FOURSCORE YEARS AGO, the American Journal of Physiology published three seminal, noteworthy papers (8, 13, 17) that focused on identifying the life-essential factor produced by the adrenal cortex. These works by Houssay (Fig. 1) and Lewis (8), Marine and Baumann (13), and Swingle and Pfiffner (17) tackled an age-old question of puzzling out what is the function of the adrenal gland. There are excellent and affectionate tales on the history of the adrenal gland (see Refs. 6, 12, 18), which the interested reader is encouraged to seek to retrace the paths of discovery. Together, the three papers presented here (8, 13, 17) established that while salt solutions are palliative, a lipid fraction derived from the adrenal cortex is necessary to stave off death in the adrenalectomized cat or dog. To today’s reader, these scientific discoveries may seem pleasant findings that are easily executed and overshadowed by the scintillating publications of today’s world. However, a lifetime after these modest findings the question of how and why the lipid fraction (namely glucocorticoids) sustains life in an adrenalectomized animal remains a subject of active research. In this intervening time span, the field has tapped on chemistry, biochemistry, pharmacology, molecular biology, and genetics. Nonetheless, even the finest gene chip of the millennium doesn’t capture the evolution of thought in chasing the life-essential essence of the adrenal cortex.

Was Science Easier a Lifetime Ago?

Surely the methods that zeroed in on insulin from the pancreas would be an effective guideline for separating out the principal factor in the adrenal gland. Imagine the high hopes when cats were acquired and the adrenals removed. There would be the methodical collection of tissue, homogenization, and painstaking fractionation of aqueous components to be followed by injection after injection, with decline and death as a dismal endpoint. Fortunately, this type of protocol is now viewed as inhumane and would no longer be permitted. In other ways, however, many of us would count aspects of that research era as appealing when scientists were not encumbered with government grant applications (in PDF), institutional board bureaucracy, and statistical constraints. Surely progress would be rapid. Perhaps investigators then were more akin to philosopher-scholars, and one wonders what they could have accomplished if armed with today’s repertoire of computer-driven technotoys and the wizardry of molecular biology. Indeed, equipped with little more than scalpels, syringes, and separatory funnels they were able to parse out effects of aqueous catecholamine extracts to target the steroid-bearing lipid fraction. Nonetheless, there are some commonalities between then and now (Fig. 2).
Fig. 2. Time spiral of select studies of negative-feedback signals in adrenalectomized rat. Reference nos. shown in parentheses.

How Would the Experiments Be Different if They Were Run Today?

A hypothetical sketch is provided in Table 1.

Is the Paradigm Outdated?

For those of us who study the rat, one ironic insight is that the approach of testing replacement therapy in an adrenalectomized animal returns to the forefront episodically. Wonderful and elegant approaches for dissecting adrenal cortical function, such as synthetic inhibitors, pharmacological receptor agonists and antagonists, behavioral tests, exquisite neural lesions, genetic mutants, and DNA/RNA interference have illuminated fascinating elements of adrenal function. Nonetheless, time and time again, administration of glucocorticoids (for rat, Kendall’s Compound B, namely corticosterone) to adrenalectomized rats yields significant nuggets of fundamental information such as physiological (10) and rate-sensitive (3) negative feedback; definition of low, replacement, and high levels for basal hypothalamic-pituitary-adrenal function (1); and regulation of circadian rhythms (9) (Fig. 2). Like a bad penny or an erratic comet, the simple “scalpel and syringe” approach has staying power.

Table 1. Comparison of how the same classic experiments were run 80 years ago and how they could be run today

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Then</th>
<th>Problem</th>
<th>How It Might Be Run Today</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Choice</td>
<td>Problem</td>
<td>Choice</td>
</tr>
<tr>
<td>Selection of animal</td>
<td>Cat or dog</td>
<td>Some are pregnant, parasite-laden</td>
<td>Transgenic mouse</td>
</tr>
<tr>
<td>Animal protocol</td>
<td>None</td>
<td>2 subjects ran away (see Ref. 8)</td>
<td>Category 3*</td>
</tr>
<tr>
<td>Surgery</td>
<td>Chloroform under a bell jar; then ether;</td>
<td>Needs delicate hand; some abscess</td>
<td>Activate promoter</td>
</tr>
<tr>
<td></td>
<td>bilateral or staged adrenalectomy</td>
<td>formation</td>
<td></td>
</tr>
<tr>
<td>Injectate</td>
<td>Slaughterhouse bovine adrenals subjected</td>
<td>Time-consuming and inconsistent impurities; made own reagents</td>
<td>Order in from pharmaceutical house</td>
</tr>
<tr>
<td></td>
<td>to 72 h of alcoholic-benzene extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and dissolved in oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endpoint</td>
<td>Death</td>
<td>Inconsistent timing</td>
<td>Reporter genes</td>
</tr>
<tr>
<td>Statistical treatment</td>
<td>Student’s t-distribution; 1908 (see Ref. 16)</td>
<td>Mostly descriptive</td>
<td>Cluster analysis</td>
</tr>
</tbody>
</table>

*Category 3 designates animal experiments that incorporate protocols with more than momentary “pain, discomfort, or stress” as defined by the National Research Council in the Guide for the Care and Use of Laboratory Animals.

Timeless Lessons

Several precepts from the founder papers endure as useful signposts even in our modern era. Given your chosen question, start simple and follow the data.

Choose the right animal. With our founder papers, the cat and dog were chosen as subjects for their availability and ideal size for operation. Of course, first, one had to catch them and keep them (8). Small matters such as the fecund state, pneumonia (13), abscess formation, outbreaks of distemper or hookworm load (17) were retrospectively weighted as possible confounds. In the current era, the ready supply of inbred and transgenic animals seems a blessing. Nonetheless, the marked endocrine variations across strains (7) and within strains derived from different vendors (19) are a grave consideration. Moreover, reports of differing behavioral outcomes from rigorously identical studies across labs (20) suggest that the experimental environment must also be weighted in any interpretation.

Really give thought to your control treatments. Whereas Marine and Baumann (13) found sodium salts and water crucial to prolonging life, Swingle and Pfiffner (17) had to further parse whether the salts in their vehicle injections for the experimental lipid injections were the de facto treatment.

Be mindful that accepted statistical analysis might lag behind the resolution of your data. In the early 1920s, Student’s t-test was not universally adopted, and the Fisher F statistic (5) was not codified until 1934 (15). Even today cluster analysis (4) is racing to evolve to the level to grapple with the complex wealth of numbers generated by microarray studies of gene expression patterns.

Why Are the Adrenal Cortices Necessary for Life?

Science has a way of spiraling back on itself. Certainly, restoration of glucocorticoids in the adrenalectomized animal is a paradigm that resurfaces with regularity. The answer to this persistent question may very well be that the adrenal cortices are necessary to maintain metabolism. Swingle and Pfiffner (17) noted that “Death invariably occurred within two or three
days from the date of first refusal of food.” In that same era, Britton and Silvette (2) had speculated that the prepotent function of the adrenals had to do with carbohydrates, which helped to fuel the “salt vs. sugar” debates at the seminal Cold Spring Harbor Symposium in 1937. Ten years later, Selye (14) named the adrenal α-ketosteroids as “glucocorticoid” for their action on mobilizing glucose from stored energy. Recently, Laugero and coworkers (11) found that, if energy stores are supported in the adrenalectomized rat, all indexes (body weight, fat depots, and ACTH and CRH content in the para-ventricular nucleus) are restored to the normal levels of the sham-adrenalectomized controls. The function of the adrenal cortex may be primarily metabolic and it has been pounding on the door for decades. A final lesson might be to mark reoccurring paradigms. Experimental designs that persistently cycle back into the forefront may be hallmarks of elegant simplicity. And while you listen for these reverberations with open ears, don’t forget to watch your back. Science may be throwing us more than one curve at a time.

REFERENCES