The Effect of Surgical Operation on the Adrenocortical Response to ACTH *

RUSSELL A. ESTABROOKS, M.D., LEON J. MARKS, M.D., JOHN C. LONERGAN, M.D., JOHN F. MURTAUGH, M.D., VERNON E. STROBL, M.D.

From the Department of Surgery and the Steroid Research Laboratory, Boston Veterans Administration Hospital, Boston, Mass.

IN PREVIOUS PAPERS of this series it was demonstrated that free plasma 17-hydroxycorticosteroid levels increase promptly in response to major operations and that these steroid elevations are in part mediated by extra-adrenal factors.[8, 12] Sandberg *et al.* observed that the administration of ACTH during the immediate postoperative period resulted in a further rise in free plasma 17-hydroxycorticosteroids regardless of the level of these steroids at the end of the surgical procedure.[16] Steenburg *et al.* demonstrated that free plasma 17-hydroxycorticosteroids rose higher in response to postoperative infusions of ACTH than to identical preoperative ACTH infusions.[18] These observations suggested the importance of evaluating the effects of varying doses of ACTH on the plasma levels of free and of conjugated 17-hydroxycorticosteroids both preoperatively and at different intervals postoperatively. In this report data dealing with these aspects of the effect of operation on the adrenal cortex will be presented.

Materials and Methods

Twenty-one male patients, ranging in age from 26 to 85 years, were selected. No patient had clinical evidence of liver disease. All major operations were performed under either ether or cyclopropane anesthesia. ACTH was administered intravenously in isotonic saline solution at the rate of either 0.25 I.U. per hour or 5.0 I.U. per hour for periods of two or of four hours. These ACTH infusions were performed three to ten days preoperatively and repeated either one hour postoperatively or during the first three postoperative days. Venous blood samples were collected before and at one, two, three, and four hours after the infusion was started.

In an additional ten preoperative surgical patients without liver disease, two successive daily six-hour infusions of 30 I.U. of ACTH were administered. In five of these patients, 40 I.U. of Zinc-ACTH * were injected intramuscularly at the completion of the first ACTH infusion. Samples of venous blood were drawn before and at two, four, and six hours after the beginning of the infusion.

All infusion studies were made with Armour ACTH. Plasma free 17-hydroxycorticosteroids (17-OHCS) were measured by a modification of the method of Nelson and Samuels.[14] Plasma conjugated 17-hydroxycorticosteroids (17-OHCS) were determined by a modification of the method of Bongiovanni *et al.*[1]

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* Cortrophin-Zinc, Organon, Inc., Orange, New Jersey.

TABLE 1. *Effect of Maximal Dose ACTH Infusions on Free Plasma 17-OHCS Levels*

Case No.*	Preoperative: Duration of Infusion, Hours	Preoperative: Control	Preoperative: Hours 1	2	3	4 (γ/100 ml.)	Postoperative: Hours Postop.†	Postoperative: Control	Postoperative: Hours 1	2	3	4 (γ/100 ml.)
1) L. A., 37 Gb, Ch	2	17	28	34	39	31	1	39	46	51	59	56
2) W. A., 47 H. H., T. H.	2	21	29	31	40	32	1	31	38	40	42	35
3) R. S., 26 Gb, Ch	2	18	24	29	28	23	1	42	51	61	54	44
4) P. S., 64 Gb, Ch	2	15	25	32	33	24	1	56	67	75	70	65
5) S. H., 85 Gb, Ch	2	19	35	45	51	46	1	44	62	68	73	81
6) W. F., 59 CaC, C.R.	2	22	32	38	34	25	1	51	63	72	66	57
Mean (1–6)		18.7	28.8	34.8	37.5	30.2		43.8	54.5	61.2	60.7	56.3
7) J. C., 37 M.T.P., P.R.	2	15	24	32	30	25	24	19	34	43	39	30
8) W. M., 66 D.U., V.	2	11	21	26	33	22	24	20	37	55	44	27
9) J. M., 48 D.U., G.R.	2	9	23	32	25	12	72	11	26	35	29	15
10) A. T., 45 D.U., G.R.	4	17	25	29	33	37	24	20	34	40	49	56
11) R. K., 42 CaC, C.R.	4	16	28	36	37	39	24	32	46	49	59	58
12) C. J., 68 CaC, C.R.	4	22	33	39		48	24	26	38	48	53	52
13) F. S., 36 Gb, Ch	4	19	29	35	42	40	24	35	53	57	55	57
14) T. C., 59 D.C., C.R.	4	13	24	31	29	33	48	24	41	57	60	64
15) R. L., 48 CaL, L.R.N.	4	18	27	34	41	45	48	21	45	56	57	56
Mean (10–15)		17.5	27.7	34.0	36.4	40.3		26.3	42.8	51.2	55.5	57.2

* The data after each case number refer to the patient's initials and age. See key to the abbreviations for diagnosis and operation.

† Time postoperatively at which ACTH infusion was commenced.

Patients 1–9 received 10 I.U. of ACTH. Patients 10–15 received 20 I.U. of ACTH.

Results

1. Effect of ACTH Infusions on Plasma 17-Hydroxycorticosteroids both Preoperatively and at Varying Intervals Postoperatively.

The free plasma 17-OHCS levels for patients who received infusions of five units of ACTH per hour for two hours and for four hours, both preoperatively and postoperatively, are presented in Table 1. The corresponding values for plasma 17-OHCS conjugates are recorded in Table 2. The free plasma 17-OHCS values for patients receiving a four hour infusion of one unit of ACTH, both preoperatively and postoperatively, are seen in Table 3.

a) The two-hour infusion of ten units of ACTH preoperatively resulted in maximal

TABLE 2. *Effect of Maximal Dose ACTH Infusions on Conjugated Plasma 17-OHCS Levels*

Case No.*	Preoperative: Duration of Infusion, Hours	Preoperative: Control	Preoperative: Hours 2 γ/100 ml.	Preoperative: Hours 4 γ/100 ml.	Postoperative: Hours Postop.†	Postoperative: Control	Postoperative: Hours 2 γ/100 ml.	Postoperative: Hours 4 γ/100 ml.
1) L. A.	2	14	18	23	1	27	31	36
2) W. A.	2	10	15	21	1	24	32	38
3) R. S.	2	20	23	30	1	21	25	29
4) P. S.	2	17	24	28	1	16	21	25
5) S. H.	2	25	30	38	1	36	42	49
6) W. F.	2	6	11	15	1	35	40	47
Mean (1–6)		15.3	20.2	25.8		26.5	31.8	37.3
7) J. C.	2	21		29	24	31		40
8) W. M.	2	9		18	24	13		23
9) J. M.	2	16		25	72	15		26
10) A. T.	4	15		23	24	17		29
11) R. K.	4	6		17	24	13		25
12) C. J.	4	8		20	24	15		26
13) F. S.	4	25		34	24	32		43
14) T. C.	4	18		27	48	23		34
15) R. L.	4	13		23	48	16		27
Mean (10–15)		14.2		24.0		19.3		30.7

* The data after each case number refer to the patient's initials and age.
† Time postoperatively at which ACTH infusion was commenced.

free plasma 17-OHCS levels at two to three hours (Table 1). By the fourth hour, free plasma steroids had fallen from their peak value. There was a progressive increase in conjugated plasma 17-OHCS levels in response to ACTH (Table 2). In the immediate postoperative period a similar pattern in the response of both the free and conjugated plasma 17-OCHS to ACTH was observed despite the fact that the postoperative pre-ACTH steroid levels were already markedly elevated (Fig. 1). Two patients who received ten units of ACTH 24 hours postoperatively developed higher values of both free and conjugated plasma 17-OHCS than they did preoperatively.

b) The four hour infusion of 20 units of ACTH resulted in a progressive increase in both free and conjugated plasma 17-OHCS preoperatively (Tables 1, 2). Identical ACTH infusions administered on the first or second postoperative day produced even higher levels of plasma steroids (Fig. 2). In several patients the peak free plasma 17-OHCS values were reached more rapidly postoperatively.

c) One unit of ACTH administered over a four hour period was capable of evoking a significant elevation in free plasma 17-OCHS preoperatively (Table 3). In the immediate postoperative pericd, three patients demonstrated moderate rises in plasma 17-OHCS levels in response to ACTH. It is difficult to interpret the small rise in free plasma steroid observed in patient P.C. because of the natural tendency of free plasma 17-OHCS to rise during the first twelve hours postoperatively.[8, 16] In two patients, free plasma 17-OHCS attained moderately higher values in response to 1 unit of ACTH 24 hours postoperatively than preoperatively.

TABLE 3. *Effect of Small Dose ACTH Infusions on Free Plasma 17-OHCS Levels*

Case No.*	Preoperative: Duration of Infusion, Hours	Preoperative: Control	Preoperative: Hours 1 γ/100 ml.	2 γ/100 ml.	3 γ/100 ml.	4 γ/100 ml.	Postoperative: Hours Postop.†	Postoperative: Control	Postoperative: Hours 1 γ/100 ml.	2 γ/100 ml.	3 γ/100 ml.	4 γ/100 ml.
1) W. O., 30 D.U., G.R.	4	19	27	33	29	28	1	47	57	62	59	57
2) P. D., 69 A.A., A.D.	4	18	23	27	35	32	1	35	37	46	45	47
3) P. C., 33 E.B., B.R.	4	20	26	28	30	33	1	42	41	45	44	48
4) M. G., 32 Tb, L.	4	15	21	22	24	27	1	29	34	37	41	42
Mean (1–4)		18	24.3	27.5	29.5	30		38.3	42.3	47.5	47.3	48.5
5) M. R., 67 CaL, L.R.N.	4	19	24	28	33	31	24	26	33	38	41	40
6) A. R., 61 A.O., A.E.	4	23	28	31	34	32	24	28	35	39	44	45

* The data after each case number refer to the patient's initials and age. See key to the abbreviations for diagnosis and operation.

† Time postoperatively at which ACTH infusion was commenced.

All patients received 1 I.U. of ACTH intravenously over a four hour period.

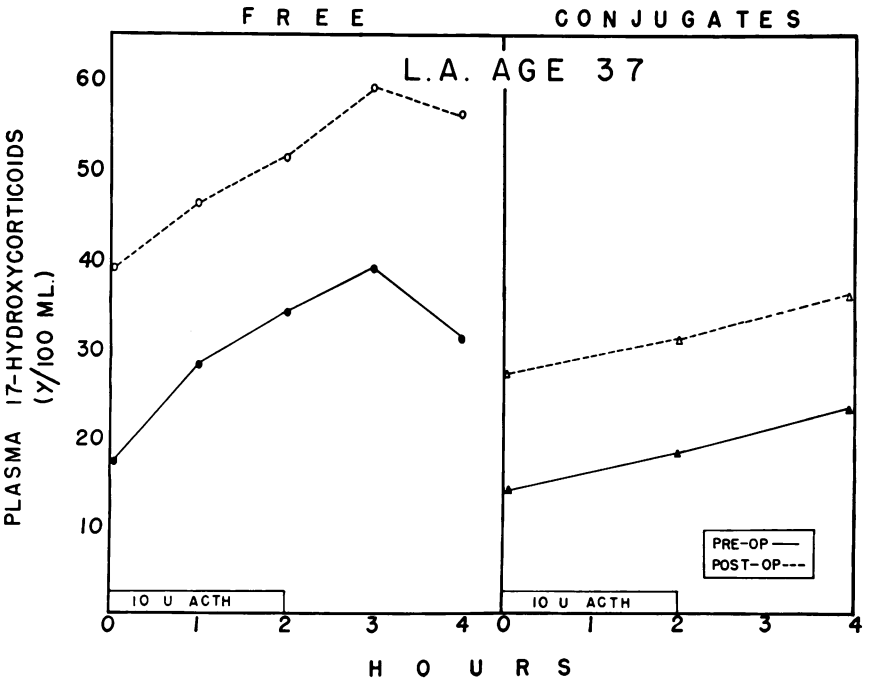

FIG. 1. Response of free and conjugated plasma 17-OHCS levels to 10 I.U. ACTH intravenously both preoperatively and immediately following major surgery.

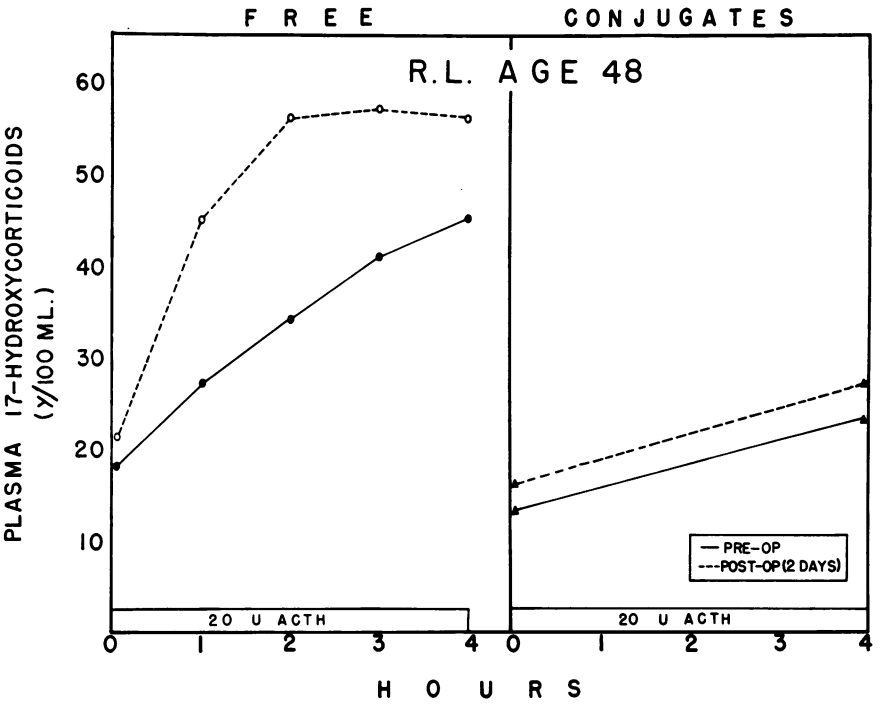

FIG. 2. Response of free and conjugated plasma 17-OHCS levels to 20 I.U. ACTH intravenously both preoperatively and on the second postoperative day.

2. Effect of Repeated Daily ACTH Infusions on Plasma 17-Hydroxycorticosteroids.

In order to evaluate the effect of the repeated administration of conventional ACTH on adrenocortical responsiveness, two daily six hour infusions of 30 units of ACTH were given to ten preoperative surgical patients who were free from liver disease. Five of these patients also received 40 units of Zinc-ACTH intramuscularly at the completion of the first infusion. The results of this study are presented in Tables 4 and 5.

During the first ACTH infusion, a progressive rise in both free and conjugated plasma 17-OHCS was observed over a six hour period. Higher levels of plasma steroids in response to ACTH were observed on the second day (Fig. 3). It is of interest that the five subjects who received Zinc-ACTH also on the first day developed higher free and conjugated plasma 17-OHCS values in response to the second infusion of ACTH than did the other five patients.

3. Direct Measurement of Adrenal 17-Hydroxycorticosteroid Output During Operation.

In a 34-year-old patient who underwent a left nephrectomy for chronic pyelonephritis and hypertension, a direct measurement of adrenocortical secretion of 17-OHCS was performed during operation. Following removal of the left kidney, the left adrenal vein was cannulated with a polyethylene catheter. Two blood samples were obtained and adrenal blood flow was estimated. Then 10 units of ACTH were injected intravenously over a one minute period. Ten to 15 minutes following completion of the ACTH injection two further samples of adrenal vein blood were collected. The results of this study are presented in Table 6.

During operation, the concentration of free 17-OHCS was 734γ per cent and 69γ

TABLE 4. *Effect of Repeated Maximal Dose ACTH Infusions on Free Plasma 17-OHCS Levels*

Case No.*	Day 1 Control	Day 1 Hours 2	4	6	Day 2 Control	Day 2 Hours 2	4	6
		γ/100 ml.				γ/100 ml.		
1) A. G., 63 CaM	25	39	48	56	27	42	51	58
2) D. K., 56 V.V.	16	33	37	42	15	40	47	55
3) R. S., 65 CaP	22	36	43	50	24	46	52	59
4) T. R., 42 I.H.	11	27	36	39	12	37	49	50
5) R. D., 36 D.U.	21	34	39	46	19	39	43	49
Mean (1–5)	19	33.8	40.6	46.6	19.4	40.8	48.4	54.2
6) F. W., 31† C.O.T.	19	31	36	41	18	38	52	58
7) J. C., 41† T.U.L.	15	28	35	34	15	37	50	53
8) N. S., 30† D.F.	20	37	41	49	30	48	53	61
9) T. O., 48† I.H.	18	34	38	45	29	45	49	52
10) M. M., 65† CaS	17	31	49	58	19	47	60	69
Mean (6–10)	17.8	32.2	39.8	45.4	22.2	43.0	52.8	58.6

* The data after each case number refer to the patient's initials and age. See key to the abbreviations for diagnosis.

† Patients 6–10 received 40 I.U. of Zinc-ACTH intramuscularly at the termination of the first ACTH infusion. All patients received 30 I.U. of ACTH intravenously over a six hour period on two consecutive days.

per cent in adrenal and peripheral venous plasma, respectively. The adrenal output of free 17-OHCS was 74γ per minute. Following administration of ACTH there was no significant change in either the adrenal output or the peripheral venous concentration of 17-OHCS. These results imply that this patient's left adrenal cortex was being maximally stimulated at the time of this study.

Discussion

From the data presented in this study it appears that major operations result in alterations in the adrenocortical capacity to respond to exogenous conventional ACTH. These results are in agreement with the previous studies of Sandberg *et al.*[16] and Steenburg *et al.*[18] The adrenocortical response to surgical trauma in animals has been shown to be dependent upon the integrity of both the hypothalamus and the anterior pituitary lobe.[4, 7, 11] Hume and Nelson assayed blood taken from dogs, anesthetized with ether, during operation and found elevated levels of circulating ACTH.[7, 13] The reaction time of the anterior pituitary with respect to ACTH secretion in response to operation in anesthetized rats is believed to be a matter of seconds.[11] The measurement of 17-OHCS in the adrenal venous blood of dogs has revealed an immediate increase in adrenocortical secretion in response to either ether anesthesia and/or surgical trauma.[5, 6] The administration

TABLE 5. *Effect of Repeated Maximal Dose ACTH Infusions on Conjugated Plasma 17-OHCS*

Case No.*	Day 1 Hours			Day 2 Hours		
	Control	4	6	Control	4	6
		γ/100 ml.			γ/100 ml.	
1) A. G.	14	23	28	16	23	29
2) D. K.	21	32	37	21	34	40
3) R. S.	10	20	29	12	23	33
4) T. R.	13	22	30	14	25	32
Mean (1–4)	14.5	24.3	31.0	15.8	26.3	33.5
6) F. W.†	10	19	25	14	22	32
7) J. C.†	16	24	29	20	31	36
8) N. S.†	21	31	34	26	36	43
9) T. O.†	17	27	28	23	32	37
10) M. M.†	13	25	36	18	33	45
Mean (6–10)	15.4	25.2	30.4	20.2	30.8	38.6

* The data after each case number refer to the patient's initials and age.
† Patients 6–10 received 40 I.U. of Zinc-ACTH intramuscularly at the termination of the first ACTH infusion.
All patients received 30 I.U. of ACTH intravenously over a six hour period on two consecutive days.

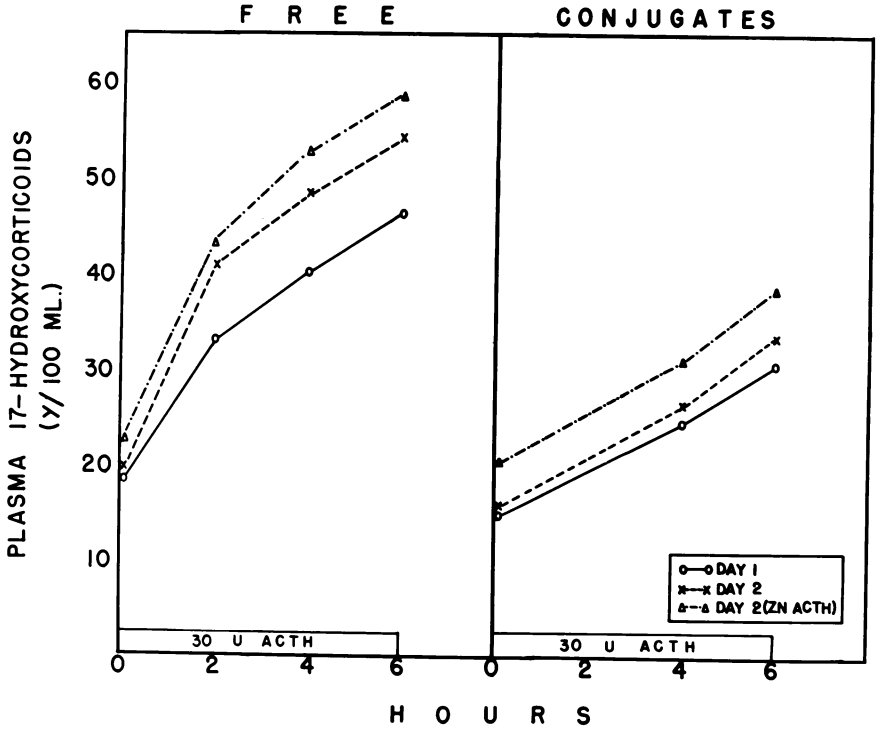

FIG. 3. Response of free and conjugated plasma 17-OHCS levels to two consecutive daily infusions of 30 I.U. of ACTH. The mean plasma 17-OHCS values of ten preoperative subjects are shown. Five of these patients received 40 I.U. of Zinc-ACTH intramuscularly at the termination of the first ACTH infusion.

of large doses of ACTH to these animals during operation did not necessarily increase adrenal 17-OHCS output.[5] Following operation and recovery from anesthesia, adrenocortical secretion diminished.[5, 6]

The inability of exogenous ACTH to increase the adrenal output of 17-OHCS during surgery in our patient is in agreement with the concept that surgical operation performed under general ether anesthesia may be a maximal adrenocortical stimulus in man. We do not mean to imply that all surgical procedures are capable of maximal adrenocortical stimulation. Minor operations are known to result in less intense adrenocortical activation, particularly those performed under spinal anesthesia.[20]

All of our "immediate" postoperative ACTH studies were commenced one hour postoperatively and were continued for four hours. During this time the patients had fully recovered from anesthesia and were in good general condition. The observation that the infusion of exogenous ACTH during this period resulted in a further increase in already markedly elevated plasma 17-OHCS levels indicated that the adrenal glands of these patients were capable of further stimulation by ACTH.

The height to which free plasma 17-OHCS values may rise during the immediate postoperative periods depends not only on adrenocortical secretion but also upon peripheral and hepatic utilization of circulating steroid.[2, 3, 17] Murray *et al.* administered standard infusions of cortisol to patients both preoperatively and postoperatively, and measured the rate of disappearance of free 17-OHCS from plasma.[12] Impaired removal of infused cortisol was observed during the immediate postoperative period in patients undergoing major surgical procedures. Tyler and associates have presented convincing evidence that impaired hepatic removal of free plasma 17-OHCS may be an important factor in maintaining an elevated 17-OHCS level in the immediate postoperative period.[19]

TABLE 6. *Adrenal and Peripheral Venous Concentration and Left Adrenal Output of 17-OHCS During Surgery, Before and After ACTH Administration*

	Free Plasma 17-OHCS		
	Adrenal Vein		Peripheral Vein
	Conc. γ/100 ml.	Output† γ/min.	Conc. γ/100 ml.
Pre-ACTH			
Period 1	734	74	69
Period 2	698	71	
Post-ACTH‡			
Period 1	750	76	
Period 2	724	73	71

† Calculation is based on assumption that 10 per cent of the free 17-OHCS in adrenal venous blood is present in the red blood cells.[10]

‡ Ten units of ACTH were given intravenously over a one minute interval. Post-ACTH samples were collected 10–15 minutes later.

In our study, the administration of standard ACTH infusions on either the first, second, or third postoperative day resulted in higher plasma 17-OHCS levels than did identical preoperative ACTH infusions. Steenburg *et al.* observed the persistence of this phenomenon for as long as ten days postoperative.[18] It is of interest that the postoperative increase in the adrenocortical capacity to respond to exogenous ACTH is not limited to maximal doses of ACTH, for we demonstrated an enhanced postoperative adrenocortical responsiveness to ACTH in patients who received infusions of only 1 unit of ACTH over a four hour period. This postoperative increase in adrenocortical capacity to respond to ACTH may be related to the fact that frequent administration of conventional ACTH results in a progressive enhancement of adrenocortical responsiveness.[15] Liddle *et al.* observed that the repeated injection of conventional ACTH over a five day period was accompanied by a stepwise increase in urinary 17-OHCS excretion.[9]

Key to the Abbreviations for Diagnosis and Operation

Gb—gall-bladder disease	Ch—cholecystectomy
H.H.—hiatus hernia	T.H.—transthoracic herniorrhaphy
CaC—carcinoma of colon	C.R.—colon resection
M.T.P.—mixed tumor of parotid	P.R.—parotid resection
D.U.—duodenal ulcer	V.—vagotomy
D.C.—diverticulitis of colon	G.R.—gastric resection
CaL—carcinoma of larynx	L., R.N.—laryngectomy and radical neck
A.A.—appendiceal abscess	A., D.—appendectomy and drainage
E.B.—emphysematous bleb	B.R.—bleb resection
Tb—tuberculoma	L.—lobectomy
A.O.—arteriosclerosis obliterans	A.E.—aortic endarterectomy
CaM—carcinoma of mouth	C.O.T.—chronic osteomyelitis of tibia
V.V.—varicose veins	T.U.L.—traumatic ulcer of leg
CaP—carcinoma of pharynx	D.F.—deformity of finger
I.H.—inguinal hernia	CaS—carcinoma of stomach

It seems logical to assume that a major surgical procedure in combination with ether anesthesia may be capable of stimulating the anterior pituitary secretion of ACTH to a maximal degree. Following operation and recovery from anesthesia, the presence of painful traumatized tissue in the postoperative patient may serve as a persistent submaximal stimulus to pituitary ACTH secretion. The continued exposure of the adrenal cortex to minimally elevated levels of circulating ACTH could render the adrenal more responsive to exogenous conventional ACTH.

Indirect evidence for the foreging thesis is furnished by the results of our studies in ten preoperative patients who received two successive daily six hour infusions of ACTH. Higher values of both free and conjugated plasma 17-OHCS were observed on the second day of ACTH administration. In the five patients who were given an injection of long-acting Zinc-ACTH at the cessation of the first ACTH infusion, even higher plasma 17-OHCS levels developed in response to the second ACTH infusion than in the other five patients. This observation suggests that the more prolonged the exposure of the adrenal cortex to increased circulating levels of ACTH, the greater will be the increase in adrenocortical capacity to respond to ACTH.

Summary

1. Free and conjugated plasma 17-hydroxycorticosteroids have been measured following standard infusions of ACTH preoperatively and at varying intervals postoperatively.

2. Major surgical operation, performed under ether anesthesia, is capable of evoking maximal pituitary-adrenocortical activation in man.

3. The administration of ACTH immediately following operation usually resulted in a further rise in plasma 17-hydroxycorticosteroid values despite the fact that the postoperative steroid levels were already markedly elevated.

4. Following operation, an increase in adrenocortical capacity to respond to ACTH has been demonstrated. This phenomenon may last as long as ten days after operation.

5. The possible relationship of the secretion of endogenous ACTH to these postoperative alterations in the adrenocortical capacity to respond to exogenous ACTH is discussed.

Bibliography

1. Bongiovanni, A. M. and W. R. Eberlein: The Determination, Recovery, Identification and Renal Clearance of Conjugated Adrenal Corticoids in Human Peripheral Plasma. Proc. Soc. Exper. Biol. & Med., **89**:281, 1955.

2. Brown, H., D. G. Willardson, L. T. Samuels and F. H. Tyler: 17-Hydrocorticosteroid Metabolism in Liver Disease. J. Clin. Invest., **33**:1524, 1954.
3. Englert, E., Jr., H. Brown, S. Wallach and E. L. Simons: Metabolism of Free and Conjugated 17-Hydroxycorticosteroids in Subjects with Liver Disease. J. Clin. Endocrinol. & Metab., **17**:1395, 1957.
4. Hume, D. M.: The Neuroendocrine Response to Injury: Present Status of the Problem. Ann. Surg., **138**:548, 1953.
5. Hume, D. M.: The Secretion of Epinephrine, Nor-epinephrine, and Corticosteroids in the Adrenal Venous Blood of the Dog Following Single and Repeated Trauma. Surgical Forum, 1957, **8**:111, 1958.
6. Hume, D. M. and D. H. Nelson: Adrenal Cortical Function in Surgical Shock. Surgical Forum, 1954, **5**:568, 1955.
7. Hume, D. M. and D. H. Nelson: Effect of Hypothalamic Lesions on Blood ACTH Levels and 17-Hydroxycorticosteroid Secretion Following Trauma in the Dog. J. Clin. Endocrinol. & Metab., **15**:839, 1955.
8. Le Femine, A. A., L. J. Marks, J. G. Teter, J. H. Leftin, M. P. Leonard and D. V. Baker: The Adrenocortical Response in Surgical Patients. Ann. Surg., **146**:26, 1957.
9. Liddle, G. W., D. Island, A. P. Rinfret and P. H. Forsham: Factors Enhancing Response of Human Adrenal to ACTH: Is There an Adrenal Growth Factor? J. Clin. Endocrinol. & Metab., **14**:839, 1954.
10. Mittelman, A. and H. G. Barker: The Distribution of Corticosteroids in the Plasma and Red Cells of Surgical Patients. Surgical Forum, 1956, **7**:133, 1957.
11. Munson, P. L. and F. N. Briggs: The Mechanism of Stimulation of ACTH Secretion. Recent Progress in Hormone Research, **11**: 83, 1955.
12. Murray, J. O. S., L. J. Marks, F. V. Colombo, B. Josephs, J. H. Leftin and M. P. Leonard: The Effect of Surgical Operation on the Plasma Clearance of Infused Cortisol. Ann. Surg., **148**:951, 1958.
13. Nelson, D. H. and D. M. Hume: A New Method for the Determination of ACTH in Blood. J. Clin. Endocrinol. & Metab., **14**:781, 1954.
14. Nelson, D. H. and L. T. Samuels: A Method for the Determination of 17-Hydroxycorticosteroids in Blood: 17-Hydroxycorticosterone in the Peripheral Circulation. J. Clin. Endocrinol. & Metab., **12**:519, 1952.
15. Renold, A. E., D. Jenkins, P. H. Forsham and G. W. Thorn: The Use of Intravenous ACTH: A Study in Quantitative Adrenocortical Stimulation. J. Clin. Endocrinol. & Metab., **12**: 763, 1952.
16. Sandberg, A. A., K. Eik-Nes, L. T. Samuels and F. H. Tyler: The Effects of Surgery on the Blood Levels and Metabolism of 17-hydroxycorticosteroids in Man. J. Clin. Invest., **33**:1509, 1954.
17. Steenburg, R. W. and W. F. Ganong: Observations on the Influence of Extra-Adrenal Factors on Circulating 17-Hydroxycorticoids in the Surgically Stressed Adrenalectomized Animal. Surgery, **38**:92, 1955.
18. Steenburg, R. W., R. Lennihan and F. D. Moore: Studies in Surgical Endocrinology. II. The Free Blood 17-Hydroxycorticoids in Surgical Patients; Their Relation to Urine Steroids, Metabolism, and Convalescence. Ann. Surg., **143**:180, 1956.
19. Tyler, F. H., C. D. Schmidt, K. Eik-Nes, H. Brown and L. T. Samuels: The Role of the Liver and the Adrenal in Producing Elevated Plasma 17-Hydroxycorticosteroid Levels in Surgery. J. Clin. Invest., **33**: 1517, 1954.
20. Virtue, R. W., M. L. Helmreich and E. Gainza: The Adrenal Cortical Response to Surgery. I. The Effect of Anesthesia on Plasma 17-Hydrocorticosteroid Levels. Surgery, **41**:549, 1957.