tively simple means for maintaining true cultures a number of days or weeks[6] without transfer; (2) the method does not require the use of a plasma coagulum (fibrin being eliminated); and (3) a monolayer of living cells, almost entirely epithelial, can be obtained for special studies.

A topographical survey of a single cell infected with vaccinia is illustrated in Fig. 1. The individual electron micrographs which make up the composite picture were taken at a magnification of 21,000; after assembly the composite picture was reduced somewhat. The opaque spherical structures occurring in the cytoplasm at the upper left corner are interpreted as elementary bodies of vaccinia seen individually and in clusters. Fig. 2 reproduces one of the micrographs containing the elementary body-like structures which make up Fig. 1. This electron micrograph was also taken at a magnification of 21,000 but has not been reduced. An idea of the reduction in size of the composite picture is obtained by comparing Fig. 2 with the individual components of Fig. 1. Spherical opaque structures such as those observed in Fig. 1 and 2 are regarded as elementary bodies since they have not been observed in similar preparations of uninfected rabbit kidney tissue culture cells. The present method adds another approach to the study of the intracellular growth of vaccine virus which has in the past been fruitfully investigated by microscopic technics employing visible light.[7,8]

Summary. A technic has been devised which provides true cultures of mammalian cells for electronmicroscopy. The usefulness of this procedure in the study of viruses is indicated.

[6] Barski, G., *Ann. Inst. Pasteur*, 1948, **74**, 312.

[7] Nauck, E. G., and Robinow, C., *Zentralbl. f. Bakt.* (Abt. 1), 1936, **135**, 437.

[8] Bland, J. O. W., and Robinow, C. F., *J. Path. and Bact.*, 1939, **48**, 381.

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Adrenalectomy and Pituitary Adrenocorticotrophic Hormone Content.*†

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Alterations in the weight and chemical composition of the adrenal gland have been used as quantitative indices for measurement of the rate of discharge of adrenocorticotrophic hormone (ACTH) from the adenohypophysis following application of stress.[1] These indices have been called "target gland indices". It is now possible to complement our knowledge of the pituitary-adrenal system gained from such indices with data on adenohypophyseal content of ACTH. The present report is one of several studies concerned with factors regulating the synthesis and discharge of pituitary ACTH, and deals especially with the changes following adrenalectomy in the rat.

Methods. Male rats from the Sprague-Dawley farm were used. Twenty rats, 230-275 g in body weight, were employed in two experiments conducted at different times. In Experiment I, 6 rats were bilaterally adrenalectomized and 4 rats served as controls; in Experiment II, 4 rats were bilaterally adrenalectomized, 3 rats were subjected to sham adrenalectomy and 3 rats served as controls.

* Supported by a grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

† With the statistical assistance of Marion A. Sayers.

‡ Fellow of the American Bureau for Medical Aid to China.

[1] Sayers, G., and Sayers, M. A., *Recent Progress in Hormone Research*, 1948, **2**, 81.

TABLE I.
Pituitary Adrenocorticotrophic Hormone Potency after Adrenalectomy.

Expt.	Donors	Dose of pituitary, μg per 100 g of recipient rats	Reduction of adrenal ascorbic acid content of recipient rat mg per 100 g adrenal tissue
I	Control (4)*	40	146,† 173, 144
		10	84, 94, 59
	Adrenex (6)	40	76, 12, 28
		10	14, 0, 17
II	Control (3)	40	180, 173, 118, 103
		10	68, 26, 18
	Sham (3)	40	150, 117, 112, 125
		10	95, 63, 18, 15
	Adrenex (4)	160	148, 107, 141, 126
		40	64, 25, 54, 81
III	Control (13)	40	147, 189, 147
		20	157, 120, 140
		10	111, 116, 63
		5	97, 26, 26
	Scald (6)	40	158, 133, 151, 147, 174
		20	88, 107
		10	69, 70, 73, 46

* No. of rats in group.
† Each value in right-hand column represents result in one recipient rat.

Twenty-four hours after removal of the adrenals, all rats in the various groups were anesthetized with sodium pentobarbital and the pituitaries removed. During the 24-hour period the animals were fasted but had free access to tap water. A third experiment (III) was conducted in order to compare the changes produced by adrenalectomy with those produced by a severe stress. Nineteen rats weighing 270 to 350 g were fasted 24 hours; 6 were then scalded and the remaining 13 served as controls. All were sacrificed after an additional 24-hour period of fasting. For scalding the rats were immersed up to the neck for 7 seconds in an aqueous detergent ("Duponal") solution at 71 ± 1°C.

The anterior lobes were carefully freed of posterior pituitary tissue, frozen and lyophilized. The pituitaries of each group of rats were pooled, and ground to a homogeneous powder. Aliquots of this powder weighing from about one-half to two mg were extracted successively with 3 portions of a solution of 0.9% NaCl made alkaline to 0.01 N with NaOH. The total volume of extraction fluid was adjusted according to the potency of the tissue and varied from 0.5 ml per 40 μg to 0.5 ml per 160 μg of dry tissue. Appropriate dilutions were prepared for the various dose levels. The extracts were injected into hypophysectomized recipient rats (0.5 ml per 100 g of body weight) and the degree of reduction of adrenal ascorbic acid was measured by the difference between the concentration of ascorbic acid in the left (control) adrenal removed immediately before administration of the pituitary extract and that of the right adrenal removed an hour after such administration.[2]

Results. The assay data from the three experiments are presented in Table I. The ability of extracts of pituitaries to deplete the adrenal ascorbic acid of hypophysectomized recipient rats is a measure of their content of ACTH. It is apparent that the content of ACTH in the adenohypophyses of adrenalectomized rats is markedly reduced. In contrast, the scalded animals exhibit only a slight reduction in ACTH content of their pituitaries.

The results of the 3 experiments have been combined for statistical analysis in Table II. Since 10 μg of pituitary tissue from adrenalectomized animals (Expt. I) failed to give a significant response, the results obtained with this dose have not been included in the analy-

2 Sayers, M. A., Sayers, G., and Woodbury, L. A., *Endocrinology*, 1948, **42**, 379.

TABLE II.
Relative Content of ACTH in Rat Adenohypophyses.

Groups compared	No. recipient rats	b	s	λ	M	Antilog of M	S_M	Antilog (M—S_M)-antilog (M+S_M)
Sham (3)*	8	130	29.7	0.228				
					$\bar{1}.861$	0.73	±0.097	0.58-0.91
Control A (7)	13	149	30.0	0.201				
Adrenex (10)	11	136	24.4	0.180				
					$\bar{1}.306$	0.20	±0.083	0.17-0.25
Control A (7)	13	149	30.0	0.201				
24-hour scald (6)	11	147	13.9	0.095				
					$\bar{1}.814$	0.65	±0.069	0.56-0.76
Control B (13)	12	125	27.3	0.218				

b = slope of the log dose response curve; s = standard deviation of all of the individual responses about this curve (a straight line fitted by the method of least squares); λ = s/b and is a measure of the accuracy of the assay method; M = logarithm of the ratio of the potencies; S_M = standard error of M; antilog (M—S_M)-antilog (M+S_M) is the range of estimate for one std. error.

* No. rats in donor groups in parentheses.

sis. The 7 control rats of Expt. I and II were combined as "Control A" in Table II. The 13 control rats of the scald experiment (III) serve as "Control B" in Table II. The analyses depicted in Table II include 3 assay pairs, as follows: sham-operated rats compared with Control A; adrenalectomized rats compared with Control A; scalded rats compared with Control B. No significant difference exists between the slopes of the log dose-response curves (b-values) of any two groups compared, a fact which indicates that the observed alterations in biological activity are quantitative and not qualitative. Antilog M, the ratio of the adrenocorticotrophic hormone potencies for each assay pair, is an expression of the relative content of ACTH in the pituitaries of the experimental as compared with the control groups. A slight reduction (27%) of the ACTH content of the pituitary took place in the sham-operated animals. The effects of the scald, acting as a continuous stress over a 24-hour period, reduced the content of ACTH in the adenohypophysis by only 35%. In contrast, adrenalectomy reduced the ACTH content by 80%. The differences in ACTH content cannot be accounted for by differences in the weights of the pituitaries. The mean dry weights of the pituitaries (expressed as mg per rat) were as follows: control A, 0.88; sham-operated, 0.58; adrenalectomized, 0.77; control B, 1.21; scalded, 1.35.

Preliminary histological studies employing the staining technic of Koneff[3] have revealed no striking cytological changes in the anterior pituitary of adrenalectomized rats associated with the marked reduction in ACTH content.

Discussion. In an attempt to elucidate the factors responsible for a reduction in the ACTH content of the pituitary following stress we have formulated the problem in its simplest terms, by assuming that two processes, synthesis (S) and discharge (D) of ACTH, can each separately undergo no change in rate ($_n$), an increase in rate (↑) or a decrease in rate (↓). These 6 variables give rise, theoretically to 9 combinations, represented symbolically as S_nD_n, $S_nD\uparrow$, $S_nD\downarrow$, $S\uparrow D_n$, $S\uparrow D\uparrow$, $S\uparrow D\downarrow$, $S\downarrow D_n$, $S\downarrow D\uparrow$ and $S\downarrow D\downarrow$. Four of these combinations S_nD_n, $S_nD\downarrow$, $S\uparrow D_n$ and $S\uparrow D\downarrow$, are eliminated at once because the problem at hand concerns only a reduction in content of ACTH.

It is possible to rule out 2 more combinations, $S\downarrow D_n$ and $S\downarrow D\downarrow$, on the basis of the fact that they cannot account for the increased rate of discharge of ACTH always associated with stress; studies in this laboratory[1] have shown that a discharge of ACTH follows the subjection of an animal to any of a variety of stresses, such as sham adrenalectomy or scald. Of the remaining 3 combinations, $S_nD\uparrow$, $S\uparrow D\uparrow$ and $S\downarrow D\uparrow$, one does

[3] Koneff, A. A., *Stain Technol.*, 1938, **13**, 49.

not seem probable, namely, S↓D↑, because it appears most unlikely that the rate of synthesis of ACTH would be depressed when there is an increased body need for the hormone. Thus only two combinations remain to be considered as possibilities, namely, (1) increased discharge of ACTH associated with no change in the rate of synthesis, and (2) increased discharge associated with an increased rate of synthesis.

It is likely that in mild stress the first possibility obtains; in severe stress, the second possibility. The pituitary stores a relatively large quantity of ACTH; there is an amount of ACTH in one rat pituitary sufficient to produce a marked reduction (50% of maximal) in the ascorbic acid content of the adrenals of 100 rats. Mild stress produces a reduction in adrenal ascorbic acid content which is approximately 50% of maximum. Therefore, the quantity of ACTH normally stored in the pituitary is 100-fold greater than the amount required to meet the immediate needs of the organism subjected to mild, short-acting forms of stress; synthesis of new hormone would not be needed in order to cope with the stressful situation. However, in the case of severe, long-acting forms of stress, for example, scald, synthesis must be accelerated so that the store of ACTH can be fairly well maintained despite its rapid and continuous discharge. In Table II, it can be seen that the ACTH content of scalded rats was maintained at 65% of the control level.

In contrast, adrenalectomy is followed by a very marked reduction in the pituitary content of ACTH. Whereas the severe continuous stress of scalding resulted in a reduction of only 35%, adrenalectomy led to a reduction of 80% in hormone content. Unfortunately, direct experimental evidence regarding rate of discharge of ACTH, such as that available in the stress experiments, was not obtainable in the adrenalectomy experiments, because adrenalectomized animals lack the target gland which provides a simple means for measuring rate of ACTH discharge. Therefore, one must consider depression of synthesis as well as increased rate of discharge as a factor responsible for the depletion of stores of ACTH in the pituitary 24 hours after adrenalectomy. However, we are inclined to favor the view that the rate of discharge of ACTH is at a maximum when there is a complete absence of the target gland secretion. This latter possibility is in keeping with other experimental data from this laboratory[4] which have been interpreted to mean that the rate of discharge of ACTH increases as the level of cortical hormones in the body fluids falls. According to this concept, adrenalectomy provides a maximum stimulus for discharge of ACTH since it produces the lowest possible titers of cortical steroids in body fluids. Determination of body fluid titers of ACTH in adrenalectomized animals by direct assay would be of considerable help in assessing the relative roles of synthesis and discharge as factors regulating the ACTH stores of the pituitary.

Summary. Twenty-four hours after adrenalectomy the content of ACTH in the pituitary of the rat is reduced by 80%. Sham adrenalectomy and scalding reduce the content of ACTH by only 27% and 35%, respectively. The significance of these observations for the problem of the regulation of rate of synthesis and discharge of pituitary ACTH is discussed.

The authors are indebted to Dr. Louis S. Goodman and Dr. Walter S. Loewe for their many helpful suggestions and criticisms during the preparation of the manuscript.

[4] Sayers, G., and Sayers, M. A., *Endocrinology*, 1947, **40**, 265.