JCI The Journal of Clinical Investigation

EFFECTS OF 17-HYDROXY-CORTICOSTERONE ("COMPOUND F") IN MAN

Paul Fourman, … , Evelyn Carroll, Joyce Alexander

J Clin Invest. 1950;29(11):1462-1473. https://doi.org/10.1172/JCI102386.

Research Article

EFFECTS OF 17-HYDROXY-CORTICOSTERONE ("COMPOUND F") IN MAN [1, 2]

By PAUL FOURMAN,[3] F. C. BARTTER,[4] FULLER ALBRIGHT, ELEANOR DEMPSEY, EVELYN CARROLL, AND JOYCE ALEXANDER

(From the Medical Service of the Massachusetts General Hospital and the Department of Medicine, Harvard Medical School, Boston)

(Submitted for publication November 30, 1949; accepted, July 31, 1950)

It is not yet established whether the adrenal cortex normally secretes several hormones or just one hormone with different actions. The results of studies on adrenocorticotropic hormone (ACTH) (1–3) have on the whole been consistent with the hypothesis that the cortex produces three types of hormone: one affecting carbohydrate metabolism ("sugar" or "S" hormone), one affecting sodium and potassium metabolism ("salt" or "Na" hormone) and one with somatotropic and androgenic properties ("nitrogen" or "androgenic" hormone).

As evidence for the first, the "sugar" hormone, ACTH produces the metabolic changes that one would expect from the combination of protoplasmic breakdown and glycogen deposition (3); it also produces glycosuria and hyperglycemia, and a fall in blood lymphocytes and eosinophils.

As evidence for the second, the "salt" hormone, on the first day of giving ACTH, and often before the other changes are apparent, there is a large loss of potassium, quite out of proportion to the breakdown of protoplasm calculated from the nitrogen loss. Extracellular fluid (ECF) is also retained, as estimated from chloride changes. Sodium is retained in amounts greater than one would expect from the amount of chloride retained, and some of it probably replaces potassium lost from the cells (3, 4).

As evidence for the third, the "nitrogen" hormone, there is, with ACTH, a rise in 17-ketosteroid excretion and sometimes acne and hirsutism.

In a study from this laboratory (3) it seemed possible that the early effect of ACTH on potassium could be attributed to "S" hormone. If this were so it would not be necessary to postulate that ACTH produces a "Na" hormone, because sodium retention with ACTH follows, and may be due to, potassium loss. Heppel (5) has shown that potassium deficiency alone may cause sodium to be retained. One might even doubt the existence of "Na" hormone as such, since there is no conclusive evidence that its prototype, desoxycorticosterone, is a normal product of the adrenal cortex. It was, therefore, decided to see whether a pure "S" hormone-like substance could reproduce the early potassuim loss found with ACTH.

Compounds A, E, and F of Kendall represent "S" hormone-like substances. The most active of these, compound F, or 17-hydroxy-corticosterone (6), was chosen for study. Relatively small amounts are available and a short experiment was designed that would not require too much hormone and yet might give the answer. We are indebted to Dr. Konrad Dobriner and Dr. M. H. Kuizenga for 100 mg. of compound F.

DESIGN OF EXPERIMENT

The quantity of compound F at our disposal was divided into two lots of 50 mg. for a replicated experiment on a normal 30 year old man weighing 72 kg. The replication provides an estimate of chance variation. The design of the experiment may be seen by examining Table VII. Each 50 mg. was given in four divided doses beginning at 7 a.m. and spread over three hours; the effects were studied in the ensuing three days. For each of these "F days" there was a corresponding control day. In the first half of the experiment three F days followed the corresponding three control days. In the

[1] The expense of these studies was partly defrayed by grants from the Rockefeller Foundation, the National Advisory Cancer Council and the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council. A bed supported by Mr. Edward Mallinckrodt, Jr., on the Metabolic Ward, was used for these studies.

[2] Presented to the Thirteenth Annual Meeting of the Canadian Physiological Society at Montreal, October, 1949.

[3] Rockefeller Traveling Fellow.

[4] Surgeon, U.S.P.H.S., National Heart Institute, on detail at Massachusetts General Hospital.

TABLE I

Mineral content of diet (in millimols)

Time	K	Na	Cl	P	Ca	Mg	Main items
7:30 a.m.	24.6	61.7	54.7	13.2	3.8	2.9	Oatmeal, bread, omelette, pear
12:15 p.m.	24.6	59.6	55.6	14.2	1.8	2.5	Minced meat, rice, pineapple
3:00 p.m.	7.4	0.4	0.3	1.3	1.0	0.8	Grapefruit juice
5:15 p.m.	24.0	60.9	58.0	16.4	2.0	3.6	Minced meat, bread, macaroni, peach
8:30 p.m.	2.3	17.0	15.8	15.1	14.3	0.8	Bread and cheese
Total	82.9	199.6	184.4	60.2	22.9	10.6	

Daily fluid intake was 3,200 ml. not including water content of foods. The composition of the diet does not include a can of beer taken with the 8:30 p.m. cheese sandwich.

second half three F days preceded the corresponding control days. The reversed sequence of the second half of the experiment (control following F as opposed to control preceding F) assured that any trend in the results with lapse of time would not be falsely attributed to the effect of F. Results in the second F periods might well be affected by the F given before, but since the second F period is compared with the second control period, which also followed an F period, comparison of each F period with its corresponding control period should give the effect of 50 mg. of F.

Each 50 mg. of F was given intramuscularly in four injections of 2 ml. of 35% alcohol without local anesthetic. Thirty-five % alcohol alone was given at the corresponding times in the control periods. In all other respects corresponding control and F days were similar. Blood counts, and blood sugar [5] determinations were done at the times indicated in Tables V and IV, respectively. Urine was collected in two-hourly periods on the days of the injections and in four-hourly periods on other days, but for all days a single eight-hour collection was made for the night. The urinary excretion of nitrogen, potassium, sodium, chloride, phosphorus, calcium and magnesium was measured.[6] The output of 17-ketosteroids was measured in 12-hour periods.

The diet was identical as to composition and time of eating from day to day, so that urinary excretion values for corresponding periods in F and control days could be compared. The subject began the diet four days before the actual experiment. For the total 16 days the diet was taken from the same original batches of food, preserved where necessary at −20° C. Tinned fruits were

[5] Blood sugar determinations were done in the second half of the experiment only.

[6] Sodium and potassium were measured by flame photometer (7); magnesium by the method of Briggs (8). For other methods see Reifenstein, Albright, and Wells (9), and Fraser and his associates (10).

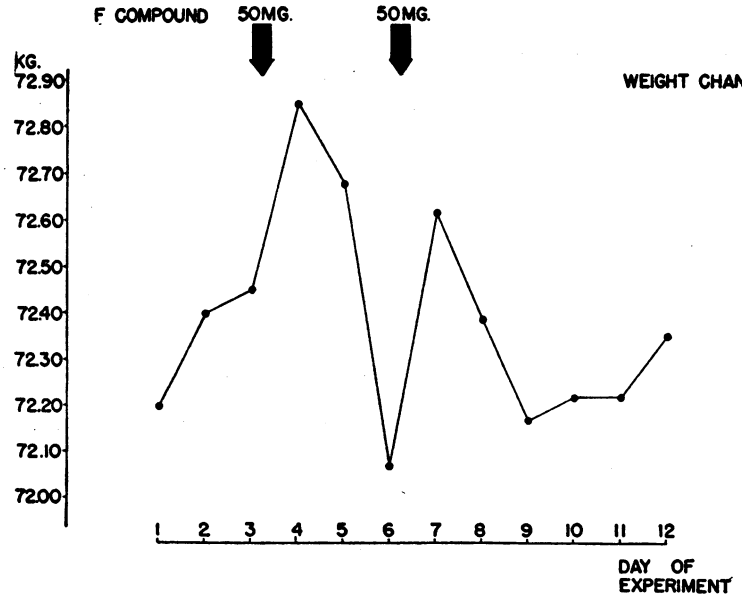

FIG. 1. WEIGHT CHANGES WITH F

TABLE II

Daily weight and 24-hour urine volume

Injections of F compound (50 mg.) given at the beginning of days 4 and 7

	End of day	Weight	Urine vol.
		kg.	*ml.*
Control$_1$	1	72.20	2,826
	2	72.40	2,791
	3	72.45	2,598
F_1	4	72.85	2,369
	5	72.68	2,541
	6	72.07	2,861
F_2	7	72.62	2,563
	8	72.39	3,000
	9	72.17	2,885
Control$_2$	10	72.22	2,593
	11	72.22	2,610
	12	72.35	2,684

from the same canning lot number. The approximate composition of the diet, which was normal for the subject, was estimated from food tables. The mineral composition is shown in Table I. The total daily caloric value was 2,910 calories, made up from 300 g. carbohydrate, 125 g. fat, 123 g. protein and 13 g. alcohol.

RESULTS

1. General effects

The subject felt in high spirits after the first series of F injections but noted no change of mood after the second series. After each series the subject gained and then again lost weight (Figure 1), but the gain and loss were small after the second series. The weight changes may largely be attributed to changes in the water content of the body because body weight and total daily urinary volume were negatively correlated. From the data of Table II,

$$\text{Weight (kg.)} = 74.81 - \text{Urine volume (L)} \times 0.9 \pm 0.3.$$

Blood pressure did not change significantly with treatment.

TABLE III

Reducing substances in urine (as glucose)

Specimen	Control$_1$	F_1	F_2	Control$_2$
7 a.m.– 9 a.m.	–	–	0.3 g.	+
9 a.m.–11 a.m.	–	–		–
11 a.m.– 1 p.m.	–	–		–
1 p.m.– 3 p.m.	–	1.9 g.		+
3 p.m.– 5 p.m.	–	+	2.4 g.	–
5 p.m.– 7 p.m.	–	0.4 g.		–
7 p.m.– 9 p.m.	–	0.3 g.		–
9 p.m.–11 p.m.	–	–		–
11 p.m.– 7 a.m.	–	–	–	–
7 a.m.–11 a.m.	–	+	+	+
11 a.m.– 3 p.m.	–	–	–	–
3 p.m.– 7 p.m.	–	–	–	–
7 p.m.–11 p.m.	–	–	–	–
11 p.m.– 7 a.m.	–	–	–	–
7 a.m.–11 a.m.	–	–	+	+
11 a.m.– 3 p.m.	–	–	–	+
3 p.m.– 7 p.m.	–	+	+	–
7 p.m.–11 p.m.	–	–	–	–
11 p.m.– 7 a.m.	–	–	–	–

+ weakly positive Benedict's test.

2. Glycosuria and blood sugar changes

Glycosuria appeared on the day of the first F injections after lunch, when 1.9 g. of glucose were lost in the urine between 1 and 3 p.m. Glycosuria recurred on that day in relation to meals (Table III). Benedict's test for sugar was weakly positive after breakfast on the following day and after lunch on the day after. With the second lot of F glycosuria was already present in the 9 a.m. specimen. It persisted in slight degree as before and continued so not only into the following control days, but for two weeks after the last dose of F. The appearance of glycosuria in this subject is especially significant in view of the known absence of glucose from a number of urine samples tested in the course of other experiments, over the six years preceding the F experiment.

TABLE IV

Blood sugar values (venous blood)

		F_2	Control$_2$
Day of injections	7 a.m.	98	85
	9 a.m.	85	81
	11 a.m.	100	84
	3 p.m.	106	108
	7 p.m.	101	119
Day after injections	7 a.m.	88	100

Blood sugar was determined only during the second F and second control periods and was no higher for F than for control values (Table IV). This raises the questions (*1*) whether the reducing substance appearing in the urine was indeed glucose and, if so, (*2*) whether glucose appeared as a result of lowered threshold or diminished tolerance. The questions are partly answered by

a glucose tolerance test done a week after the last F injection, when reducing substances were still being intermittently excreted. On the morning of the test Benedict's solution was not reduced; reduction occurred after taking glucose and was, therefore, presumably due to glucose. The tolerance curve was normal and was not higher than curves done three years earlier or three months later.

As far as the present evidence goes, therefore, the glycosuria after F was due to a lowered renal threshold. Over long periods of administration the carbohydrate-active steroids do, of course, impair glucose tolerance (11).

3. Blood counts

Absolute eosinophil and total and differential white cell counts were done on oxalated venous blood. The method for eosinophil counts followed that of Hills, Forsham and Finch (12) except that the blood was diluted 1 : 10 instead of 1 : 20 to facilitate counting more cells. Counts were done on a total volume of 1.28 mm³. of undiluted blood, so that the sampling error was reasonably small. White cell counts were done on a total volume of .09 mm³. of blood. Differential counts were based on at least 200 cells for each count.

It is now well established that the carbohydrate-active adrenal steroids cause a fall in the circulating eosinophils. This was shown by Forsham and his colleagues (2) for single doses of 20 mg. of compound F, and by Hills, Forsham and Finch (12) for single doses of 25 mg. of ACTH. The latter data (12) showed that the count four hours after giving ACTH depends on the control count:

$$\text{four-hour count} = \text{control count} \times 0.33 \pm 0.06.$$

In the present experiment the fall in the eosinophil count from the pre-injection level was of the same order (Figure 2). When counts on F were compared with the corresponding control counts (Table V), the effect of 50 mg. of F (given in four injections over three hours beginning at 7 a.m.) was apparent at 11 a.m. and maximal at 3 p.m. By 7 p.m. the loss had been partly made up and on the following day the count did not differ significantly from control values. The second series of

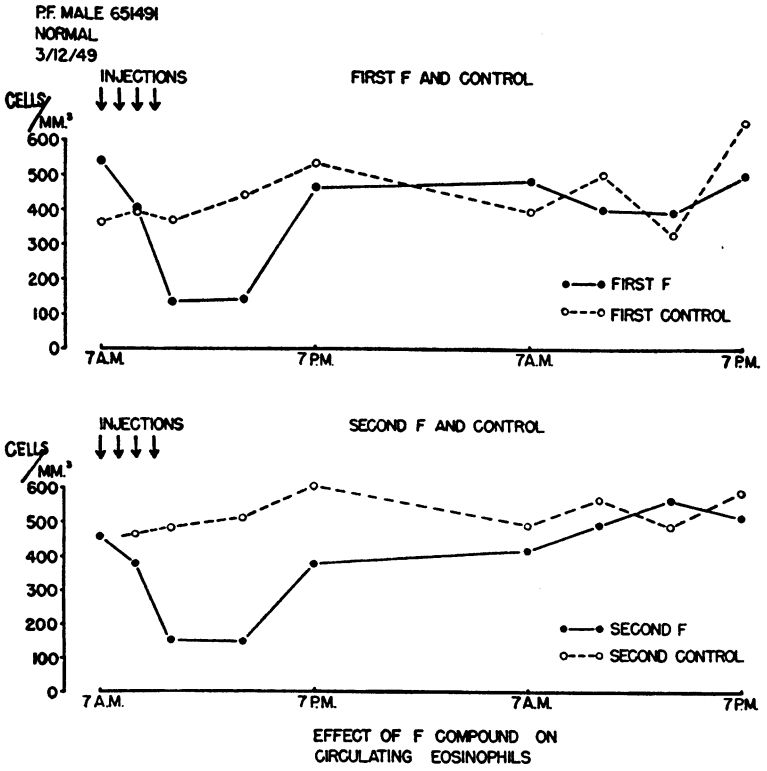

FIG. 2. EFFECT OF F ON CIRCULATING EOSINOPHILS

TABLE V

Effect of compound F on eosinophil count

	Time	Control −F (mean of two differences)
Day of injections	9 a.m.	36
	11 a.m.	232
	3 p.m.	331
	7 p.m.	148 (Standard error of mean difference at one period: ± 46)*
	7 a.m.	−7
Following day	11 a.m.	88
	3 p.m.	−68
	7 p.m.	64

* From an analysis of variance of the eosinophil data, omitting the 7 a.m. pre-injection values.

control counts were significantly higher than the first.

There was a fall in lymphocytes at about the same time as the fall in eosinophils, but it should be noted that on the second day of the second control period lymphocytes also fell (Figure 3). In this experiment at least changes in lymphocytes with F were not as marked as changes in eosinophils. Polymorphs were not significantly affected.

In the course of counting 9,800 white cells for the differential counts a possible effect of F on the basophil count was noted. On the days of F injection 15 basophils were seen out of 2,900 cells counted; on the other days for which there are counts, only 16 basophils were seen out of 6,900 cells counted. The difference is just significant ($\chi^2 = 4.34$; $p < 0.05$). The basophils seen with F included a clump of five and one of two. While this is in itself an unusual finding it could invalidate the χ^2 test.

4. Changes in nitrogen excretion with F (see Figure 4)

Data for the urinary excretion of nitrogen, as well as of potassium, sodium, chloride and water, are given in Tables VI–X. The effect of F is given by the difference between corresponding control and F data. In the charts these differences are presented cumulatively, because in consecutive short collections variations in excretion in one period may be canceled in the next.[7]

F caused slight nitrogen loss both times. The loss continued through the day after F was given and reached a total of 2.0 and 1.3 g. 48 hours after

[7] Of the 3–7 p.m. specimen on the last day of the experiment, the portion for the first hour, the volume of which was 400 ml., was lost. The data for the three-hour collection are given in brackets in the tables. For the cumulative data the value of the incomplete specimen has somewhat arbitrarily been taken as ⅔ of that measured.

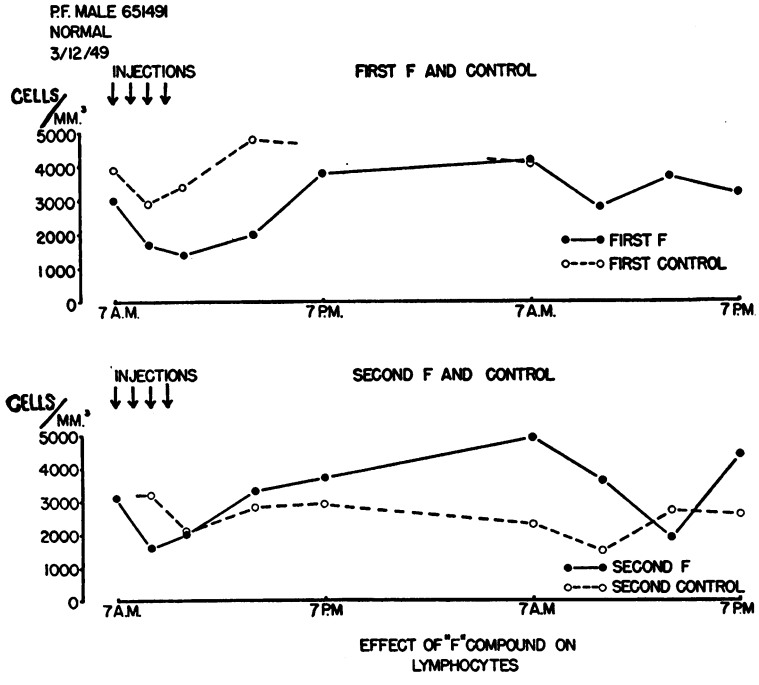

FIG. 3. EFFECT OF F ON CIRCULATING LYMPHOCYTES

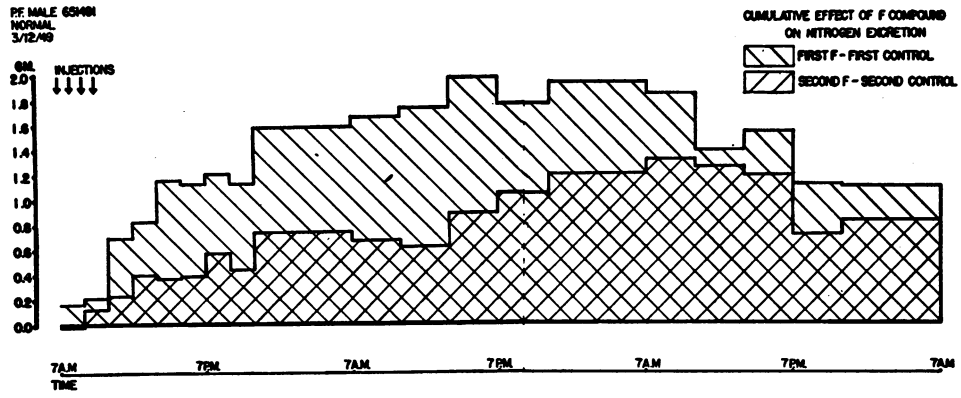

FIG. 4. CUMULATIVE EFFECT OF F ON NITROGEN EXCRETION

In this and succeeding charts, the effect of F was obtained by difference between F and control data.

the injection. Retention did not begin until the third day, and the loss was not made up by the end of that day. The changes were barely significant ($p \doteq .05$).

5. Changes in potassium excretion with F (see Figure 5)

F caused loss of potassium quite out of proportion to the breakdown of protoplasm calculated from the nitrogen loss. The changes in K excretion were the most clear-cut of the effects of F on electrolyte excretion. Thus, uncontrolled variation was small (standard deviation for the excretion in one period = 1.29 meq.); the treatment effect was similar in both halves of the experiment; and the changes were relatively large.

K loss began between 9 and 11 a.m. on the days of F injections. The rate of loss was highest in the next two hours. By 5 p.m. retention of K had begun, after about 20 meq. of K had been lost. K loss was finally made up after breakfast on the day following F injection. Breakfast contained 25

TABLE VI

Nitrogen excretion

(grams)

Day	Time	Control (1)	F (1)	F (2)	Control (2)	F-Control (1)	F-Control (2)
I	7 a.m.	1.34	1.50	1.42	1.44	+.16	−.20
	9 a.m.	1.63	1.68	1.40	1.26	+.05	+.14
	11 a.m.	1.22	1.71	1.59	1.48	+.49	+.11
	1 p.m.	1.69	1.82	1.83	1.66	+.13	+.17
	3 p.m.	1.49	1.82	1.39	1.42	+.33	−.03
	5 p.m.	1.62	1.59	1.65	1.63	−.03	+.02
	7 p.m.	1.79	1.87	1.76	1.58	+.08	+.18
	9 p.m.	1.84	1.76	1.68	1.81	−.08	−.13
	11 p.m.	5.13	5.58	5.70	5.40	+.45	−.30
II	7 a.m.	2.83	2.91	2.83	2.90	+.08	−.07
	11 a.m.	3.23	3.30	3.21	3.27	+.07	−.06
	3 p.m.	3.07	3.31	3.29	3.02	+.24	+.27
	7 p.m.	3.50	3.29	3.73	3.57	−.21	+.16
	11 p.m.	5.08	5.25	5.47	5.32	+.17	+.15
III	7 a.m.	3.03	2.94	2.90	2.78	−.09	+.12
	11 a.m.	3.28	2.82	3.10	3.16	−.46	−.06
	3 p.m.	3.19	3.34	3.21	(2.46)*	+.15	(−.07)
	7 p.m.	3.80	3.38	3.53	4.00	−.42	−.47
	11 p.m.	5.11	5.09	5.40	5.29	−.02	+.11

Analysis of variance†

Effect	Mean square	P
Treatment (T)	480	>.1
Period (P)	72,118	
P-T interaction	350	≐.05
Replication (R)	34	
R-T interaction	10	
R-P interaction	264	188
R-P-T interaction	111	

* Value of 3.28 = 2.46 × 4/3 substituted in analysis.

† See appendix.

TABLE VII

Potassium excretion

(milliequivalents)

Compound F was given in four doses (12.5 mg. per dose) at 7, 8, 9, and 10 a.m. on days F_1 and F_2

Day	Time	Control (1)	F (1)	F (2)	Control (2)	F-Control (1)	F-Control (2)
I	7 a.m.	2.5	3.9	4.4	4.8	+1.4	−0.4
	9 a.m.	6.6	12.1	14.0	6.6	+5.5	+7.4
	11 a.m.	7.2	19.7	23.0	8.8	+12.5	+14.2
	1 p.m.	11.2	15.0	21.6	15.1	+3.8	+6.5
	3 p.m.	9.0	9.5	9.5	8.0	+0.5	+1.5
	5 p.m.	10.0	6.7	6.8	8.9	−3.3	−2.1
	7 p.m.	6.2	3.5	4.4	6.4	−2.7	−2.0
	9 p.m.	3.5	2.6	2.6	3.8	−0.9	−1.2
	11 p.m.	8.0	6.8	6.1	9.3	−1.2	−3.2
II	7 a.m.	10.7	7.4	6.2	13.0	−3.3	−5.8
	11 a.m.	27.9	13.9	16.1	30.6	−14.0	−14.5
	3 p.m.	13.3	14.2	16.3	13.9	+0.9	+2.4
	7 p.m.	9.0	8.7	8.8	8.0	−0.3	+0.8
	11 p.m.	9.4	7.2	8.8	8.6	−2.2	+0.2
III	7 a.m.	11.4	8.9	10.2	12.5	−2.5	−2.3
	11 a.m.	25.5	14.4	20.6	22.0	−11.1	−1.4
	3 p.m.	15.5	10.7	11.1	[12.9]	−4.8	[−1.8]
	7 p.m.	7.8	8.5	6.1	9.7	+0.7	−3.6
	11 p.m.–7 a.m.	9.2	8.1	9.9	9.0	−1.1	+0.9

Analysis of variance*

Effect	Mean square	P
Treatment (T)	9.90	<.05
Period (P)	103.14	
P-T interaction	30.22	<.001
Replication (R)	13.91	
R-T interaction	3.70	
R-P interaction	2.06	2.14
R-P-T interaction	2.22	

* The analysis of variance was done with the data expressed to two decimal places, whereas in this table the data are "rounded" to one decimal place.

meq. of K, or 30% of the daily K intake. Apparently K deficiency could not be made up until an excess was available in the diet.

On the third day a remarkable thing happened: there was K retention of the same order as the previous K loss, and this happened after that loss had already been made up.

Now if F has its counterpart in the normal secretion of the adrenal cortex, the apparent effect of F injected will be a resultant of the amount absorbed

TABLE VIII

Sodium excretion

(milliequivalents)

Day	Time	Control (1)	F (1)	F (2)	Control (2)	F-Control (1)	F-Control (2)
I	7 a.m.	11.4	14.7	18.1	17.2	+3.3	+.9
	9 a.m.	29.0	16.3	24.1	19.6	−12.7	+4.5
	11 a.m.	22.6	15.6	18.9	25.3	−7.0	−6.4
	1 p.m.	38.7	20.0	29.0	28.9	−18.7	+.1
	3 p.m.	24.7	16.2	13.3	20.8	−8.5	−7.5
	5 p.m.	30.0	17.9	19.2	20.0	−12.1	−.8
	7 p.m.	20.6	12.6	17.8	18.3	−8.0	−.5
	9 p.m.	20.1	11.8	11.5	11.6	−8.3	−.1
	11 p.m.	28.4	22.4	24.8	23.3	−6.0	+1.5
II	7 a.m.	29.6	29.0	26.6	40.5	−.6	−13.9
	11 a.m.	62.0	64.1	61.6	56.0	+2.1	+5.6
	3 p.m.	47.1	51.6	53.8	38.9	+4.5	+14.9
	7 p.m.	36.4	35.8	50.2	33.6	−.6	+16.6
	11 p.m.	20.3	26.8	31.8	31.2	+6.5	+.6
III	7 a.m.	29.0	39.8	47.8	31.3	+10.8	+16.5
	11 a.m.	55.5	59.7	66.7	51.6	+4.2	+15.1
	3 p.m.	37.9	58.8	53.1	[26.6]	+20.9	[+26.5]
	7 p.m.	39.4	46.1	36.0	34.6	+6.7	+1.4
	11 p.m.–7 a.m.	24.5	27.6	30.8	25.1	+3.1	+5.7

Analysis of variance

Effect	Mean square	P
Treatment (T)	47.84	
Period (P)	773.88	
P-T interaction	75.89	<.001
Replication (R)	.27	
R-T interaction	134.49	
R-P interaction	19.57	
RPT interaction		

TABLE IX

Chloride excretion
(milliequivalents)

Day	Time	Control (1)	F (1)	F (2)	Control (2)	F-Control (1)	F-Control (2)
I	7 a.m.	9.9	15.7	20.2	21.7	+5.8	−1.5
	9 a.m.	31.5	25.2	33.1	23.9	−6.3	+9.2
	11 a.m.	25.6	29.6	33.9	31.4	+4.0	+2.5
	1 p.m.	38.7	23.6	36.7	32.5	−15.1	+4.2
	3 p.m.	25.1	18.5	14.9	19.4	−6.6	−4.5
	5 p.m.	28.1	15.7	16.7	21.4	−12.4	−4.7
	7 p.m.	19.2	10.5	13.7	16.6	−8.7	−2.9
	9 p.m.	13.5	6.8	5.5	8.2	−6.7	−2.7
	11 p.m.	15.9	10.3	11.1	11.2	−5.6	−0.1
II	7 a.m.	36.5	33.2	29.4	44.0	−3.3	−14.6
	11 a.m.	74.4	65.3	64.4	63.1	−9.1	+1.3
	3 p.m.	42.6	47.5	51.6	35.2	+4.9	+16.4
	7 p.m.	26.9	29.3	40.4	27.5	+2.4	+12.9
	11 p.m.	8.6	14.3	19.3	16.0	+5.7	+3.3
III	7 a.m.	33.9	42.0	51.4	41.5	+8.1	+9.9
	11 a.m.	64.8	60.8	72.6	59.5	−4.0	+13.1
	3 p.m.	38.2	50.4	45.4	[26.9]	+12.2	[+18.5]
	7 p.m.	31.4	39.7	28.4	27.0	+8.3	+1.4
	11 p.m.–7 a.m.	12.3	15.2	19.6	14.9	+2.9	+4.7

Analysis of variance		
Effect	Mean square	P
Treatment (T)	24.22	
Period P	1082.74	
P-T interaction	47.41	<.05
Replication (R)	5.00	
R-T interaction	106.34	
R-P interaction	17.45	
RPT interaction		

from the injection site, the amount destroyed, and the amount of corresponding hormone produced by the adrenal cortex. One might expect the effect of injected F to be maximal at first, when it is added to the normal output of the gland. The injected steroid would, however, suppress ACTH production (13) and after the maximal effect there would be a period of no apparent effect while the suppressed normal output offset the injected F. Finally the effect of F might wear off before normal secretion was resumed. In this way might be explained the three phases—loss, restoration, and retention of K—after each series of F injections.

TABLE X

Water excretion
(milliliters)

Day	Time	Control (1)	F (1)	F (2)	Control (2)	F-Control (1)	F-Control (2)
I	7 a.m.	185	271	298	246	+86	+52
	9 a.m.	421	322	282	143	−99	+139
	11 a.m.	250	248	348	396	− 2	−48
	1 p.m.	496	395	543	522	−101	+21
	3 p.m.	238	150	124	200	−88	−76
	5 p.m.	353	167	189	405	−186	−216
	7 p.m.	178	150	141	139	−28	+ 2
	9 p.m.	277	218	175	208	−59	−33
	11 p.m.	428	448	463	334	+20	+129
II	7 a.m.	480	470	445	510	−10	−65
	11 a.m.	1022	771	790	864	−251	−74
	3 p.m.	504	633	820	451	+129	+369
	7 p.m.	446	314	445	371	−132	+74
	11 p.m.	339	353	500	414	+14	+86
III	7 a.m.	412	646	544	369	+234	+175
	11 a.m.	812	565	816	838	−247	−22
	3 p.m.	585	815	746	680	+230	+66
	7 p.m.	435	460	270	359	+26	−89
	11 p.m.–7 a.m.	354	375	509	438	+21	+71

Analysis of variance		
Effect	Mean square	P
Treatment (T)	3,517	
Period (P)	1,423,930	
P-T interaction	18,674	<.05
Replication (R)	34	
R-T interaction	25,974	
R-P interaction	9,386	6,431
RPT interaction	3,476	

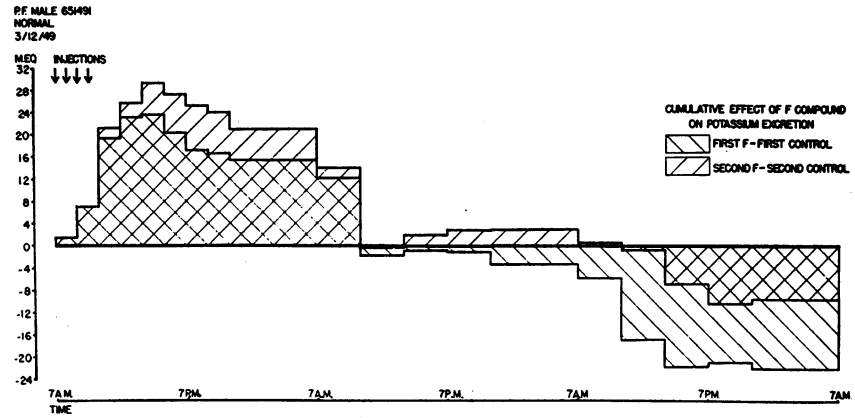

FIG. 5. CUMULATIVE EFFECT OF F ON POTASSIUM EXCRETION

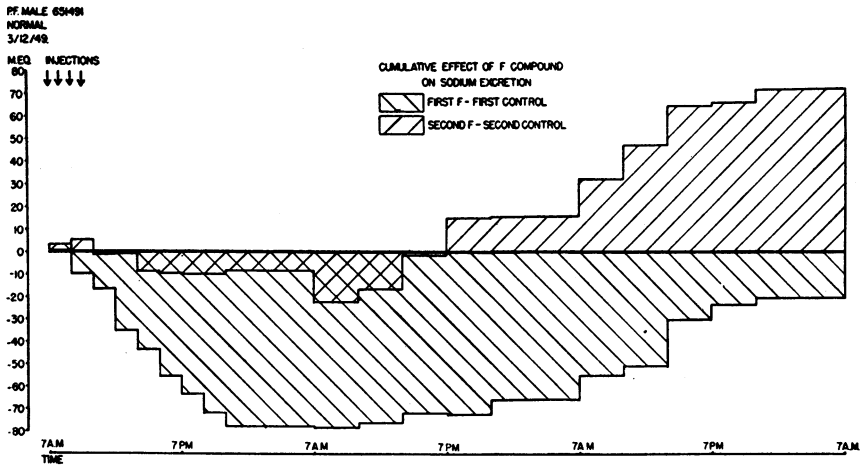

FIG. 6. CUMULATIVE EFFECT OF F ON SODIUM EXCRETION

6. Changes in water, sodium and chloride excretion with F (see Figures 6 and 7)

The data for water, sodium and chloride are difficult to interpret because of a difference in the corresponding control days of the two halves of the experiment. Less Na, Cl and water were excreted during the first day of the second control period than during the first day of the first control period. F days on the other hand were very similar. For the first half of the experiment, if the first control is compared with the first F (Tables VIII–X), water, Na and Cl were retained on the day of injection. After some delay the retained water, Na and Cl were lost, at least in part. In the second half of the experiment, on the other hand, while the ultimate loss of Na, Cl and water is again apparent, the preceding retention is masked because on the corresponding control day as well salt and water were retained. The difference between the early and late F effect for both halves is significant, and it seems that with F water and salt are retained, later to be lost. Since, however, the water content of the body may vary appreciably without treatment, the total amount of retention due to F cannot be determined with any confidence in a short experiment.

Relation between water, chloride, sodium and potassium changes (see Figure 8)

The effect of F on water and Cl excretion was very similar. The effect on sodium was only roughly similar to that on the other two. Early after F more Na was retained (or less lost) than Cl; late after F less Na was retained (or more lost) than Cl (compare Figure 6 with Figure 7).

TABLE XI

Effect of compound F on excretion of 17-ketosteroids (in mg.)

Day of expt.	1	2	3	4*	5	6	7*	8	9	10	11	12
12 hrs. day	11.7	10.8	11.2	9.2	8.8	9.0	8.7	10.0	11.3	8.6	11.1	10.0
12 hrs. night	6.9	7.2	7.8	5.1	5.2	6.8	4.8	6.6	6.2	5.6	5.3	5.7
24 hrs. total	18.6	18.0	19.0	14.3	14.0	15.8	13.5	16.6	17.5	14.2	16.4	15.7

* F given.

The changing relation between Na and Cl after giving F corresponds to changes in K shown in Figure 5. When, early after F, the body was deficient in K, Na was being retained in relation to Cl. When, late after F, K was retained, Na was lost in relation to Cl. The relation between the cumulative Na, Cl, and K changes due to F has been calculated by the method of partial regression. For each half of the experiment in milliequivalents,

$$(1)\quad \text{Na} = 5.73 \pm 0.96 + (\text{Cl} \times 1.18 \pm 0.02) - (\text{K} \times 0.67 \pm 0.03)$$

$$(2)\quad \text{Na} = 0.20 \pm 0.92 + (\text{Cl} \times 1.13 \pm 0.03) - (\text{K} \times 0.55 \pm 0.05).$$

In these equations, the positive correlation of Na with Cl suggests that Na goes with Cl and the negative correlation with K suggests that in addition Na replaces K. For the two equations, the weighted mean of the coefficient of Cl is 1.17 ± 0.15. This approaches the relation of Na to Cl in ECF; Na changes beyond those correlated with Cl presumably represent changes in intracellular fluid composition. Sodium changes "corrected"

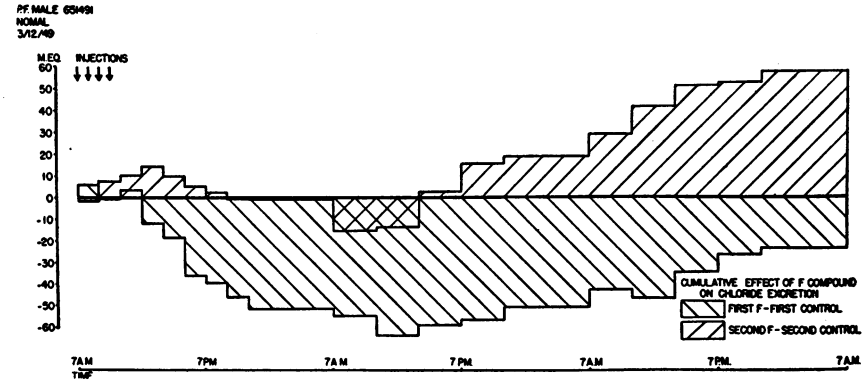

FIG. 7. CUMULATIVE EFFECT OF F ON CHLORIDE EXCRETION

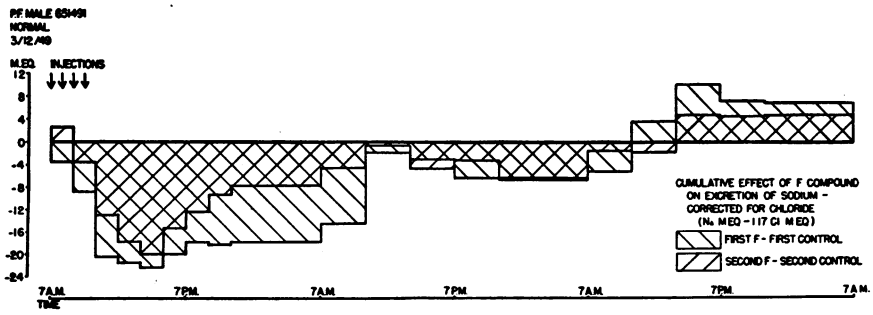

FIG. 8. CUMULATIVE EFFECT OF F ON EXCRETION OF SODIUM CORRECTED FOR CHLORIDE CHANGES (NA MEQ. − 1.17 CL MEQ.)

The corrected Na changes presumably represent changes in intracellular sodium, and are negatively correlated with potassium changes (Figure 6). The factor for Cl is based on the data of this experiment (see text).

for Cl changes are shown in Figure 8, which gives the values of (Na − 1.17 Cl). They have a reasonably close inverse relation to the K changes shown in Figure 6. It would seem that Na partly, but not completely, replaces K lost from cells.

The relation between Na, Cl and K found with F resembles that found with desoxycorticosterone under similar conditions (14).

7. Changes in phosphorus, calcium and magnesium with F

These were not significant.

8. Changes in 17-ketosteroid excretion (see Table XI)

17-Ketosteroid excretion was consistently less for the 12-hour night periods (7 p.m.–7 a.m.) than for the 12-hour day periods. After the first series of F injections 17-ketosteroid excretion fell slightly and remained at the new level during the rest of the experiment.

DISCUSSION

The recognized effects of F were produced in this experiment: fall of eosinophils and lymphocytes, glycosuria, loss of nitrogen. As with ACTH, the earliest and most impressive metabolic change was a loss of potassium. This loss amounted to some 20 meq. for each lot of 50 mg. of F given. The potassium loss was in part made up by sodium retained in excess of chloride. Changes in ECF were equivocal: retention of water and chloride apparently occurred but changes of similar magnitude occur without treatment.

These results are like those obtained on the first day of ACTH therapy. If they can be confirmed, there would be no reason to postulate that ACTH liberates a separate sodium hormone, in addition to an "S" hormone, since F, with "S" hormone-like action, reproduced those effects of ACTH that have been ascribed to the sodium (DOCA-like) hormone. (In the rat, too, F reproduces the effects of ACTH, in that apparently neither hormone has DOCA-like effects [15, 16].)

"S" hormone is thought to reproduce some of the features of the alarm reaction of Selye. If the "alarm" state is due in part to "S" hormone liberation, one might expect potassium deficiency in the alarm state. Thus might be explained the failure of some ill people to conserve potassuim (17).

SUMMARY

Whether carbohydrate-active steroids of the adrenal cortex have a desoxycorticosterone-like effect on sodium and potassium metabolism has been investigated for 17-hydroxy-corticosterone (compound F).

A normal male subject was twice observed for three days after the intramuscular injection of 50 mg. of F, and twice for three corresponding control days. The effect of F on the urinary excretion of N, K, Na, Cl, water, P, Ca and Mg was measured by the difference between F and control data. The results were evaluated statistically by analysis of variance.

With F (as with adrenocorticotropic hormone [ACTH]), circulating lymphocytes and eosinophils fell. About 1.6 g. of nitrogen were lost. Glycosuria appeared and was apparently due to a lowered renal threshold.

With F (as with ACTH), potassium was lost (about 20 meq. of potassium on the day F was given); this loss of K was restored by the next morning after breakfast; there was a "rebound" retention after that. There were sodium changes beyond those associated with chloride which were inversely related to changes in potassium. It is suggested that sodium entered potassium deficient cells, although this interpretation cannot be made with confidence in the absence of measurements of ECF electrolytes. These electrolyte changes resemble changes that occur with DOCA under similar conditions.

Other electrolytes were not significantly affected.

CONCLUSIONS

1. Since F reproduces the changes in K, Na and Cl characteristic of ACTH there is no reason to postulate that a separate DOCA-like substance is secreted when the adrenals are stimulated with ACTH.

2. If carbohydrate-active steroids are liberated in the alarm reaction, then the K deficiency of ill patients may be in part due to the alarm reaction, and not simply a result of diminished intake. To be sure, even after the effect of the carbohydrate-active steroid has worn off, the body will not retain K unless there is an excess in the diet.

APPENDIX

Analysis of data

The urinary excretion data have been assessed statistically by analyses of variance (18). With 76 data for each substance measured the analysis takes this form:

Effect	Degrees of freedom	
Treatment	1	
Period	18	
Replication	1	
Interactions		
Period-treatment	18	
Replication-treatment	1	
Period-replication	18	"Error" $\sqrt{\text{"Error" mean square}} =$ standard deviation
Period-replication-treatment	18	
Total	75	

The question to be answered in each case is whether the total effect of treatment over three days and the effect of treatment at different periods are significant. This is done by comparing the treatment and period-treatment interaction mean squares with the error mean square (variance ratio) in the analysis of variance. Essentially the test of significance is based on a comparison of the amount of variation attributable to different treatments with the amount of variation found within similar treatments from period to period.

BIBLIOGRAPHY

1. Mason, H. L., Power, M. H., Rynearson, E. H., Ciaramelli, L. C., Li, C. H., and Evans, H. M., Results of administration of anterior pituitary adrenocorticotropic hormone to a normal human subject. J. Clin. Endocrinol., 1948, **8**, 1.
2. Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., Clinical studies with pituitary adrenocorticotropin. J. Clin. Endocrinol., 1948, **8**, 15.
3. Bartter, F. C., Fourman, P., Albright, F., Forbes, A. P., Jefferies, W. M., Griswold, G., Dempsey, E., Bryant, D., and Carroll, E., Effect of adrenocorticotropic hormone in panhypopituitarism. J. Clin. Invest., 1950, **29**, 950.
4. Prunty, F. T. G., Forsham, P. H., and Thorn, G. W., Desoxycorticosterone-like activity induced by adrenocorticotrophin in man. Clin. Sc., 1948, **7**, 109.
5. Heppel, L. A., Electrolytes of muscle and liver in potassium-depleted rats. Am. J. Physiol., 1939, **127**, 385.
6. Pabst, M. L., Sheppard, R., and Kuizenga, M. H., Comparison of liver-glycogen deposition and work performance tests for bio-assay of adrenal cortex hormones. Endocrinology, 1947, **41**, 55.
7. Berry, J. W., Chappell, D. G., and Barnes, R. B., Improved method of flame photometry. Indust. & Engin. Chem. (Analyt. Ed.), 1946, **18**, 19.
8. Briggs, A. P., Some applications of the colorimetric phosphate method. J. Biol. Chem., 1924, **59**, 255.
9. Reifenstein, E. C., Albright, F., and Wells, S. L., The accumulation, interpretation and presentation of data pertaining to metabolic balances, notably those of calcium, phosphorus and nitrogen. J. Clin. Endocrinol., 1945, **5**, 367.
10. Fraser, R. W., Forbes, A. P., Albright, F., Sulkowitch, H., and Reifenstein, E. C., Jr., Colorimetric assay of 17-ketosteroids in urine. J. Clin. Endocrinol., 1941, **1**, 234.
11. Long, C. N. H., A discussion of the mechanism of action of adrenal cortical hormones on carbohydrate and protein metabolism. Endocrinology, 1942, **30**, 870.
12. Hills, A. G., Forsham, P. H., and Finch, C. A., Changes in circulating leucocytes induced by the administration of pituitary adrenocorticotropic hormone in man. Blood, 1948, **3**, 755.
13. Cheng, Chi-Ping, Regulation of pituitary adrenocortitropic activity by adrenal cortical hormones. J. Clin. Endocrinol., 1949, **9**, 656.
14. Fourman, P., Kepler, E. J., Reifenstein, E. C., Jr., and Dempsey, E. F., Effect of a single dose of desoxycorticosterone acetate on electrolyte metabolism. J. Clin. Endocrinol., 1949, **9**, 660.
15. Ingle, D. J., Li, C. H., and Evans, H. M., The effect of adrenocorticotropic hormone on the urinary excretion of sodium, chloride, potassium, nitrogen and glucose in normal rats. Endocrinology, 1946, **39**, 32.
16. Ingle, D. J., Sheppard, R., Oberle, E. A., and Kuizenga, M. H., A comparison of the acute effects of corticosterone and 17-hydroxycorticosterone on body weight and the urinary excretion of sodium, chloride, potassium, nitrogen, and glucose in the normal rat. Endocrinology, 1946, **39**, 52.
17. Tarail, R., and Elkinton, J. R., Potassium deficiency and the role of the kidney in its production. J. Clin. Invest., 1949, **28**, 99.
18. Fisher, R. A., Design of Experiments. Oliver & Boyd, Edinburgh, 1947, 4th edition.