 µU/ml—10 times greater than their estimate of the least ACTH concentration required for maximal secretion. The discrepancy between their findings and ours probably relates to the different initial ACTH concentrations.

The surprisingly rapid decline in cortisol output following reduction in ACTH concentration bears on the long-standing postulate that adrenal tissue binds ACTH in a form which is prolongedly active. The relatively slow decline in adrenocortical secretion rate after the removal of ACTH, reported by Macchi and Hechter (13), supported this idea, as did later studies on incubated adrenal fragments (2), which showed that even brief exposure of adrenal tissue to ACTH sufficed to elevate the rate of steroidogenesis for relatively long periods of time. The responses to the short-pulse inputs, shown in Fig. 1, are also consistent with this view. However, a quite different conclusion is suggested by the results with stepwise decreases in ACTH concentration (Figs. 2-4; Tables 3 and 4). An important feature in the design of the experiments was to separate in time the increases and decreases of ACTH concentration by an interval of well-defined steady-state secretion. It was thus evident that about threefold more time (30-35 min) was needed to reach a steady rate of secretion after an increase in ACTH concentration than after a decrease (10-12 min). When the adrenal exposure to ACTH was only brief, the prolonged secretory response seems paradoxically to have been largely the result of the relatively slow dynamics associated with the onset of ACTH action. These findings do not negate the conclusion of ACTH binding in some fashion to adrenal tissue, but if so, its dynamic consequences are slight.

The data show cortisol secretion rate to be quite tightly coupled to ACTH concentration within the 0-2 µU/ml concentration range. The rates of cortisol secre-
tion associated with this range of ACTH concentration correspond to those of intact, undisturbed dogs (6; personal observations), and so the coupling characteristics at these relatively low input levels have special physiological interest. It is often convenient to think of a continuous relation between ACTH concentration and cortisol secretion rate, but statistical uncertainties of the coupling effectively map a continuous range of ACTH concentrations into some relatively small number of statistically defined intervals of cortisol secretion rate, between its least and greatest possible values. This quantization is a well-known limitation on the use of adrenocortical secretory responses in the bioassay of ACTH; here it explicitly concerns information transfer. The data provide an initial estimate of the number of quantization intervals, which specifies the number of different levels of ACTH detectable from observations on the corresponding rates of cortisol secretion. The estimate is based in part on the total range over which cortisol secretion rate can vary. A mean value for this total range is 8.2 \( \mu g/min \), and is derived from the increments in cortisol secretion rate produced by increasing ACTH concentration from 0 to values in excess of 150 \( \mu U/ml \) (Tables 3 and 4). The data in Table 2 indicate the size of one of the quantization intervals that comprise this total range. The interval corresponds to four standard deviations of steady-state cortisol secretion rate, and would thus include 95% of the values. The over-all mean value of this interval in seven experiments with a steady 2 \( \mu U/ml \) input was 0.88 \( \mu g/min \) (Table 2). This estimate of course includes the errors in the radiochemical assay of cortisol. If one assumes that all the error is due to the gland, thereby overestimating its variability, the result is 8 or 9 quantization intervals, or roughly three bit accuracy. This estimate depends on the untested assumption that variations in cortisol secretion rate, over and above those due to errors in the assay, are independent of secretion rate. Statistics on the two responses to high input levels (Fig. 5) are too disparate to challenge the validity of this assumption, since the quantization intervals were 1 and 4.3 \( \mu g/min \), respectively. A direct experimental measure of the quantization intervals over the whole range of cortisol secretion rate is both feasible and desirable.

The present results emphasize the large discrepancy between the dynamic range of the adrenal and that of the distribution and disposal process for cortisol, from which results the dynamic relation between cortisol secretion rate and the concentration of cortisol in systemic blood. In the dog, this distribution and disposal process has a time constant of about 40 min (14). If valid, this time constant specifies the frequency dependence of the coupling between the rate of secretion and the systemic blood concentration of cortisol. A time constant of 40 min signifies that the coupling will progressively attenuate as frequency increases beyond \((40)^{-1} \) rad/min, or about 1 cycle/4 hr. In contrast, the coupling between ACTH concentration and cortisol secretion rate does not attenuate until frequency exceeds 1 cycle/20 min. A tentative conclusion about the physiological significance of this disparity arises from the following considerations. The data in Fig. 7 show that the adrenal introduces approximately 150° of phase lag at 0.05 cycles/min. This large amount of phase lag is a potential threat to the stability of negative-feedback regulation of pituitary adrenal function, for it signifies the possibility that the adrenal may introduce, or contribute significantly to, a frequency-dependent sign reversal in the system, with resulting positive, rather than negative feedback. However, the small dynamic range of the distribution and disposal process for cortisol would markedly attenuate or filter signals in this frequency range, and so insure stability, albeit at the expense of dynamic range. The limits of stability of this
nonlinear system are of interest from the standpoint of disease, but they pose a complex issue which can only be resolved as it becomes possible to study and model the dynamics of other parts of the pituitary-adrenal neuroendocrine system (cf. 21, 22).

The present results indicate a previously unsuspected complexity in the mechanisms of action of ACTH. Most previous discussions of ACTH action on steroidogenesis focused attention on this hormone acting at a single rate-limiting step in the steroidogenic pathway, probably the step of cholesterol conversion to 20α-hydroxysteroids (cf. 7). If this is so, we face an important but unanswered question raised by the present results: how the rate parameter of cholesterol hydroxylation depends in time upon ACTH. If that parameter overshoots its final steady state value when ACTH concentration is increased stepwise, it would provide a limited explanation of the observed secretory responses, but would imply unsuspected complexities in the coupling between ACTH and the rate parameter. It is noteworthy that cortisol secretion rate overshoots following a stepwise increase in the concentration of the nucleotide, cyclic 3',5'-AMP in the perfusate, and that the time course of this secretory response, and the extent of its overshoot, does not differ from that seen with ACTH (20). Thus, the complexities which give rise to the overshoot in particular, and the dynamic asymmetry generally, would be expected to reside in the mechanisms which couple cyclic AMP to cholesterol hydroxylation (cf. 8). On the other hand, it may be that the rate parameter for cholesterol hydroxylation does not overshoot, but instead rises monotonically when ACTH concentration is increased. In that event, the present results require us to postulate additional mechanisms in the steroidogenic pathway, over and above those already surmised, in order to account for our findings. Tentatively, we have adopted this latter view, and offer two dynamic models which can be related to current knowledge of the pathways of steroidogenesis, but include additional unsubstantiated mechanisms necessary to give the models the dynamic characteristics of the perfused adrenal.

The models are shown in Fig. 8, and their equations, which provide the information necessary to represent them with a computer, are given in the APPENDIX. Common to both models is a cascade of reactions, symbolized \( e \rightarrow d \rightarrow e \ldots \), which represents a truncated version of the steroidogenic pathway from cholesterol to cortisol (cf. 7). This pathway is known to include at least seven reactions, but we have represented them with only four for relative simplicity, since each intermediate compound entails a conservation equation. The point at issue is not to represent meticulously every known aspect of steroidogenesis but to identify features of steroidogenesis and of ACTH action which are salient in the dynamics of adrenocortical secretion.

Further rationale of these models and the basis of choice of parameters are given elsewhere (20). In the present context three points should be made explicit. The first is that either set of equations functions with an input-output relation which simulates, within tolerable limits, the experimental results. Thus each model compactly represents the results of the present study. It remains for future study to explore in detail the predictions which either model makes about the adrenocortical response to inputs other than those tested experimentally. The second point is that there are two quite different biochemical mechanisms represented in the two models. In the first model, an input change from one steady level to another forces every intermediate "compound" to a new steady-state quantity of concentration. However, in the second model, the three intermediates, \( c', b, \) and \( c'' \), undergo change only as the input level increases, and to an extent related only to the rate of increase of the input level; but these three variables do not respond to decreases in input level, and their steady-state levels are independent of the steady-state level of input. Thus, the second model has phenomenological attributes of a unidirectional responsivity to the rate of change of the input signal. This is a dynamic feature common to a number of biological systems (cf. 5), and our data support a description of the adrenal cortex in these terms, namely that it responds both to the absolute concentration of ACTH and to its rate of increase. The third, and final, point about these models is that they are mechanistically quite dissimilar, yet they both simulate the experimental results. This emphasizes the existence of a whole family of biochemically plausible (by contemporary standards) steroidogenic mechanisms equally consistent with the experimental results. Only further study can narrow the limits of plausibility, and it is to that end that we model the gland with systems of conservation equations rather than with arbitrary mathematical expressions.

APPENDIX

The purpose of this appendix is to set forth the details of the two models in Fig. 8 of the text. Each model is presented as a set of first-order differential equations of conservation for each of the hypothetical chemical species shown in Fig. 8.

The equations of model I are:

\[
\begin{align*}
\dot{a} &= I - 3 (a) \\
\dot{b} &= 0.1 (I) + 0.022 - 0.0342 (b) \\
\dot{c} &= 0.233 (b)(d) + 1.98 (a) \\
\dot{d} &= 0.233 (b)(e) - 3 (f) \\
\dot{e} &= 3 (f/g) \\
\dot{f} &= 3 (g/h) \\
\dot{g} &= 3 (h) \\
\dot{h} &= 3 (g/h) \\
\text{output} &= 3 (h) \\
\text{input} &= I
\end{align*}
\]

We model cholesterol conversion to \( d \) as governed by a zero-order rate parameter, which is dependent on the input via \( a \). This input dependence is the "fast" action of ACTH depicted in Fig. 8. The adrenal content of cholesterol is large, and in response to ACTH stimulation declines slowly in relation to the events we are modeling (17). Thus, there is no conservation equation for \( e \) (cholesterol) in the model. The state variable, \( a \), can either be regarded as a
chemical entity other than ACTH which mediates ACTH action
on the zero-order parameter, or else as a representation of a
dynamic lag in the parametric action of ACTH itself. The same com-
ments apply to the state variable, \( b \), which mediates the "slow"
actions of ACTH. Satisfactory scaling is achieved if an input
magnitude of 1 \( \mu \text{U/ml} \) is made equivalent to unity.

The equations of model 2 are:

\[
\dot{x} = 0.205 - 0.374 (x) + 0.35 \left( [0.13 I + 0.1] (xa) - (\phi (a)) \right)
\]

\[
\dot{a} = 0.35 \left( [0.13 I + 0.1] (xa) - (\phi (a)) \right)
\]

\[
\dot{\phi} = 0.446 - 0.04 (\dot{\phi}) + \phi (a) (c (b) (0.0075))
\]

\[
\dot{c} = 0.0047 (x) (b) - 0.0062 (\dot{\phi} (c (b))(0.0075))
\]

\[
\dot{b} = \phi (a) (c (b) (0.0075)) - 0.0006 (\dot{\phi} (c (b))(0.0075))
\]

\[
\phi = 0.333 (x - f (f))
\]

\[
\dot{f} = 0.333 (x - f)
\]

\[
\dot{g} = 0.333 (x - f)
\]

\[
\text{output} = \phi (x)
\]

\[
\text{input} = I
\]

\[
0.5 \leq \phi (x) = 30 (x) - 17.8 \leq 10
\]

Satisfactory scaling is achieved if an input magnitude of 1 \( \mu \text{U/ml} \) is made equivalent to unity. Initial conditions of 10 are applied to
\( a \) or \( (xa) \), and of 160 to \( b \) or \( (\dot{\phi}c) \) at the time the model is first set
into operation. Time should be allowed for the model to settle before applying the first input. The model operates indefinitely in
response to varying inputs without a respecification of initial condi-
tions, although if analog multipliers are used their inaccuracies may
require the initial conditions to be respecified from time to time.

The term 0.205 in the equation for \( x \) represents the source shown
in Fig. 8. There is no conservation equation for the substance \( \epsilon \)
which represents cholesterol. Instead, we model the sink for cho-
lesterol as two zero-order rate parameters. One appears as the
term 0.35 in the equation for \( a \), and the other, which is input
dependent, appears as the term 0.0047 in the equation for \( \dot{\phi} \).

Figures 9 and 10 display the operation of the two models in
response to a series of input signals which correspond to some of
the data shown in Fig. 8. There is no conservation equation for the substance \( \epsilon \), which represents cholesterol. Instead, we model the sink for cholesterol as two zero-order rate parameters. One appears as the term 0.35 in the equation for \( a \), and the other, which is input dependent, appears as the term 0.0047 in the equation for \( \dot{\phi} \).

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