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Research Article

STUDIES CONCERNING THE ROLE OF THE ADRENAL CORTEX IN THE PATHOLOGIC PHYSIOLOGY OF DIABETIC ACIDOSIS

II. THE IDENTIFICATION OF ADRENAL-CONDITIONED FACTORS IN THE PHYSIOLOGIC REACTION TO THE STRESS OF INSULIN DEPRIVATION [1]

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In a descriptive study designed to correlate changes in the level of adrenal cortical function with other metabolic phenomena occurring during the evolution of experimental diabetic acidosis it was observed (1) that eosinopenia and an increase in the rate of corticosteroid excretion are comparatively late features of ketoacidosis induced by the omission of insulin. A close temporal relation was found to obtain between evidences of increased adrenal activity and the following metabolic events: (a) An acceleration in the rate of catabolism of protoplasm; (b) a loss of potassium in excess of nitrogen; and (c) a decreased sensitivity to injected insulin. In some, but not all, experiments it appeared that adrenal activation was related in time to the occurrence of increased lipemia and ketonemia as well.

In this paper are presented the results of experiments designed to ascertain whether the above events, temporally linked to increased adrenal activity, can, in fact, be regarded as adrenal conditioned. The postulates underlying these experiments are that metabolic reactions dependent upon increased adrenal activity for their initiation or maintenance should be susceptible to: (a) Premature induction by exogenous stimulation of the adrenal cortex during mild stress; and (b) diminution or suppression by maintenance of a fixed supply of adrenal cortical hormones during severe stress.

Accordingly, the experiments comprise a comparison between the metabolic changes which characterize the stressful terminal phases of insulin deprivation in the depancreatized dog and: (a) The changes which accompany the induction of a comparable degree of eosinopenia by the administration of ACTH to a depancreatized dog deprived of insulin for a relatively brief period; and (b) the changes which accompany the withdrawal of insulin from an adrenalectomized-depancreatized animal maintained upon a fixed dose of adrenal cortical hormones.

EXPERIMENTAL PROCEDURE

Response of the depancreatized dog to exogenous stimulation of the adrenal cortex. A balance study was performed upon an animal (Frisky) whose metabolic response to insulin deprivation had been well established by previous withdrawal experiments (1). After being stabilized on the same diet as had been employed for previous balance studies, the animal was deprived of insulin for a period of 36 hours. The eosinophil fall [2] ordinarily occurring from the 48th to the 60th hour after insulin withdrawal was anticipated by administering ACTH intramuscularly in a dose of 20 mg. every three hours between the 24th and 36th hours of deprivation. This particular interval was chosen for adrenal stimulation in order that

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[2] The eosinophil count, rather than the rate of urinary corticosteroid excretion, was employed for determination of the ACTH dosage necessary to simulate the degree of adrenal cortical activity observed in the original insulin deprivation experiments. A reduced clearance of corticosteroids, due to a depression of the glomerular filtration rate during the terminal stages of ketoacidosis, is believed to have resulted in spuriously low levels of corticosteroid excretion.

TABLE I

Blood, plasma and serum constituents preceding and accompanying the administration of ACTH to a depancreatized dog

Date and time	Beginning of period	Clinical notes	Total WBC	Neutrophils	Lymphocytes	Eosinophils	Sugar	CO_2 content	Hgb.
			Cells/mm.³				*mg. %*	*mEq./L.*	*Gm. %*
May 27—9 am	C I		11,560	9,248	1,156	594	340	26.8	16.3
28—9 am			10,400	7,800	1,560	609			
29—9 am	C II		—	—	—	—	270	24.9	16.5
30—9 am			12,460	9,983	1,246	650			
31—9 am	E I	Insulin omitted	11,060	8,516	1,659	594	235	23.6	15.7
9 pm	E II		9,880	7,114	1,482	538	307	22.1	15.6
June 1—9 am	E III	ACTH	10,680	8,117	1,495	562	262	19.5	15.6
12 noon		20 mg.	18,720	14,602	2,059	466			
3 pm		q. 3 h.×	19,220	15,760	1,922	272			
6 pm		12 h.	23,220	20,666	929	190			
9 pm			20,580	18,728	617	94	275	14.7	15.0

a clear delineation of the effect of exogenous ACTH upon the loss of excess potassium might be obtained. From the results of previous experiments it was known that a loss of excess potassium, presumably associated with hepatic glycogenolysis, would occur during the first 12 hours and that the losses of nitrogen and potassium would be in protoplasmic proportion during the succeeding 24 hours.

Response of the adrenalectomized-depancreatized dog to insulin deprivation. A balance study was performed upon an adrenalectomized-depancreatized dog (Lady) during a 60-hour period of insulin deprivation, a constant supply of adrenal hormones being furnished the animal throughout. The subject was a depancreatized bitch ordinarily requiring 50 to 60 units of crystalline insulin daily for control of the diabetes. Adrenalectomy was performed in two stages, and at autopsy was demonstrated to have been complete. There was no alteration in the severity of the diabetes upon removal of the first adrenal; following the removal of the second adrenal there was little immediate change in the insulin requirement, and desoxycorticosterone acetate was the sole adrenal supplement employed. However, over a period of three weeks the insulin requirement gradually declined to 15 units per 24 hours. Cortisone was then added in amounts sufficient to restore the pre-adrenalectomy insulin requirement. The maintenance regime upon which the animal was ultimately stabilized comprised: (a) 12-hourly feedings containing 30 mEq. of sodium and 65 mEq. each of potassium and chloride, and (b) 12-hourly administration of 25 to 30 units of crystalline insulin subcutaneously, and of 2.25 mg. of desoxycorticosterone acetate and 10 mg. of cortisone acetate intramuscularly. On this regime the dog's weight and serum electrolytes were maintained and the animal exhibited its customary vigor. However, it was vulnerable to all forms of stress. During such episodes there was a marked increase in its sensitivity to insulin, necessitating careful regulation of dosage in order to prevent the occurrence of hypoglycemic reactions. Quantitative tests of insulin sensitivity were not made in this animal inasmuch as the stress of insulin deprivation was accompanied by an increase, rather than a decrease, in sensitivity to injected insulin.

The above maintenance regime was employed throughout the balance study, insulin being withdrawn after two three-day control periods and balance measurements be-

TABLE II

Metabolic balance data preceding and accompanying the administration of ACTH to a depancreatized dog

		INTAKE							OUTPUT						
									Urine						
Date and time	Period	N_2	Na	Cl	K	Ca	Mg	P	Vol.	N_2	Na	Cl	K	Ca	Mg
		Gm.	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*	*cc.*	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq*
May 27, 9 am–May 29, 9 am	C I	35.5	273.1	295.4	109.8	82.4	34.0	2584	3095	32.8	251.0	266.7	103.5	8.9	16.4
May 29, 9 am–May 31, 9 am	C II	35.5	273.1	295.4	109.8	82.4	34.0	2584	1987	29.8	243.0	257.7	87.5	5.0	12.0
May 31, 9 am–9 pm	E I	8.9	68.5	73.9	27.4	20.6	8.5	646	1915	9.4	76.6	72.4	59.4	1.7	2.9
May 31, 9 pm–June 1, 9 am	E II	8.9	68.5	73.9	27.4	20.6	8.5	646	1390	11.0	55.5	36.8	32.8	2.4	3.9
June 1, 9 am–9 pm	E III	8.9	68.5	73.9	27.4	20.6	8.5	646	1565	12.4	77.3	20.8	62.6	3.7	4.6

TABLE I—*Continued*

Blood, plasma and serum constituents preceding and accompanying the administration of ACTH to a depancreatized dog

Hct.	NPN	Creatinine	Total ketones	Beta hydroxybutyric acid	Acetoacetic acid	Na	Cl	K	Ca	P	Mg
%	mg. %	mg. %		mM/L.		mEq./L.	mEq./L.	mEq./L.	mg. %	mg. %	mEq./L.
54	36	0.61				144	98	4.7	10.3	3.8	1.8
54	43	0.62				142	102	4.7	10.0	3.2	1.7
51	41	0.59	0.8	0.7	0.1	141	104	4.7	9.9	2.8	1.7
50	54	0.70	1.7	1.3	0.4	139	99	3.6	9.8	4.9	1.6
53	52	0.59	3.1	2.0	1.1	142	101	3.9	9.9	4.7	1.5
52.4	53	0.63	4.6	2.3	2.3	141	97	4.0	9.1	4.7	1.8

ing continued throughout five 12-hour experimental periods. It had been hoped that the balance data could be obtained under conditions of fasting, inasmuch as control of food intake presents serious difficulty in anorexic and vomiting animals. However, fasting proved to constitute such a severe auxiliary stress to the depancreatized-adrenalectomized dog that the animal could not withstand insulin deprivation long enough to permit collection of the necessary data. The dog was therefore fed, and the proportion of each feeding retained estimated from the weight of the rejected food and the nitrogen content of any vomitus. By good fortune, the mean percentage of each feeding retained by this dog (59.6) proved almost identical with that retained by a depancreatized animal[3] (62.4), the early vomiting of the adrenalectomized-depancreatized dog being counterbalanced by its lack of anorexia. The corrected food intakes of these two animals during insulin deprivation are presented in Table VI, together with the gross balances of nitrogen, phosphorus, and potassium. Nitrogen balances corrected for changes in blood non-protein nitrogen are also shown; potassium balances have not been similarly corrected since serum charges were negligible.

[3] See metabolic data on Frisky (1).

RESULTS

A. Response of the Depancreatized Dog to Exogenous Stimulation of the Adrenal Cortex

1. *Clinical course.*

The administration of ACTH produced a striking acceleration in the animal's rate of progress toward diabetic acidosis. Polyuria became extremely severe and vomiting began prematurely. When, after a total insulin deprivation period of only 36 hours the experiment was terminated, the dog was moribund.

2. *General metabolic measurements.*

(a) *Blood, plasma and serum constituents.* The changes in the blood, plasma and serum constitu-

TABLE II—*Continued*

Metabolic balance data preceding and accompanying the administration of ACTH to a depancreatized dog

	OUTPUT																		
	Urine						Feces							Vomitus					
P	Glucose	Creatinine	Total ketones	Beta hydroxybutyric acid	Acetoacetic acid	N_2	Na	Cl	K	Ca	Mg	P	N_2	Na	Cl	K	Ca	Mg	P
mg.	Gm.	mg.		mM		Gm.	mEq.	mEq.	mEq.	mEq.	mEq.	mg.	Gm.	mEq.	mEq.	mEq.	mEq.	mEq.	mg.
1695	175.6	1117	0.5	0.4	0.1														
618	79.4	1037	0.4	0.3	0.1														
596	119.7	283	2.3	1.4	0.9	11.9	27.0	3.7	4.2	183.0	17.7	1570							
686	83.4	342	17.1	11.7	5.4														
960	66.1	397	53.3	37.4	15.9								3.4	42.7	59.0	10.4	3.7	9.2	217

TABLE III

Summary of balance data presented in Table II

	Nitrogen (*Gm.*)			Sodium (*mEq.*)			Chloride (*mEq.*)			Potassium (*mEq.*)		
Period	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance
C I	35.5	37.2	−1.7	273.1	261.0	+12.1	295.4	267.9	+27.5	109.8	105.1	+4.7
II	35.5	34.2	+1.3	273.1	253.0	+20.1	295.4	258.9	+36.5	109.8	89.1	+20.7
E I	8.9	10.5	−1.6	68.5	79.1	−10.6	73.9	72.7	+ 1.2	27.4	59.8	−32.4
II	8.9	12.1	−3.2	68.5	58.0	+10.5	73.9	37.1	+36.8	27.4	33.2	− 5.8
III	8.9	16.9	−8.0	68.5	122.5	−54.0	73.9	80.1	− 6.2	27.4	73.4	−46.0

ents which occurred during this experiment are summarized in Table I. Except for a rise in the level of blood ketones and a reciprocal fall in serum CO_2 content which took place during the administration of ACTH these changes were slight.

(b) *Metabolic balances.* The metabolic balance data are presented in detail in Table II and in summary form in Table III. In Figure 1 the balances of nitrogen, potassium, and phosphorus are shown graphically. During the 24 hours of insulin deprivation prior to the administration of ACTH, the balance changes did not differ materially from those observed during comparable phases of previous insulin withdrawal experiments (1). During the 12-hour period of ACTH administration the negativity of the nitrogen, potassium, and phosphorus balances increased abruptly. Nitrogen and phosphorus were lost in roughly pro-

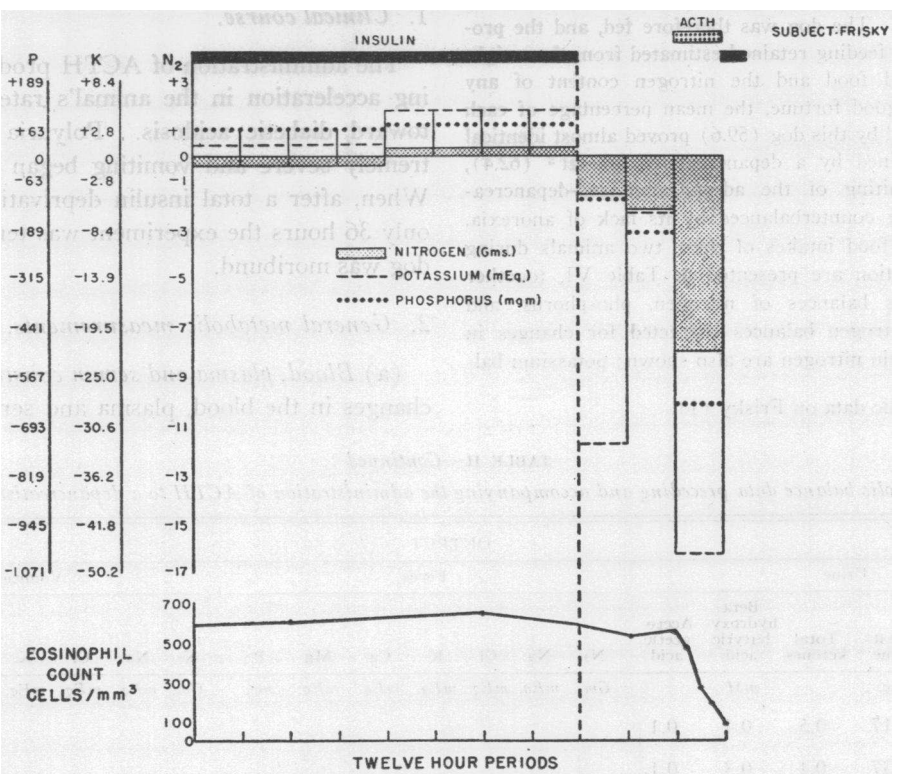

FIG. 1. METABOLIC BALANCES OF NITROGEN, POTASSIUM AND PHOSPHORUS AS RELATED TO THE EOSINOPHIL COUNT BEFORE AND FOR 36 HOURS AFTER THE WITHDRAWAL OF INSULIN FROM A DEPANCREATIZED DOG

During the last 12 hours of insulin deprivation ACTH was administered intramuscularly in a dosage of 20 mg. every three hours.

TABLE III—*Continued*

Summary of balance data presented in Table II

Calcium (*mEq.*)			Magnesium (*mEq.*)			Phosphorus (*mg.*)				
Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Theoretical balance based on Ca	Protoplasmic balance (Total P minus P based on Ca)
82.4	76.9	+5.5	34.0	22.8	+11.2	2584	2267	+317	+63	+254
82.4	73.0	+9.4	34.0	18.4	+15.6	2584	2190	+394	+98	+296
20.6	18.7	+1.9	8.5	4.5	+ 4.0	646	739	− 93	+20	−113
20.6	19.4	+1.2	8.5	5.5	+ 3.0	646	829	−183	+14	−197
20.6	24.4	−3.8	8.5	15.4	− 6.9	646	1320	−674	−30	−644

toplasmic proportion, whereas potassium was lost in amounts significantly in excess of nitrogen. The decline in urinary sodium excretion ordinarily beginning 24 hours after the withdrawal of insulin and persisting until the termination of the deprivation experiments failed to take place; rather, an increase in urinary sodium excretion occurred with the resultant development of a strongly negative sodium balance. The chloride, calcium and magnesium balances exhibited a premature swing from positive to negative.

(c) *Renal clearance studies.* The renal clearance rates of creatinine, sodium, and potassium are presented in Table IV and those of total ketones, acetoacetic and beta hydroxybutyric acids in Table V. During the administration of ACTH the endogenous creatinine clearance and the potassium clearance increased significantly whereas the renal clearance of sodium remained essentially unchanged. The clearance rate of acetoacetic acid increased somewhat and that of beta hydroxybutyric acid increased markedly. These increases in ketone clearance are, in all probability, responsible for the comparatively small rise in blood ketone levels which occurred during the administration of ACTH. A large increment in urinary ketone excretion occurred during this period indicating that ketosis was, in fact, substantially increased.

B. Response of the Adrenalectomized-Depancreatized Dog to Insulin Deprivation

1. *Clinical course.*

In some respects, the clinical course of the depancreatized-adrenalectomized dog following insulin withdrawal was milder than that of the depancreatized animal. Although vomiting began earlier (during the second experimental period), the animal did not become anorexic and appeared less prostrated at the end of the experiment than did depancreatized animals subjected to insulin deprivation for the same length of time.

On the other hand, tachycardia became alarming during the third period and was associated with an upward trend in the hemoglobin, hematocrit, and the blood level of non-protein nitrogen.[4] It

[4] The rapidity with which these findings, which were regarded as indications of adrenal insufficiency, appeared occasioned some surprise. It will be recalled that in depancreatized dogs the stress of insulin deprivation was not accompanied by an increased secretion of cortical hormones, as gauged by eosinopenia or increased corticosteroid excretion, until 36 to 48 hours had elapsed following the withdrawal of insulin. One possible explanation for the

TABLE IV

Renal clearances of creatinine, sodium and potassium as related to the eosinophil count preceding and accompanying the administration of ACTH to a depancreatized dog

Period		Creatinine clearance (GFR)	Sodium clearance	Potassium clearance	$\frac{\text{K clearance} \times 100}{\text{GFR}}$	Eosinophil count at end of period
		cc. per min.	*cc. per min.*	*cc. per min.*		*Cells/mm.*[3]
C	I	64	0.60	7	12	—
	II	52	0.59	6	12	594
E	I	60	0.74	20	32	538
	II	73	0.54	12	16	562
	III	90	0.74	22	24	94

TABLE V

Renal clearances of total ketones, acetoacetic and beta hydroxybutyric acids preceding and accompanying the administration of ACTH to a depancreatized dog

Period	Creatinine clearance (GFR)	Total ketone clearance	Acetoacetic acid clearance	Beta hydroxybutyric acid clearance
	cc./min.	*cc./min.*	*cc./min.*	*cc./min.*
C I	64	2	4	1
II	52	1	2	1
E I	60	3	5	2
II	73	10	10	10
III	90	·19	13	32

was felt that the repeated withdrawal of blood for chemical analysis was hastening circulatory collapse, and the animal was therefore transfused with 250 cc. of blood at the end of 36 hours of insulin deprivation. It was calculated that this volume of blood would replace that withdrawn during the control period and the previous experimental periods plus that to be withdrawn during the remainder of the experiment. No further rise in hemoglobin, hematocrit, non-protein nitrogen nor pulse rate occurred following the transfusion, and the clinical condition of the animal improved.

earlier collapse of the adrenalectomized animal is that desoxycorticosterone and cortisone, despite their ability to restore normal vigor, normal serum electrolytes, and a normal insulin requirement did not constitute complete hormonal replacement in the dosage administered.

2. *General metabolic measurements.*

(a) *Blood, plasma, and serum constituents.* The changes in blood, plasma and serum constituents which occurred during this experiment are summarized in Table VII. It is worthy of note that both eosinophil and lymphocyte counts began to fall at the end of 36 hours of insulin deprivation and continued to decline until the end of the experiment. There was, however, no reciprocal elevation in the neutrophil count; all types of leukocytes underwent a simultaneous and proportional decline.

Except for the early rise in indices of hemoconcentration the overall trends in blood constituents differ in only one respect from those observed in deprivation experiments upon depancreatized animals. The decline in serum CO_2 content which occurred during the periods immediately subse-

TABLE VI

The food intakes of an adrenalectomized-depancreatized dog (Lady) and of a depancreatized dog (Frisky) before and after the withdrawal of insulin, together with net balances of nitrogen (corrected for changes in blood NPN), phosphorus and potassium

A. Adrenalectomized-depancreatized dog (body weight 18.4 Kg.)

Period	N_2 ingested	N_2 lost in vomitus	N_2 retained	% feeding retained	N_2 balance (*Gm.*) Gross	N_2 balance (*Gm.*) Net‡	P balance	K balance
	Gm.	*Gm.*	*Gm.*				*mg.*	*mEq.*
C I*	11.9	0	11.9	100	+1.7	+0.9	+ 98	+ 8.8
II*	11.9	0	11.9	100	+1.2	+1.2	+ 87	+ 6.6
E I	11.9	0	11.9	100	−1.2	−2.4	− 2	−12.3
II	11.9	2.9†	9.0	76	−2.1	−3.4	− 21	+18.6
III	11.9	4.1	7.8	66	−2.1	−1.8	+ 28	+ 8.5
IV	11.9	13.4†	0	0	−4.9	−5.2	−341	− 2.5
V	11.9	5.2	6.7	56	−5.4	−3.4	−302	− 1.8

B. Depancreatized dog (body weight 17.1 Kg.)

C I*	10.1	0	10.1	100	+1.9	+2.5	+ 35	− 0.4
II*	10.1	0	10.1	100	−0.3	+0.2	−110	− 2.7
E I	10.1	0	10.1	100	−1.1	−1.4	−268	−21.9
II	10.1	0	10.1	100	−2.0	−1.9	−157	− 1.7
III	7.8	0	7.8	77	−2.5	−2.3	−253	− 5.2
IV	0.4	0.4	0	0	−8.1	−7.9	−579	−30.1
V	4.9	3.2	1.7	35	−7.3	−8.1	−522	−44.0

* Data for control periods are expressed as mean values per 12-hour period.
† Vomitus contaminated with urine.
‡ Corrected for changes in blood non-protein nitrogen by assuming body water to be 53 per cent of body weight and non-protein nitrogen to be distributed uniformly therein.

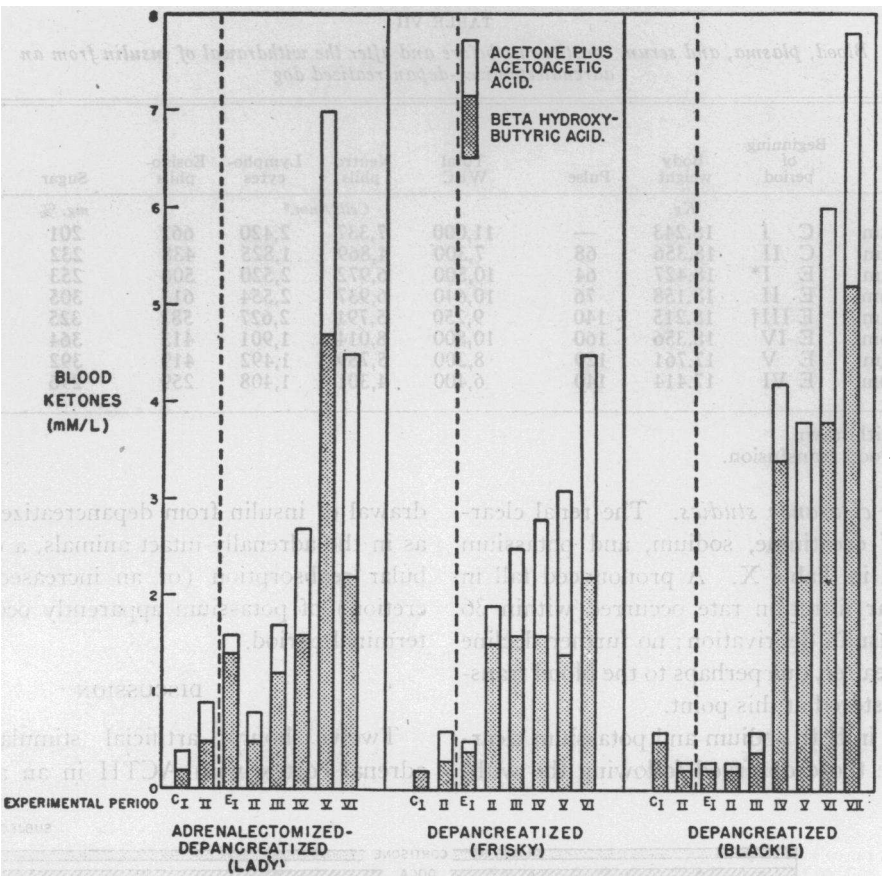

FIG. 2. CHANGES IN THE LEVELS OF BLOOD KETONES BEFORE AND AFTER THE WITHDRAWAL OF INSULIN FROM AN ADRENALECTOMIZED-DEPANCREATIZED DOG AND FROM TWO DEPANCREATIZED ADRENALLY-INTACT ANIMALS

quent to insulin withdrawal was eventually arrested and ultimately reversed. It seems probable that this reversal was due to the early and repeated vomiting, and the disproportionate loss of chloride with respect to sodium which it entailed.

The serum was observed to become lipemic (chylomicron counts and lipokrits were not determined in this study) after only 12 hours of insulin deprivation; thereafter, lipemia rapidly increased. Ketonemia was unequivocal after 36 hours of deprivation and ultimately equalled that observed in depancreatized dogs during terminal acidosis (Figure 2).

(b) *Metabolic balances.* The metabolic balance data is presented in detail in Table VIII and in summary form in Table IX. In Figure 3 the balances of nitrogen, potassium and phosphorus are shown graphically. Although the negativity of both nitrogen and phosphorus balances increased considerably during the last 24 hours of insulin deprivation, the net losses of these substances were much smaller than those observed during comparable periods of deprivation in depancreatized dogs whose adrenals were intact (1). Potassium was lost in excess of nitrogen only during the first 12 hours following insulin withdrawal, the loss thereafter being negligible. Magnesium remained in equilibrium until the final experimental period, when the urinary excretion rate increased sufficiently to render the balance negative. The calcium balance, initially positive, became negative as insulin deprivation continued.

The severe glycosuria which occurred upon the withdrawal of insulin was accompanied initially by heavy losses of sodium and chloride; thereafter, urinary sodium and chloride were well conserved. The external balance of chloride became negative only terminally as vomiting increased.

TABLE VII

Blood, plasma, and serum constituents before and after the withdrawal of insulin from an adrenalectomized-depancreatized dog

Date and time	Beginning of period	Body weight	Pulse	Total WBC	Neutrophils	Lymphocytes	Eosinophils	Sugar	CO_2 content	Total protein
		Kg.			*Cells/mm.³*			*mg. %*	*mEq.*	*Gm. %*
February 20—am	C I	18.243	—	11,000	7,337	2,420	662	201	27.3	6.6
23—am	C II	18.356	68	7,300	4,869	1,825	438	232	29.4	6.9
26—am	E I*	18.427	64	10,500	6,972	2,520	506	253	29.5	5.9
26—pm	E II	18.158	76	10,640	6,937	2,554	613	305	23.8	5.8
27—am	E III†	18.215	140	9,250	5,791	2,627	582	325	17.4	5.2
27—pm	E IV	18.356	160	10,800	8,014	1,901	413	364	16.9	5.8
28—am	E V	17.761	120	8,200	5,789	1,492	419	392	22.6	5.7
28—pm	E VI	17.414	140	6,400	4,301	1,408	259	296	23.3	5.0

* Insulin withdrawn.
† 250 cc. blood transfusion.

(c) *Renal clearance studies.* The renal clearance rates of creatinine, sodium, and potassium are presented in Table X. A pronounced fall in the glomerular filtration rate occurred within 36 hours after insulin deprivation; no further decline occurred thereafter, due perhaps to the blood transfusion administered at this point.

The trends in both sodium and potassium clearance resemble those observed following the withdrawal of insulin from depancreatized dogs; and, as in the adrenally-intact animals, a decreased tubular reabsorption (or an increased tubular secretion) of potassium apparently occurred in the terminal period.

DISCUSSION

Twelve hours' artificial stimulation of the adrenal cortex with ACTH in an animal previ-

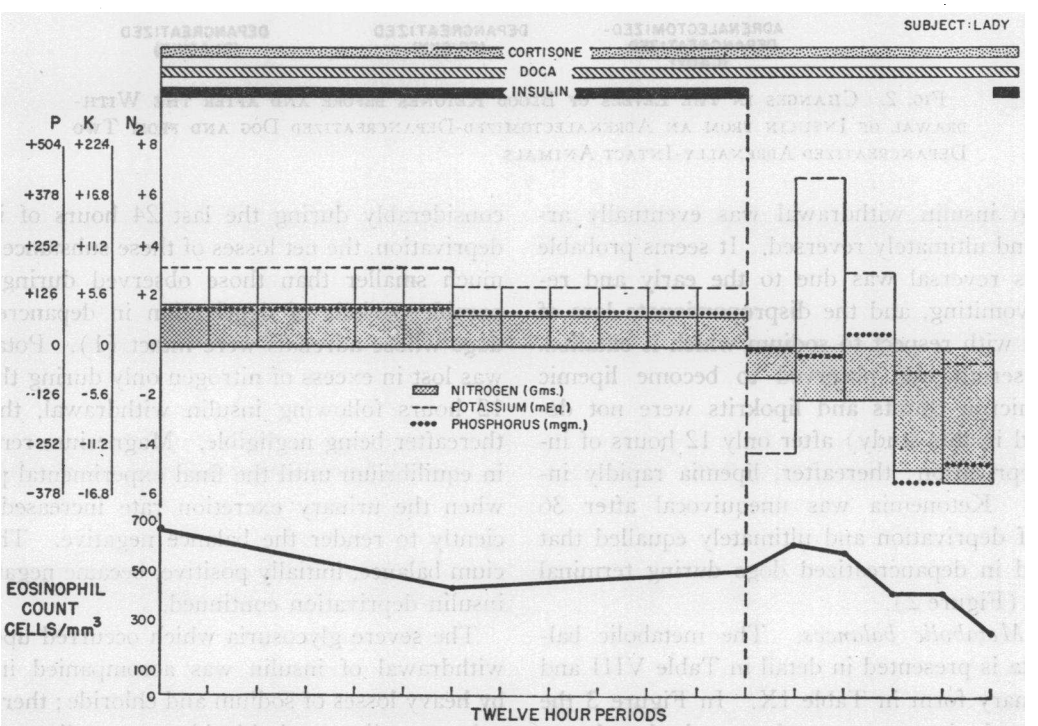

FIG. 3. METABOLIC BALANCES OF NITROGEN, POTASSIUM AND PHOSPHORUS AS RELATED TO THE EOSINOPHIL COUNT BEFORE AND AFTER THE WITHDRAWAL OF INSULIN FROM A DEPANCREATIZED-ADRENALECTOMIZED DOG

TABLE VII—*Continued*

Blood, plasma, and serum constituents before and after the withdrawal of insulin from an adrenalectomized-depancreatized dog

Hgb.	Hct.	NPN	Creatinine	Total ketones	Beta hydroxybutyric acid	Acetoacetic acid	Na	Cl	K	Ca	P	Mg
Gm. %	*%*	*mg. %*	*mg. %*		*mm./L.*		*mEq./L.*	*mEq./L.*	*mEq./L.*	*mg. %*	*mg. %*	*mEq./L.*
14.9	47.6	27	0.33	0.4	0.2	0.2	150.0	104.2	4.0	9.2	2.6	1.2
15.5	50.3	35	0.33	0.9	0.5	0.4	138.0	99.0	4.0	—	2.4	1.3
15.7	49.2	35	0.33	1.6	1.4	0.2	142.4	102.5	4.0	9.4	2.5	1.2
16.3	50.8	47	0.43	0.8	0.5	0.3	139.0	104.0	4.0	8.4	4.0	1.2
16.8	52.3	60	0.54	1.7	1.2	0.5	134.4	98.2	4.4	6.9	4.4	1.5
17.0	50.0	57	0.82	2.7	1.6	1.1	126.0	92.0	4.4	7.1	5.2	—
15.6	47.0	60	0.87	7.0	4.7	2.3	129.0	87.4	4.3	6.8	4.8	2.0
17.0	51.5	40	1.06	4.5	2.2	2.3	133.6	81.4	3.7	6.2	4.7	1.8

ously deprived of insulin for only 24 hours resulted in a premature exacerbation of the diabetic state and in the appearance of a complex of metabolic events ordinarily observed to occur spontaneously only after 48 to 60 hours of insulin deprivation. Events common to pure insulin-deprivation acidosis and ACTH-accelerated acidosis include the following: (a) Increased catabolism of protoplasm; (b) loss of potassium in excess of nitrogen; and (c) increased ketosis. In view of the limitations of the eosinophil count as an index of adrenal cortical activation it is surprising that the use of this index for determination of ACTH dosage resulted in metabolic alterations which were similar not only in direction but in magnitude to those observed in pure insulin-deprivation acidosis.

In most respects, the effects produced by ACTH under the condition of this experiment resemble those previously described in non-diabetic individuals (2–8). Net losses of nitrogen, excess potassium, phosphorus, and calcium resulted from administration of the hormone and the glomerular filtration rate, as estimated from the endogenous creatinine clearance, was increased. The ketosis and the vomiting resulting from the animal's accelerated progress toward diabetic acidosis appeared to introduce a number of modifications in the metabolic pattern ordinarily resulting from ACTH administration. Thus, negative rather than positive balances of sodium and chloride were obtained. Obligatory excretion of sodium in combination with the ketone bodies took place, and large amounts of chloride were lost in the vomitus. Magnesium was also lost, a result in disagreement with that reported by other investigators (6) in a non-diabetic individual. Whether this loss reflects a general drain on fixed bases imposed by the ketonuria, or some other mechanism, is not clear. With respect to ketone metabolism the effects produced by ACTH agree with those reported in diabetic patients and animals by several other investigators (9–12).

Except for the rise in the glomerular filtration rate and the increase in the rate of sodium excretion, the metabolic pattern resulting from ACTH administration was virtually identical with that previously found to occur 24 hours later in this animal during the terminal phase of insulin-deprivation acidosis. In the absence of pertinent data, it seems reasonable to assume that the dehydration from which the animal suffered during the final phases of the insulin deprivation experiments modified the renal response to ACTH. It can, indeed, be argued that had ACTH stimulation not occurred in this critical situation the glomerular filtration rate might have fallen further. The increase in sodium excretion seems logically attributable to the suddenness with which severe ketosis developed during ACTH treatment, the renal ammonia-producing mechanism being thereby denied an opportunity to become fully activated.

In fulfilling the first postulate, the results of this experiment are compatible with the hypothesis that increased adrenal cortical activity is a factor conditioning the occurrence of the following components of acidosis resulting from insulin deprivation: (a) Increased catabolism of protoplasm; (b) loss of potassium in excess of nitrogen; and (c) increased ketosis. However, these results alone do not suffice to establish the correctness of the hypothesis and an examination of the manner in

TABLE VIII

Metabolic balanse data before and after the withdrawal of insulin from an adrenalectomized-depancreatized dog

Date	Period	INTAKE							OUTPUT									
									Urine									
		N_2	Na	Cl	K	Ca	Mg	P	Vol.	N_2	Na	Cl	K	Ca	Mg	P	Glucose	Creatinine
		Gm.	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*	*cc.*	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*	*Gm.*	*mg.*
February 20—am 23—am	C I	71.4	182.4	392.4	387.6	139.2	27.6	4747	3747	56.4	148.3	370.9	335.0	3.5	1.4	3035	42.8	1499
February 23—am 26—am	C II	71.4	182.4	392.4	387.6	139.2	27.6	4747	4085	59.3	148.6	351.3	348.0	3.5	1.8	3103	81.7	1420
February 26—am pm	E I	11.9	30.4	65.4	64.6	23.2	4.6	791	2067	12.2	55.8	62.0	76.9	0.4	1.1	631	88.4	272
February 26—pm 27—am	E II	11.9	30.4	65.4	64.6	23.2	4.6	791	1106	9.8	7.2	16.1	42.4	0.3	1.0	424	65.1	220
February 27—am pm	E III	11.9	30.4	65.4	64.6	23.2	4.6	791	979	9.0	0.8	0.7	42.7	0.1	0.1	426	51.6	217
February 27—pm 28—am	E IV	11.9	30.4	65.4	64.6	23.2	4.6	791	257	2.5	0.3	0.0	8.4	0.0	0.4	173	8.3	76
February 28—am pm	E V	11.9	30.4	65.4	64.6	23.2	4.6	791	1230	11.2	5.0	0.2	56.3	0.2	3.3	646	48.7	312

TABLE VIII—*Continued*

Metabolic balance data before and after the withdrawal of insulin from an adrenalectomized-depancreatized dog

Date	Period	OUTPUT																							
		Urine plus vomitus									Vomitus								Feces						
		Vol.	N_2	Na	Cl	K	Ca	Mg	P	Glucose	Vol.	N_2	Na	Cl	K	Ca	Mg	P	N_2	Na	Cl	K	Ca	Mg	P
		cc.	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*	*Gm.*	*cc.*	*Gm.*	*Eq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*
February 20—am 23—am	C I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10.0	0	0	0	199	20.9	1600
February 23—am 26—am	C II	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
February 26—am pm	E I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
February 26—pm 27—am	E II	285	3.3	7.1	11.4	3.6	1.3	1.0	237	12.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
February 27—am pm	E III	—	—	—	—	—	—	—	—	—	670	4.1	26.8	61.6	13.4	11.0	1.2	271	4.7	0	0	0	93.2	13.7	618
February 27—pm 28—am	E IV	1150	6.5	0	20.7	29.9	4.9	0.2	503	37.8	710	6.9	21.0	65.0	28.8	12.6	2.8	448	—	—	—	—	—	—	—
February 28—am pm	E V	—	—	—	—	—	—	—	—	—	1440	5.2	25.9	93.6	10.0	10.5	3.6	378	—	—	—	—	—	—	—

which the more rigorous demands of the second postulate are fulfilled is required.

Components of pure insulin-deprivation acidosis which were diminished or suppressed in the adrenalectomized-depancreatized animal maintained on a fixed dose of adrenal cortical hormones during insulin deprivation and which, in fulfilling the second postulate, appear to qualify as adrenal-conditioned, include the following: (a) Hematologic changes comprising increased neutrophil and total white blood cell counts and markedly decreased eosinophil and lymphocyte counts; (b) increased catabolism of protoplasm; (c) loss of potassium in excess of nitrogen; and (d) decreased sensitivity to injected insulin.

Both qualitative and quantitative differences obtained between the hematologic responses of the adrenalectomized-depancreatized and the depancreatized dogs to insulin deprivation. The neutrophil and total white blood cell counts showed a decrease rather than an increase in the doubly-operated animal, and the decrease in the eosinophil and lymphocyte counts was less marked in the adrenalectomized than in the adrenally-intact dog. These observations are in accord with those reported by Muehrcke, Staple, and Kark (13) and Herlant (14). In the light of the meager knowledge currently available regarding the factors which control the levels of the various circulating leukocytes, less interest attaches to the differences between the responses of the two types of animals than to their similarities. The fact that both eosinopenia and lymphopenia occurred to some extent in an adrenalectomized animal maintained on a constant supply of adrenal cortical hormones during stress agrees with the concept of Selye (15) and Engel (16) that these hematologic reactions may be more correctly viewed as conditioned responses than as pure effects of adrenal hormone action.

The finding that less protoplasm was catabolized by the adrenalectomized-depancreatized than by the depancreatized animals during the stress of insulin deprivation is in harmony with many previous reports (17–24). The adrenalectomized animal's failure to excrete excess potassium seems logically attributable to a failure of cellular potassium release; inasmuch as the level of serum potassium remained stationary throughout, the conservation of potassium cannot be ascribed to impaired renal excretion resulting from adrenal insufficiency. The observation that stress increased the insulin sensitivity of the adrenalectomized dog is in accord with the finding of de Bodo, Sinkoff, and Kiang (25) that insulin sensitivity is enhanced in adrenalectomized dogs suffering from adrenal insufficiency following the prolonged deprivation of desoxycorticosterone.

Although the finding that ACTH administration increased ketosis in the depancreatized adrenally-intact animal suggested that ketosis is an adrenal-conditioned event, this expectation was not borne out in the experiment on the adrenalectomized-depancreatized animal. The finding that the level of blood ketones attained by the adrenalectomized-depancreatized dog at the conclusion of the period of insulin deprivation did not differ materially from that previously observed in depancreatized animals is, however, in accord with a number of observations made by other investigators. The ability of anterior pituitary extracts to elevate the levels of blood ketones to the same extent in adrenalectomized animals as in adrenally-intact animals has been clearly demonstrated (26–29). Ketonuria has often been noted to be minimal in adrenalectomized animals treated with ketogenic extracts, giving rise to the impression that ketogenesis has not been stimulated. However, studies comprising determinations of blood as well as urine ketone levels have shown that the blood levels attained differ little from those observed in intact animals (30).

In view of the results indicating that increased adrenal activity is of little or no significance in the genesis of ketonemia, it is not surprising that the same conclusion is suggested regarding changes in the concentration of blood fats. In the adrenalectomized-depancreatized dog lipemia became visible after only 12 hours of insulin deprivation; in the depancreatized animals lipemia did not become evident until a somewhat longer time had elapsed after the withdrawal of insulin and it was less marked. If, as is suggested by a number of studies (31, 32), the corticosteroids participate significantly in fat mobilization, the concentration of cortical hormones required to condition this reaction must be comparatively low.

The adrenalectomized-depancreatized dog proved capable of conserving sodium and chloride as quickly and efficiently as did the depancreatized

TABLE IX

Summary of balance data presented in Table VIII

	Nitrogen (*Gm.*)			Sodium (*mEq.*)			Chloride (*mEq.*)			Potassium (*mEq.*)		
Period	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance
C* I	11.9	10.2	+1.7	30.4	24.7	+ 5.7	65.4	61.8	+ 3.6	64.6	55.8	+ 8.8
II	11.9	10.7	+1.2	30.4	24.8	+ 5.6	65.4	58.5	+ 6.9	64.6	58.0	+ 6.6
E I	11.9	13.1	−1.2	30.4	55.8	−25.4	65.4	62.0	+ 3.4	64.6	76.9	−12.3
II	11.9	14.0	−2.1	30.4	14.3	+16.1	65.4	27.5	+37.9	64.6	46.0	+18.6
III	11.9	14.0	−2.1	30.4	27.6	+ 2.8	65.4	62.3	+ 3.1	64.6	56.1	+ 8.5
IV	11.9	16.8	−4.9	30.4	21.3	+ 9.1	65.4	85.7	−20.3	64.6	67.1	− 2.5
V	11.9	17.3	−5.4	30.4	30.9	− 0.5	65.4	93.8	−28.4	64.6	66.4	− 1.8

* Aggregate data for control periods are here expressed as mean values per 12-hour period, for readier comparison with the 12-hour experimental periods.

animal. Moreover, the renal tubular absorption of potassium decreased (or the tubular secretion increased) terminally in the adrenalectomized as well as in adrenally-intact animals. These findings agree with the observation (33) that bilaterally adrenalectomized patients are able to reduce sodium excretion when subjected to salt depletion, and with that of Ingle, Prestrud, Li, and Evans (34) that adrenalectomized rats maintained on a constant intake of adrenal cortical hormones are capable of "physiologically economical adaptations in sodium, chloride and potassium balance." They suggest that intrinsic renal mechanisms, rather than hormonal influences, predominate in the control of electrolyte excretion.

The concept that an exacerbation of the diabetic state is a logical result of stress in the organism with defective pancreatic islet cell function is supported by general physiologic considerations. An increased need for carbohydrate may be the first effect of stress in general, as Engel (35) points out, since stress regularly results in hypoglycemia and death in adrenalectomized or hypophysectomized animals in which the activation of the adrenal cortex and increased protein catabolism which follow stress in intact animals do not occur. As stores of carbohydrate begin to be depleted, the counteraction of increased protein catabolism, gluconeogenesis and decreased insulin sensitivity takes place in the presence of a normally responsive adrenal cortex. In the diabetic organism, preserved from carbohydrate starvation only by exogenous insulin, stress-induced activation of these adrenal-conditioned mechanisms fails of its physiologic purpose. Stores of preformed carbohydrate are only partially utilized due to decreasing effectiveness of injected insulin and together with newformed carbohydrate are excreted in the urine, carrying with them water and electrolytes.

TABLE X

Renal clearances of creatinine, sodium, and potassium before and after the withdrawal of insulin from an adrenalectomized-depancreatised dog

Period	Creatinine clearance (GFR)	Sodium clearance	Potassium clearance	K clearance ×100 / GFR
	cc./min.	*cc./min.*	*cc./min.*	
C I	105	0.23	19	18
II	100	0.24	20	20
E I	99	0.54	26	26
II	—	—	—	—
III	44	0.01	13	30
IV	—	—	—	—
V	45	0.05	19	43

The studies of Long and Lukens (36) demonstrated that an intact pituitary-adrenal axis is essential for full manifestation of pancreatic diabetes. The present studies suggest that the adrenal cortical hormones, in conditioning a number of the components of the non-specific alarm reaction to the stress of insulin deprivation, may contribute significantly to the pathologic physiology of experimental diabetic acidosis as well.

SUMMARY

1. The metabolic changes which characterized the stressful terminal phases of insulin deprivation in the depancreatized dog have been compared with: (a) The changes which accompany the induction of a comparable degree of eosinopenia by

TABLE IX—*Continued*

Summary of balance data presented in Table VIII

Calcium (*mEq.*)			Magnesium (*mEq.*)			Phosphorus (*mg.*)				
Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Theoretical based on Ca	Protoplasmic balance (total P minus P based on Ca)
23.2	17.2	+ 6.0	4.6	2.0	+2.6	791	639	+152	+ 54	+ 98
23.2	17.2	+ 6.0	4.6	2.0	+2.6	791	650	+141	+ 54	+ 87
23.2	19.0	+ 4.2	4.6	3.8	+0.8	791	755	+ 36	+ 38	− 2
23.2	20.2	+ 3.0	4.6	4.7	−0.1	791	785	+ 6	+ 27	− 21
23.2	29.7	− 6.5	4.6	4.0	+0.6	791	821	− 30	− 58	+ 28
23.2	36.1	−12.9	4.6	6.1	−1.5	791	1248	−457	−116	−341
23.2	29.3	− 6.1	4.6	9.6	−5.0	791	1148	−357	− 55	−302

the administration of ACTH to a depancreatized dog deprived of insulin for a comparatively brief period; and (b) the changes which accompany the withdrawal of insulin from an adrenalectomized-depancreatized dog maintained upon a fixed dose of adrenal cortical hormones.

2. The administration of ACTH under these circumstances was found to result in a premature exacerbation of the diabetic state and in the appearance of a complex of metabolic phenomena characteristic of experimental diabetic acidosis resulting from prolonged insulin deprivation.

3. The withdrawal of insulin from an adrenalectomized-depancreatized dog maintained on a fixed dose of adrenal cortical hormones resulted in diminution or suppression of a number of components of pure insulin-deprivation acidosis.

4. It is concluded that those constituents of pure insulin deprivation acidosis which are: (a) Temporally related to the occurrence of evidences of increased adrenal activity; (b) prematurely induced by exogenous stimulation of the adrenal cortex during mild stress; and (c) diminished or suppressed by maintenance of a fixed supply of adrenal cortical hormones during severe stress are, in fact, adrenal-conditioned.

These constituents include: (1) Hematologic changes comprising increased neutrophil and total white blood cell counts and markedly decreased eosinophil and lymphocyte counts; (2) increased catabolism of protoplasm; (3) loss of potassium in excess of nitrogen; and (4) decreased sensitivity to injected insulin.

5. It would appear that the increase in adrenal cortical activity which occurs in response to the stress of insulin deprivation, by conditioning or sustaining a number of the other constituents of the alarm reaction to this stress, contributes significantly to the pathologic physiology of experimental diabetic acidosis.

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