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Research Article

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

I. TEMPORAL RELATIONS BETWEEN THE METABOLIC EVENTS OF EXPERIMENTAL DIABETIC ACIDOSIS AND THE LEVEL OF ADRENAL CORTICAL FUNCTION [1]

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An increase in adrenal activity, often of considerable magnitude, has been found to accompany a large number of clinical disorders including such varied entities as meningococcal meningitis, perforated peptic ulcer and myocardial infarction. Adrenal activation under these circumstances is thought to be an important constituent of a non-specific pattern of response to trauma, the so-called "general adaptation syndrome" of Selye (1), whose acute phase has been termed the "alarm reaction." Among other components of the alarm reaction are a negative nitrogen balance, a negative potassium balance out of proportion to the negativity of the nitrogen balance, an impairment of carbohydrate metabolism with a decreased sensitivity to insulin, and an increased mobilization of fat from the periphery to the liver with a tendency to fasting ketosis (2). It is believed that an important function of this complex of events is the conservation of carbohydrate stores and the utilization of body fat and protein as sources of energy.

While this response is presumably a homeostatic mechanism, possessing survival value for the normal organism, it seems unlikely that the reaction is of equal physiological appropriateness under all circumstances. Conceivably, in an organism with pre-existing disease of tissues participating in the reaction, activation of this response pattern might even be detrimental. The outcome of a given stress situation might then be expected to be a biological resultant between the deleterious action of the specific stress and the various effects of the non-specific response to trauma, certain of which would tend to support the organism and others to operate to its disadvantage.

Inasmuch as the adrenal cortical hormones apparently perform an important function in supporting certain components of the general bodily reaction to damage, an increase in adrenal activity might be expected to accompany those metabolic events dependent upon the cortical hormones for their initiation or maintenance during stress. Therefore, the inclusion of serial estimates of the level of adrenal cortical function in a detailed metabolic description of an organism's response to a specific stress might be expected to assist in the identification of the adrenal-conditioned components of that response. Metabolic phenomena found to occur concomitantly with an increase in the level of adrenal cortical activity could then be subjected to closer scrutiny in order to determine whether they were, in fact, dependent upon increased adrenal activity for their initiation or maintenance. Separation of the beneficial from the harmful effects of the alarm reaction would thus be facilitated.

Diabetic acidosis appeared to be a state whose pathologic physiology might be illuminated by such an investigation, inasmuch as the severity of diabetes is known to be increased, and coma occasionally precipitated, by a variety of types of trauma. Experimental diabetic acidosis was the form of

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stress chosen for these studies the first of which, described in this paper, is a description of the metabolic concomitants of increased adrenal activity during the evolution of experimental ketoacidosis. This has been obtained by correlating estimations of blood and urine chemical constituents, balance measurements and changes in insulin sensitivity with blood counts and urinary corticosteroid excretion rates in depancreatized dogs rendered ketotic by insulin deprivation.

EXPERIMENTAL PROCEDURE

Three parasite-free mongrel bitches selected for their gentleness and adaptability were employed as subjects. Females were used in preference to males because of the greater ease of catheterization, which was further facilitated by dorsal slitting of the perineum. Diabetes was produced by total pancreatectomy and several months were allowed to elapse post-operatively in order to permit subsidence of the adrenal reaction to the surgical trauma. During the recovery period the dogs were gradually accustomed to all of the various procedures to be employed during the performance of the metabolic experiments in order to minimize the stressful effects of the procedures themselves. Care was taken to perform the metabolic experiments only during dioestrus.

Prior to the performance of the metabolic experiments the animals were stabilized on a constant dietary regime together with such insulin as permitted a moderate glycosuria and insured the avoidance of hypoglycemia. The diet employed for the first animal studied (Brownie) consisted of horse meat, raw beef pancreas, bread crumbs, and Brewer's yeast. A number of inadequacies became apparent in this regime: (1) Steatorrhea could not be controlled, even by the administration of raw pancreas in amounts of 200 Gm. per day; (2) The calcium balance was consistently negative; and (3) The liver of the animal became enlarged and at autopsy was found to be infiltrated with fat. Subsequent diets were composed of horse meat and bread crumbs plus Brewer's yeast, choline, cod liver oil, sodium chloride, and calcium lactate. Desiccated pancreas substance (Viokase) was substituted for raw pancreas with abolition of the steatorrhea. Hepatic enlargement no longer occurred, and the absence of fatty degeneration in the liver was established in one animal (Blackie) who came to autopsy.

The whole of the food required for the control and experimental periods was intimately mixed before the study was begun. It was then divided into accurately weighed portions each of which was placed in a separate jar and kept frozen until just before use. The nitrogen and mineral contents of these diets were determined separately for each experiment; these values are summarized in Table III–A, B, C.

Crystalline insulin and food were given every twelve hours to all of the animals except one (Brownie, who was given protamine zinc insulin, crystalline insulin, and food every twelve hours). In the experiments designed to obtain a description of the changes in blood and urine chemical constituents and in metabolic balances, the withdrawal of insulin was preceded by a number of control periods, each of which was three days in length (except in Brownie, whose control periods were twelve hours in length). Food was withdrawn simultaneously from one of the animals (Brownie), but was offered at twelve hour intervals to the other two dogs. The three animals had access to water at all times. Because of the rapidity with which acidosis developed, the length of the subsequent experimental periods was shortened to twelve hours.

The dogs were confined in metabolism cages and all urine, feces, vomitus and uneaten food collected. When, during its decline into acidosis the animal became nauseated, it was lightly harnessed in the cage and a bipartite collecting pan inserted beneath the grated cage floor so that urine and vomitus could be accumulated separately. Jugular blood samples were taken under oil at the beginning of each period before food was offered. The urine collected was stored at 5° C.; all except that destined for corticosteroid analysis was preserved under toluene. The animals were weighed at the beginning of each period on scales with an accuracy of ± 10 grams.

The experiments designed to obtain a description of the changes in insulin sensitivity were, of necessity, conducted separately since it was found that performance of serial sensitivity tests in an animal deprived of its maintenance insulin postponed the onset of ketoacidosis almost indefinitely. Determinations of insulin sensitivity were therefore made in successive experiments upon the same animal (Frisky) after 12, 24, 36, 48, and 60 hours of total insulin deprivation. Upon the completion of each experiment, time was allowed for the dog to regain metabolic equilibrium and insulin sensitivity, if it had been impaired, before its maintenance insulin was again withdrawn.

The insulin sensitivity tests were performed by administering intravenously 10 units of a special preparation of Lilly insulin [3] which was free from the hyperglycemic factor. This comparatively large dose (0.6 units per kilogram) was selected in order that an unequivocal fall in blood sugar might be obtained after prolonged insulin deprivation, when the occurrence of insulin resistance was anticipated. To minimize errors in dosage the insulin was diluted with physiological saline so that one cubic centimeter contained one unit. Blood was drawn for determination of its sugar content just prior to the injection of the insulin and 20, 60, and 90 minutes, thereafter.

It was decided to terminate the observations at 90 minutes because of the demonstration (3, 4) that for a period varying from 30 to 90 minutes after the injection of insulin the blood sugar falls at a virtually uniform rate which bears no close relation to the nutritional condition of the subject. After this period the blood sugar rises much more rapidly in glycogen-rich than in glycogen-depleted subjects, the retarded rate of recovery in the latter

[3] Special insulin No. 2756, for generous supplies of which the authors are indebted to Dr. W. R. Kirtley of the Lilly Research Laboratories.

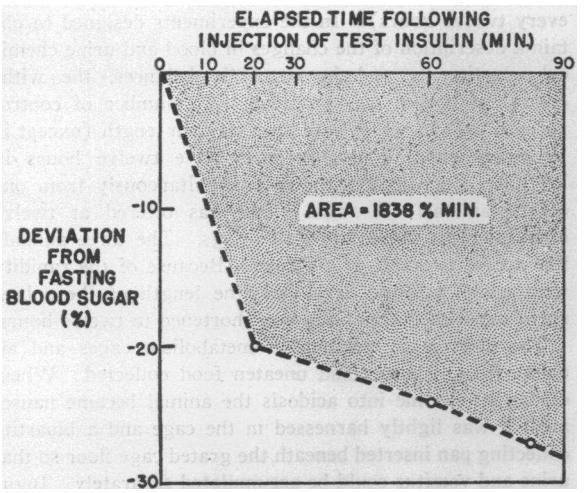

FIG. 1. THE METHOD EMPLOYED FOR COMPUTING THE INDEX OF INSULIN SENSITIVITY, UTILIZING THE RESULTS OF THE SENSITIVITY TEST PERFORMED AFTER 60 HOURS OF INSULIN DEPRIVATION FOR ILLUSTRATION

Elapsed time following injection of test insulin	Blood sugar	Deviation from fasting blood sugar
(*min.*)	(*mg.* %)	(%)
0	570	—
20	458	−20
60	434	−24
90	408	−28

presumably reflecting the occurrence of gluconeogenesis. While it thus appeared that hepatic glycogen depletion would tend to minimize rather than to exaggerate any tendency toward insulin resistance, it was hoped to reduce the influence of this variable by employing a comparatively brief period of observation.

For estimation of the insulin sensitivity upon any given occasion the percentage fall from the fasting level was ascertained for each blood sugar value, this percentage plotted on graph paper, and the area enclosed by the resulting curve determined planimetrically. This area, expressed numerically in "per cent minutes," constitutes an index of sensitivity to the injected insulin (see Figure 1). The area varies directly with insulin sensitivity, and were there an entirely adequate means of correcting for variation in the level of the fasting blood sugar there might be justification for regarding the area as a measure rather than as an index of sensitivity. In computing the area enclosed by the curve, the role of the fasting blood sugar level as a variable has been reduced, but not negated, by expressing subsequent blood sugar values in terms of percentage fall from the fasting level for the given test.

METHODS

Sodium was determined by the method of Butler and Tuthill (5), chloride by a modification of the method of Wilson and Ball (6), potassium by the method of Fiske and Litarczek (7), calcium by the method of Fiske and Logan (8), phosphorus by the method of Fiske and Subbarrow (9), magnesium by a modification of the method of Briggs (10), total nitrogen by the macro-Kjeldahl method of Peters and Van Slyke (11) and glucose and non-protein nitrogen by the methods of Folin (12, 13). Total protein of the serum was estimated by the method of Lowry and Hunter (14), CO_2 content by the method of Van Slyke and Neill (15) and pH by the method of Hastings and Sendroy (16). Measurements of serum fat content were made during the insulin sensitivity studies, the indices employed being the lipokrit (17) which provides a measure of changes in the total lipoid content of the serum, and the chylomicron index (18) which presumably reflects changes in serum neutral fat.

Creatinine was determined by a modification of the method of Bonsnes and Taussky (19). A correction factor has been applied to all of the serum creatinine values reported in Parts I and IIb so as to render them comparable to the serum creatinine values obtained in Part IIa by the use of Hare's (20) method. Hare's method appears to be more specific for creatinine than that of Bonsnes and Taussky, in that it eliminates substances other than creatinine which may give a color with picrate.

Total ketones were measured by a modification of the method of Nanavutty (21) in the experiment on Brownie. In the other experiments acetoacetic acid and acetone were separated from beta hydroxybutyric acid and both fractions subsequently assayed by the salicylaldehyde method of Behre (22). Acetone and acetoacetic acid were removed from a Somogyi $ZnSO_4$–$Ba(OH)_2$ filtrate by acidification and distillation into 2 per cent $NaHSO_3$ for 15 minutes at low heat. Beta hydroxybutyric acid, which still remained, was then oxidized to acetone by refluxing for 15 minutes with acid dichromate and the acetone collected by distillation into a fresh solution of 2 per cent $NaHSO_3$ for 20 minutes. Recoveries from standard solutions of these constituents were 70 per cent for acetone and acetoacetic acid and 50 per cent for beta hydroxybutyric acid; recoveries from standards added to a filtrate of normal human blood were 66 per cent for acetone and acetoacetic acid and 52 per cent for beta hydroxybutyric acid. All values reported have been corrected to compensate for these losses, which have been assumed to be 30 per cent for acetone plus acetoacetic acid and 50 per cent for beta hydroxybutyric acid. The acetone derived from the acetoacetic acid-acetone fraction has been expressed as millimols of acetoacetic acid; that from the beta hydroxybutyric acid fraction as millimols of beta hydroxybutyric acid.

Hematologic changes and alterations in urinary corticosteroid excretion were employed as indices of the level of adrenal cortical function. Circulating blood eosinophils and total white cells were counted in the double-celled Fuchs-Rosenthal chamber by a modification of the method of Randolph (23). A solution of 0.025 per cent phloxine B in propylene glycol was employed as the diluent and all eosinophils in both cells of two chambers

enumerated. Lymphocyte and neutrophil counts were made from a Wright-stained blood smear, a total of 500 cells being counted. The urinary corticosteroids were measured by the method of Talbot, Saltzman, Wixom, and Wolfe (24).

RESULTS

I. *General Metabolic Experiments*

(a) *Clinical course*

The clinical course of all three animals following the discontinuation of insulin therapy was very similar. Hedon's serial photographs (25) of his dog as he lapsed into acidosis after insulin withdrawal are an admirable representation of the changes in physical appearance. Our animals differed from Hedon's only in their more rapid decline during insulin deprivation. Polydipsia and polyuria were noted to begin within a few hours after insulin withdrawal, anorexia and muscular weakness in 48 hours, vomiting in 60 hours, somnolence and Kussmaul respiration in 72 hours. If treatment were delayed until 72 hours had elapsed following the omission of insulin, death ensued in spite of vigorous efforts to save the animals with insulin and intravenous fluids. One animal (Frisky), in whom therapy was begun after 60 hours of insulin deprivation, survived. Despite their serious condition in the later stages of ketosis, the dogs did not become comatose until just before death, a fact which obscured their urgent need for treatment. An incidental observation of some interest was the occurrence of hemolytic crises [4] in two of the dogs during last stages of ketoacidosis.

(b) *Indices of adrenal cortical function*

The hematologic data and urinary corticosteroid excretion values are summarized in Table I and are presented in graphic form for one animal in Figure 2. Changes in these indices immediately after insulin withdrawal were slight. However, as insulin deprivation continued, the eosinophil count tended to rise somewhat.

Terminally, when the animals were prostrate, vomiting, and rapidly approaching death, all the indices pointed to an increase in the level of adrenal cortical function. Both eosinophil and lymphocyte counts fell, and the neutrophil count rose abruptly. The resultant of these changes was a pronounced increase in the total white cell count. It seems probable that the eosinopenia and lymphopenia and the leukemoid reaction (26, 27) sometimes reported in diabetic coma patients not suffering from infection are analogous phenomena, ascribable to the alarm reaction. The rate of corticosteroid excretion underwent a terminal increase in two of the three animals and declined just prior

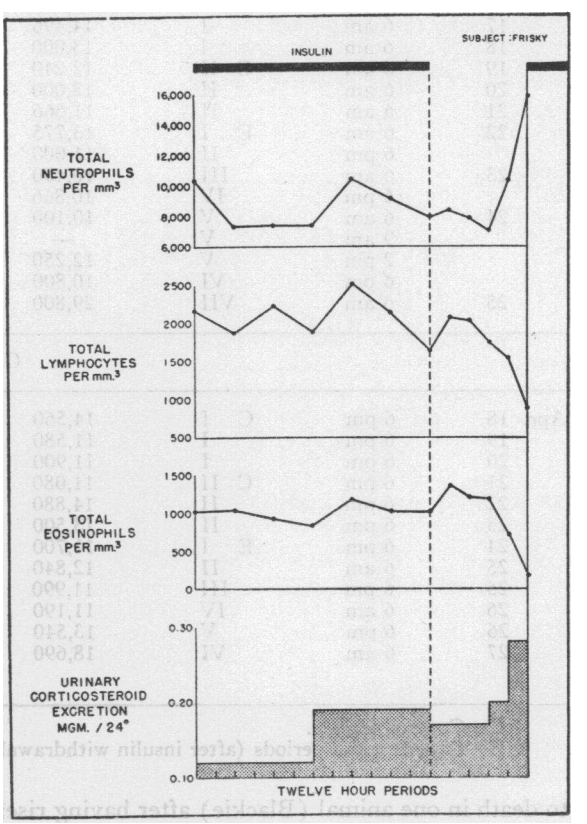

FIG. 2. CHANGES IN THE NUMBER OF CIRCULATING NEUTROPHILS, LYMPHOCYTES AND EOSINOPHILS AND IN THE RATE OF URINARY CORTICOSTEROID EXCRETION BEFORE AND AFTER WITHDRAWAL OF INSULIN FROM A DEPANCREATIZED DOG

[4] In one animal (Brownie) hemoglobinemia and hemoglobinuria were observed before treatment had been started; in the other animal (Frisky), they appeared shortly after the institution of insulin and saline therapy and continued for 24 hours. Methemalbumin was demonstrated spectroscopically in the serum of both animals, indicating that the site of hemolysis was, in all probability, intravascular. Similar hemolytic crises were reported by Hedon to have occurred in his dog during two acidotic episodes; they have also been observed by Long (28) in diabetic cats permitted to become ketotic. Although frank hemolysis has never, to our knowledge, been observed in human beings suffering from diabetic acidosis, Guest (29) has found the osmotic fragility of the erythrocytes of such patients to be increased.

TABLE I

Changes in hematologic and chemical indices of adrenal cortical function before and after the withdrawal of insulin from depancreatized dogs

Date	Time	Period*	Blood				Urinary corticosteroids	Clinical notes
			Total WBC	Neutrophils	Lymphocytes	Eosinophils		
			per mm.³				mg./24 hrs.	
A. Brownie								
Nov. 12	6 am	C I				844		
	6 pm	II				625	0.10	
13	6 am	III				475		
	6 pm	IV				862		
14	6 am	V				638	0.17	
	6 pm	E I				294		
15	6 am	II				694	0.03	Insulin omitted
	6 pm	III				762		
16	6 am	IV				956	0.09	
	6 pm	V				406		
17	6 am	VI				37	0.32	
	6 pm	VII				12		Death
B. Blackie								
Mar. 16	6 am	C I	12,600			981		
17	6 am	I	14,496			1,231	0.14	
18	6 am	I	13,000			950		
19	6 am	C II	12,240			1,075		
20	6 am	II	12,000			1,250	0.13	
21	6 am	II	11,666			1,150		
22	6 am	E I	13,775			1,050		
	6 pm	II	11,000			1,350	0.20	Insulin omitted
23	6 am	III	10,450			1,725		
	6 pm	IV	10,866			1,659	0.33	
24	6 am	V	10,100			1,741		
	9 am	V	—			2,428	0.26	
	2 pm	V	12,250			1,891		
	6 pm	VI	10,800			1,564	0.16	
25	6 am	VII	29,800			388		Death
C. Frisky								
Apr. 18	6 pm	C I	14,560	10,367	2,155	1,033		
19	6 pm	I	11,580	7,341	1,853	1,047	0.12	
20	6 pm	I	11,900	7,449	2,213	928		
21	6 pm	C II	11,080	7,424	1,884	843		
22	6 pm	II	14,880	10,327	2,500	1,175	0.19	
23	6 pm	II	13,500	8,999	2,133	1,027		
24	6 pm	E I	11,700	7,839	1,638	1,012		
25	6 am	II	12,840	8,346	2,054	1,360	0.17	Insulin omitted
25	6 pm	III	11,990	7,805	2,026	1,215		
26	6 am	IV	11,190	7,195	1,746	1,190	0.20	
26	6 pm	V	13,540	10,399	1,516	712	0.28	
27	6 am	VI	18,690	15,887	897	197		Treatment begun

* C—Control periods.
E—Experimental periods (after insulin withdrawal).

to death in one animal (Blackie) after having risen previously. In no case did the corticosteroid excretion rate increase to the extent previously observed (30) in human beings. In the earlier study it was found that the rate of corticosteroid excretion by patients suffering from diabetic acidosis of moderate severity was two to eight times as rapid as that following recovery; in this study the rate during acidosis was, at most, slightly more than twice as rapid as the control rate. At least

two factors appear to be responsible for the discrepancy: (*1*) The great rapidity with which the animals declined into ketoacidosis, denying to the dogs' adrenals sufficient time to achieve maximal activity prior to death; and (*2*) the renal impairment manifested by all the animals in the terminal phases of ketoacidosis. As Marks and Leaf (31) have shown, experimental reduction in the glomerular filtration rate of the dog is associated with a pronounced reduction in renal clearance of corticosteroids.

(c) *General metabolic observations*

(1) *Blood, plasma, and serum constituents.* The changes in the blood, plasma and serum constituents which occurred in the three animals during the evolution of ketoacidosis are summarized in Table II. Additional data concerning changes in plasma CO_2 content, blood ketones and serum lipoids were secured in the experiments concerned with changes in insulin sensitivity and are shown in Table VIII.

The blood changes bear a qualitative, but a not always quantitative, resemblance to those classically established in human subjects. The blood sugar increased to a level between 200 and 300 mg. per cent immediately after insulin withdrawal and remained in this range until the condition of the animal became critical, when again it rose abruptly. The serum CO_2 content and pH declined as vomiting began. However, the depression was not comparable to that observed in human diabetic acidosis of equivalent clinical severity. An increase in the level of circulating lipoids preceded the rise in blood ketones; the ketone levels finally attained were roughly comparable to those found clinically in diabetic coma. It should be noted that there is a considerable difference between the range of ketone values obtained by the Behre and Nanavutty methods, particularly with respect to urinary ketone values. The Behre method yielded results in good agreement with those obtained by investigators (32) using the method of Van Slyke (33) and by Dixon, Comfort, Lichtman, and Benson (34) using the method of Greenberg and Lester (35). However, these values are only about half as high as those obtained by the Nanavutty method. No reason for the discrepancy is apparent; it appears to reflect the unsatisfactory state of ketone methodology.

(2) *Metabolic balances.* The metabolic balance data are presented in detail in Table III and in summary form in Table IV. All of the balances (except that of sodium) were similar to those reported to occur after the withdrawal of insulin from human subjects.

The balances of nitrogen, potassium, phosphorus, and magnesium became negative as soon as insulin was withdrawn. The negativity of the nitrogen, phosphorus, and potassium balances underwent a further increase terminally, in association with evidences of increased adrenal activity. The rate of loss of magnesium, on the other hand, remained much the same throughout the experiment. In Figure 3–A, B, C the balances of nitrogen, potassium, and phosphorus are represented graphically. The ordinate scales of the figures are so correlated that all of the columns coincide when these substances are lost or retained in the proportions in which they exist in protoplasm, 2.78 mEq. of potassium and 63 mg. of phosphorus being

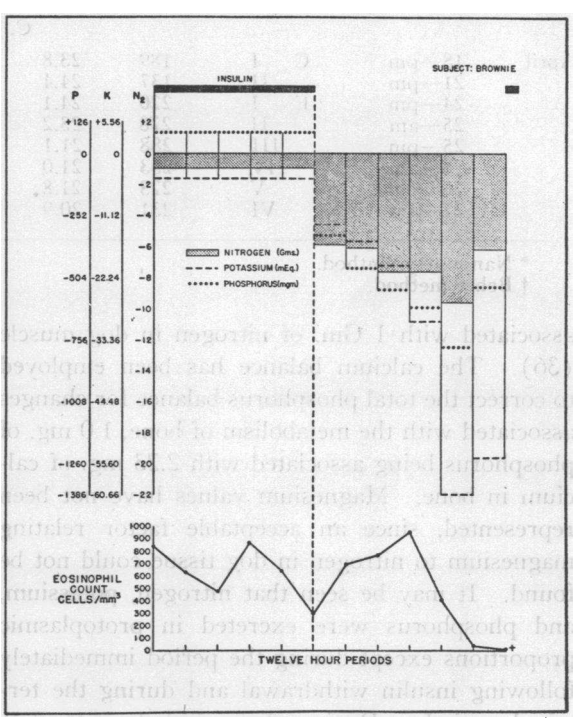

FIG. 3–A. METABOLIC BALANCES OF NITROGEN, POTASSIUM, AND PHOSPHORUS AS RELATED TO THE EOSINOPHIL COUNT BEFORE AND AFTER THE WITHDRAWAL OF INSULIN FROM A DEPANCREATIZED DOG

TABLE II

Blood, plasma and serum values before and after the withdrawal of insulin from depancreatized dogs

Date and time	Beginning of period	Sugar	CO_2 content	pH	Hgb.	Hct.	Total protein	NPN	Creatinine	Total ketones
		mg. %	*mEq./L.*		*Gm. %*	*%*	*Gm. %*	*mg. %*	*mg. %*	*mM/L.*
				A. Brownie						
November 12—am	C I	53	27.1	7.40	15.7	48	6.8	72	—	0*
13—am	III	54	26.2	7.38	14.2	48	6.5	59	—	0
14—am	V	217	24.5	7.42	13.3	45	6.7	74	—	0
14—pm	E I	303	26.4	7.44	14.4	45	6.8	45	—	0
15—am	II	286	25.0	7.40	13.3	45	6.9	70	—	0
15—pm	III	274	22.0	7.39	13.7	45	6.7	83	—	4.0
16—am	IV	247	19.7	7.40	13.5	45	6.7	96	—	8.8
16—pm	V	258	16.7	7.35	14.3	47	6.1	98	—	11.6
17—am	VI	320	12.3	7.32	13.3	44	5.9	109	—	18.0
17—pm	VII	388	12.9	7.27	14.6	47	6.7	141	—	24.8
18—am	(Post mort.)	513	—	—	—	—	—	149	—	26.4
				B. Blackie						
March 16—am	C I	202	25.4	7.45	16.5	52	6.8	44	0.42	0.6†
19—am	II	238	26.0	7.47	16.3	51	6.7	39	0.48	0.3
22—am	E I	189	25.5	7.47	15.5	49	6.6	38	0.50	0.3
22—pm	II	238	22.7	7.47	15.7	49	6.4	44	0.48	0.3
23—am	III	288	22.2	7.48	14.9	45	6.4	40	0.60	0.6
23—pm	IV	310	24.1	7.40	14.4	43	5.8	44	0.52	4.2
24—am	V	282	21.7	7.48	13.5	41	5.7	34	0.47	3.8
24—pm	VI	263	20.9	7.45	13.7	—	5.7	34	0.49	6.0
25—am	VII	364	11.4	7.40	15.2	51	6.0	56	0.78	7.8
25—am	(Post mort.)	392	5.5	7.18	16.5	46	6.5	94	1.56	52.1
				C. Frisky						
April 18—pm	C I	189	23.8	7.43	17.0	51	7.0	42	0.58	0.2†
21—pm	II	137	24.4	7.43	16.5	52	6.7	35	0.63	0.6
24—pm	E I	220	24.1	7.43	16.3	50	6.7	29	0.63	0.5
25—am	II	278	23.2	7.43	16.1	49	5.9	32	0.56	1.9
25—pm	III	288	21.1	7.40	16.3	48	5.7	31	0.55	2.5
26—am	IV	253	21.0	7.41	15.7	44	5.1	29	0.53	2.8
26—pm	V	223	21.8	7.39	14.9	44	4.8	27	0.63	3.1
27—am	VI	231	20.9	7.38	15.5	47	4.7	36	0.69	4.3

* Nanavutty method.
† Behre method.

associated with 1 Gm. of nitrogen in dog muscle (36). The calcium balance has been employed to correct the total phosphorus balance for changes associated with the metabolism of bone, 1.0 mg. of phosphorus being associated with 2.23 mg. of calcium in bone. Magnesium values have not been represented, since an acceptable factor relating magnesium to nitrogen in dog tissue could not be found. It may be seen that nitrogen, potassium, and phosphorus were excreted in protoplasmic proportions except during the period immediately following insulin withdrawal and during the terminal periods. During these periods potassium was excreted in excess of nitrogen.

The initial loss of excess potassium, which occurred in association with a transient intensification of the glycosuria, seems logically attributable to the breakdown of hepatic glycogen. In Brownie, an animal whose liver glycogen stores were presumably reduced as a consequence of dietary inadequacies and steatorrhea, both glucose and potassium were excreted in comparatively small amounts upon the withdrawal of insulin.

The terminal loss of potassium, presumably from the cells of the body as a whole, occurred in association with evidences of adrenal activation. The temporal relation between the two events was an intimate one. Eosinopenia appeared during a different experimental period in each of the dogs and was accompanied in each instance by a loss of excess potassium.

The dogs appeared to differ materially from hu-

TABLE II—*Continued*

Blood, plasma and serum values before and after the withdrawal of insulin from depancreatized dogs

Beta hydroxy-butyric acid	Aceto-acetic acid	Na	Cl	K	Ca	P	Mg	Eosinophil count	Clinical notes
mM/L.		*mEq./L.*	*mEq./L.*	*mEq./L.*	*mg. %*	*mg. %*	*mEq./L.*	*cells/mm.*[3]	
				A. Brownie					
—	—	154	109	4.7	8.6	4.9	1.7	844	
—	—	150	108	4.4	9.1	5.1	1.7	475	
—	—	147	107	4.5	9.1	4.3	1.7	638	
—	—	144	99	4.7	8.8	4.5	1.5	294	Insulin omitted
—	—	145	102	4.7	8.8	4.5	1.6	694	
—	—	145	105	5.0	9.3	4.5	1.3	762	
—	—	147	99	5.3	8.8	6.2	1.1	956	Vomiting begins
—	—	143	97	5.1	8.6	4.5	1.3	406	
—	—	133	86	5.3	7.2	5.7	1.9	37	
—	—	129	79	4.1	5.6	8.7	2.4	12	Treatment instituted
—	—	109	65	26.3	6.8	32.8	4.7	—	Death
				B. Blackie					
0.5	0.1	141	99	5.8	8.8	3.2	1.5	981	
0.2	0.1	141	101	5.6	8.6	3.1	1.4	1075	
0.2	0.1	143	101	5.5	9.1	3.2	1.5	1050	Insulin omitted
0.2	0.1	141	103	5.1	8.6	4.2	1.5	1350	
0.4	0.2	140	100	5.5	8.6	3.9	1.4	1725	
3.4	0.8	141	104	6.0	8.2	4.0	1.0	1659	
2.2	1.6	141	104	4.9	8.1	4.9	0.9	1741	Vomiting begins
3.8	2.2	141	103	4.4	7.2	4.6	0.9	1564	
5.2	2.6	131	88	5.9	4.1	8.3	2.3	388	Treatment instituted
45.5	6.6	126	88	8.2	3.2	16.7	2.9	47	Death
				C. Frisky					
0.2	0	139	105	5.4	8.9	4.5	1.7	1033	
0.3	0.3	145	107	5.1	9.1	4.5	1.7	843	
0.4	0.1	142	105	4.8	9.6	3.4	1.7	1012	Insulin omitted
1.5	0.4	143	105	4.8	9.4	3.8	1.5	1360	
1.6	0.9	138	102	4.5	9.6	4.0	1.5	1215	
1.6	1.2	138	103	5.0	8.0	3.2	0.9	1190	Vomiting begins
1.4	1.7	136	97	3.9	7.8	3.6	2.8	712	
1.8	2.5	134	89	4.2	7.1	4.2	2.0	197	Treatment instituted

man beings in their capacity to conserve sodium. Despite mounting ketosis, two of the three [5] animals were able to effect a marked reduction in urinary sodium and chloride excretion. Herein would appear to lie the explanation of the comparative mildness of canine acidosis. To what extent the dog's capacity to spare base depends upon the rapidity with which it can achieve maximal ammonia production (37) and to what extent it depends upon other factors cannot be stated at present.

Calcium balances became negative late in the experiment in the two animals (Frisky and Blackie) whose calcium intake was sufficiently generous to allow this phenomenon to become apparent.

(3) *Renal clearance studies.* Measurement of blood creatinine levels and of urinary creatinine excretion were made in two of the animals studied. This permitted calculation of the endogenous creatinine clearance, which in the dog can be regarded as a valid index of the glomerular filtration rate. Electrolyte clearances could then be derived and certain inferences regarding tubular, as well as glomerular, function made.

In Tables V–A and B, the renal clearances of creatinine, sodium and potassium are presented together with the eosinophil count at the beginning of each metabolic period. In Frisky, an animal deprived of insulin for only 60 hours, the glomerular

[5] Brownie, who manifested an increasing rate of sodium loss as the rate of ketone excretion increased, had an elevated level of blood non-protein nitrogen even during the control periods which was unexplained; she may have had impairment of the renal ammonia-producing mechanism as well.

TABLE III

Metabolic balance data obtained before and after the withdrawal of insulin from depancreatized dogs

Date	Period	Insulin dosage	Body weight	INTAKE: Food N_2	Na	Cl	K	Ca	Mg	P
		units	*Kg.*	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*
November			A. Brownie							
12 am–pm	C I	—	18.77	—	—	—	—	—	—	—
12 pm–13 am	II	P27, R48	—	22.8	80.8	86.5	65.0	9.2	21.6	2200
13 am–13 pm	III	—	18.07	—	—	—	—	—	—	—
13 pm–14 am	IV	P26, R40	—	22.8	80.8	86.5	65.0	9.2	21.6	2200
14 am–14 pm	V	—	18.32	—	—	—	—	—	—	—
14 pm–15 am	E I	—	17.78	—	—	—	—	—	—	—
15 am–15 pm	II	—	17.14	—	—	—	—	—	—	—
15 pm–16 am	III	—	17.19	—	—	—	—	—	—	—
16 am–16 pm	IV	—	16.83	—	—	—	—	—	—	—
16 pm–17 am	V	—	16.76	—	—	—	—	—	—	—
17 am–17 pm	VI	—	16.70	—	—	—	—	—	—	—
17 pm	Treatment begun		16.64							
March			B. Blackie							
16–19	C I	R61	13.12	64.2	421.8	407.4	198.0	178.1	53.8	4737
19–22	II	R61	13.35	64.2	421.8	407.4	198.0	178.1	53.8	4737
22 am–22 pm	E I	—	13.38	10.7	70.3	67.9	33.0	29.7	9.0	789
22 pm–23 am	II	—	13.30	10.7	70.3	67.9	33.0	29.7	9.0	789
23 am–23 pm	III	—	12.95	10.7	70.3	67.9	33.0	29.7	9.0	789
23 pm–24 am	IV	—	13.04	7.7	50.6	48.9	23.7	21.4	6.5	568
24 am–24 pm	V	—	12.84	1.2	7.8	7.5	3.7	3.3	1.0	87
24 pm–25 am	VI	—	12.60	0.4	2.9	2.8	1.4	1.2	0.4	33
25 am	Treatment begun		12.47							
April			C. Frisky							
18–21	C I	R40	17.24	60.8	341.3	392.8	135.4	95.3	41.9	3918
21–24	II	R35	17.24	60.8	341.3	392.8	135.4	95.3	41.9	3918
24 pm–25 am	E I	—	17.25	10.1	56.9	65.5	22.6	15.9	7.0	653
25 am–25 pm	II	—	17.15	10.1	56.9	65.5	22.6	15.9	7.0	653
25 pm–26 am	III	—	17.28	7.8	43.7	50.3	17.3	12.2	5.4	503
26 am–26 pm	IV	—	17.16	0.4	2.2	2.5	0.9	0.6	0.3	25
26 pm–27 am	V	—	16.92	4.9	27.7	31.9	11.0	7.7	3.4	319
27 am	Treatment begun		16.73							

‡ Weight obtained by extrapolation.

TABLE III—*Continued*

Metabolic balance data obtained before and after the withdrawal of insulin from depancreatized dogs

Date	Period	OUTPUT: Urine Vol.	N_2	Na	Cl	K	Ca	Mg	P	Glucose	Creatinine	Total ketones	Beta hydroxybutyric acid	Acetoacetic acid
		cc.	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*	*Gm.*	*mg.*		*mM*	
November					A. Brownie									
12 am–pm	C I	155	5.0	26.8	20.8	18.0	0.0	0.1	439	0.8	—	0*	—	—
12 pm–13 am	II	750	18.5	35.0	42.0	61.7	0.2	7.2	1822	22.5	—	0	—	—
13 am–13 pm	III	135	4.4	21.3	19.7	18.1	0.0	2.0	382	1.3	—	0	—	—
13 pm–14 am	IV	1210	17.8	45.2	43.2	66.0	0.0	7.4	1705	60.5	—	0	—	—
14 am–14 pm	V	220	4.9	20.9	33.4	11.0	0.3	0.6	226	7.7	—	0	—	—
14 pm–15 am	E I	195	3.6	2.3	6.7	11.0	0.1	3.0	287	19.5	—	0	—	—
15 am–15 pm	II	195	3.8	3.3	3.5	14.2	0.1	2.2	412	17.6	—	1.7	—	—
15 pm–16 am	III	280	5.9	1.8	4.7	18.4	0.1	3.0	529	19.6	—	15.5	—	—
16 am–16 pm	IV	320	5.9	8.2	4.0	29.9	0.2	2.9	605	19.2	—	43.1	—	—
16 pm–17 am	V	910†	7.5	28.2	31.5	59.6	1.1	2.0	766	31.8	—	79.3	—	—
17 am–17 pm	VI	1450†	4.5	31.9	51.5	54.1	1.1	2.1	527	27.5	—	53.5	—	—
17 pm	Treatment begun													
March					B. Blackie									
16–19	C I	3125	60.0	345.0	380.0	179.2	2.9	4.9	2710	52.5	1295	1.4‡	0.9	0.5
19–22	II	2555	55.9	315.0	356.0	168.8	2.6	4.0	2320	23.6	1266	1.5	1.1	0.4
22 am–22 pm	E I	1160	10.8	80.8	81.4	49.4	0.3	0.2	606	47.4	281	0.5	0.3	0.2
22 pm–23 am	II	1456	13.9	76.7	78.8	47.3	0.4	0.3	661	81.8	327	1.0	0.7	0.3
23 am–23 pm	III	1420	11.9	33.3	49.2	39.9	0.3	1.2	632	86.0	263	3.7	2.4	1.3
23 pm–24 am	IV	885	10.1	29.7	34.9	31.1	0.2	0.8	319	59.8	258	5.7	3.1	2.6
24 am–24 pm	V	300	5.1	9.3	5.2	15.5	0.2	0.3	382	14.5	196	4.8	2.9	1.9
24 pm–25 am	VI	410	5.1	5.1	5.8	29.8	0.5	0.2	426	20.2	207	5.6	3.4	2.2
25 am	Treatment begun													
April					C. Frisky									
18–21	C I	1585	42.7	287.0	306.0	137.0	3.9	14.0	2412	16.6	1476	1.1‡	0.8	0.3
21–24	II	1975	55.6	357.0	380.0	150.5	5.6	18.5	3288	29.2	1984	1.4	1.1	0.3
24 pm–25 am	E I	965	10.4	53.4	58.2	44.3	1.3	2.7	736	58.2	359	0.4	0.2	0.2
25 am–25 pm	II	1410	11.3	56.2	55.0	24.1	1.7	2.8	629	84.6	342	2.7	1.2	1.5
25 pm–26 am	III	810	9.5	5.0	8.9	22.3	1.2	2.8	603	48.6	330	5.1	1.5	3.6
26 am–26 pm	IV	870	7.3	11.7	8.7	27.8	0.9	2.5	540	34.8	283	10.0	4.3	5.7
26 pm–27 am	V	800	8.2	8.6	0.8	43.6	0.9	2.0	660	43.0	340	10.3	3.7	6.6
27 am	Treatment begun													

* Nanavutty method. † Urine mixed with vomitus. ‡ Behre method.

TABLE III—*Continued*

Metabolic balance data obtained before and after the withdrawal of insulin from depancreatized dogs

Date	Period	OUTPUT: Feces: Dry weight	Feces: N_2	Feces: Na	Feces: Cl	Feces: K	Feces: Ca	Feces: Mg	Feces: P	Vomitus: Vol.	Vomitus: N_2	Vomitus: Na	Vomitus: Cl	Vomitus: K	Vomitus: Ca	Vomitus: Mg	Vomitus: P
		Gm.	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg.*	*cc.*	*Gm.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	*mg*
November							A. Brownie										
12 am–pm	C I	—								—	—	—	—	—	—	—	—
12 pm–13 am	II	—								—	—	—	—	—	—	—	—
13 am–13 pm	III	—								—	—	—	—	—	—	—	—
13 pm–14 am	IV	—	13.5	39.6	3.9	9.2	49.8	29.4	780	—	—	—	—	—	—	—	—
14 am–14 pm	V	—								—	—	—	—	—	—	—	—
14 pm–15 am	E I	—								—	—	—	—	—	—	—	—
15 am–15 pm	II	—								—	—	—	—	—	—	—	—
15 pm–16 am	III	—								—	—	—	—	—	—	—	—
16 am–16 pm	IV	—	6.9	16.3	1.6	3.1	24.2	16.8	320	—	—	—	—	—	—	—	—
16 pm–17 am	V	—								—	—	—	—	—	—	—	—
17 am–17 pm	VI	—								37	0.4	1.6	3.7	0.5	0.9	0.3	9
17 pm	Treatment begun																
March							B. Blackie										
16–19	C I	169.4	13.9	28.9	5.3	6.1	242.0	47.1	2910	—	—	—	—	—	—	—	—
19–22	II									—	—	—	—	—	—	—	—
22 am–22 pm	E I									—	—	—	—	—	—	—	—
22 pm–23 am	II									—	—	—	—	—	—	—	—
23 am–23 pm	III	49.5	5.5	3.1	1.3	2.5	67.8	19.8	870	—	—	—	—	—	—	—	—
23 pm–24 am	IV									—	—	—	—	—	—	—	—
24 am–24 pm	V									tr	0.2	0.7	9.1	1.3	1.2	0.6	45
24 pm–25 am	VI									665	1.0	17.5	32.8	3.3	1.6	4.5	167
25 am	Treatment begun																
April							C. Frisky										
18–21	C I	154.0	13.0	12.7	3.1	2.1	82.8	49.7	1703	—	—	—	—	—	—	—	—
21–24	II									—	—	—	—	—	—	—	—
24 pm–25 am	E I									—	—	—	—	—	—	—	—
25 am–25 pm	II									—	—	—	—	—	—	—	—
25 pm–26 am	III	16.1	4.2	3.5	0.7	1.0	19.0	15.3	440	—	—	—	—	—	—	—	—
26 am–26 pm	IV									450	0.4	10.8	28.3	3.0	1.2	1.1	24
26 pm–27 am	V									1410	3.2	19.3	57.2	11.2	7.1	2.6	130
27 am	Treatment begun																

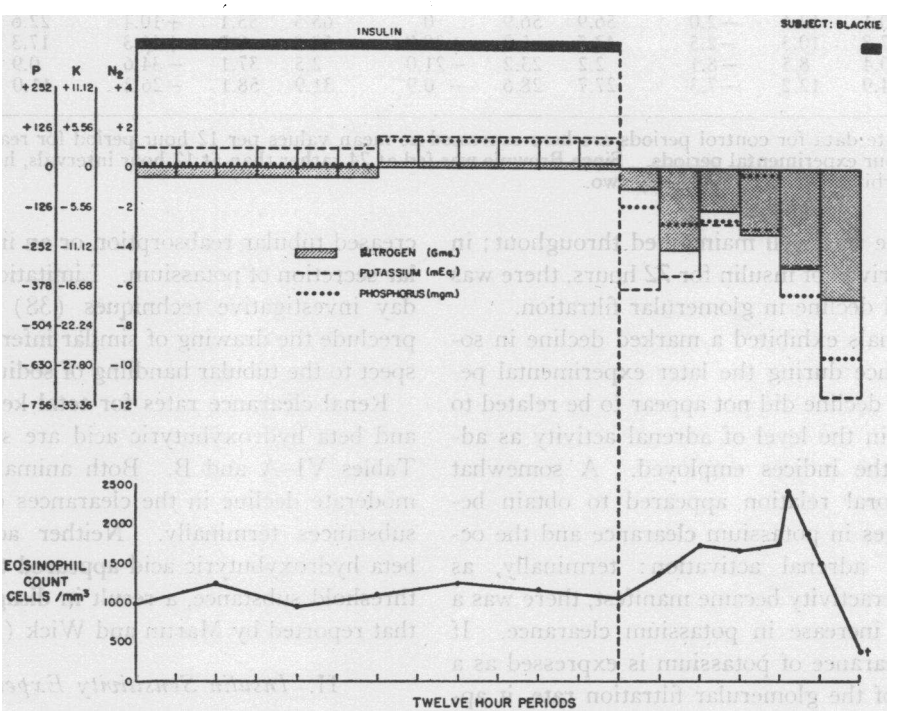

FIG. 3-B. METABOLIC BALANCES OF NITROGEN, POTASSIUM, AND PHOSPHORUS AS RELATED TO THE EOSINOPHIL COUNT BEFORE AND AFTER THE WITHDRAWAL OF INSULIN FROM A DEPANCREATIZED DOG

TABLE IV

Summary of balance data presented in Table III *

Period	Nitrogen (*Gm.*)			Sodium (*mEq.*)			Chloride (*mEq.*)			Potassium (*mEq.*)		
	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance
A. Brownie												
C I	11.4	12.3	−0.9	40.4	36.4	+ 4.0	43.2	32.5	+10.7	32.5	36.5	− 4.0
II	11.4	12.3	−0.9	40.4	36.4	+ 4.0	43.2	32.5	+10.7	32.5	36.5	− 4.0
III	11.4	12.3	−0.9	40.4	36.4	+ 4.0	43.2	32.5	+10.7	32.5	36.5	− 4.0
IV	11.4	12.3	−0.9	40.4	36.4	+ 4.0	43.2	32.5	+10.7	32.5	36.5	− 4.0
V	11.4	12.3	−0.9	40.4	36.4	+ 4.0	43.2	32.5	+10.7	32.5	36.5	− 4.0
E I	0	5.9	−5.9	0	8.9	− 8.9	0	7.4	− 7.4	0	12.5	−12.5
II	0	6.1	−6.1	0	9.9	− 9.9	0	4.2	− 4.2	0	15.7	−15.7
III	0	7.6	−7.6	0	5.9	− 5.9	0	5.1	− 5.1	0	19.2	−19.2
IV	0	7.6	−7.6	0	12.3	−12.3	0	4.4	− 4.4	0	30.7	−30.7
V	0	9.6	−9.6	0	33.9	−33.9	0	35.6	−35.6	0	60.9	−60.9
VI	0	6.2	−6.2	0	36.0	−36.0	0	51.9	−51.9	0	54.9	−54.9
B. Blackie												
C I	10.7	11.2	−0.5	70.3	59.9	+10.4	67.9	63.7	+ 4.2	33.0	30.4	+ 2.6
II	10.7	10.5	+0.2	70.3	54.9	+15.4	67.9	59.7	+ 8.2	33.0	28.6	+ 4.4
E I	10.7	11.8	−1.0	70.3	81.3	−11.0	67.9	81.6	−13.7	33.0	49.8	−16.8
II	10.7	14.8	−4.1	70.3	77.2	− 6.9	67.9	79.0	−11.1	33.0	47.7	−14.7
III	10.7	12.8	−2.1	70.3	33.8	+36.5	67.9	49.4	+18.5	33.0	40.3	− 7.3
IV	7.7	11.0	−3.3	50.6	30.2	+20.4	48.9	35.1	+13.8	23.7	31.5	− 7.8
V	1.2	6.2	−5.0	7.8	10.5	− 2.7	7.5	14.5	− 7.0	3.7	17.2	−13.5
VI	0.4	7.0	−6.6	2.9	23.1	−20.2	2.8	38.8	−36.0	1.4	33.5	−32.1
C. Frisky												
C I	10.1	8.2	+1.9	56.9	48.9	+ 8.0	65.5	51.3	+14.2	22.6	23.0	− 0.4
II	10.1	10.4	−0.3	56.9	60.6	− 3.7	65.5	63.6	+ 1.9	22.6	25.3	− 2.7
E I	10.1	11.2	−1.1	56.9	54.1	+ 2.8	65.5	58.3	+ 7.2	22.6	44.5	−21.9
II	10.1	12.1	−2.0	56.9	56.9	0	65.5	55.1	+10.4	22.6	24.3	− 1.7
III	7.8	10.3	−2.5	43.7	5.7	+38.0	50.3	9.0	+41.3	17.3	22.5	− 5.2
IV	0.4	8.5	−8.1	2.2	23.2	−21.0	2.5	37.1	−34.6	0.9	31.0	−30.1
V	4.9	12.2	−7.3	27.7	28.6	− 0.9	31.9	58.1	−26.2	11.0	55.0	−44.0

* Aggregate data for control periods are here expressed as mean values per 12 hour period for readier comparison with the 12-hour experimental periods. Since Brownie was fed at 24 rather than at 12-hour intervals, her control intake values have arbitrarily been divided by two.

filtration rate was well maintained throughout; in Blackie, deprived of insulin for 72 hours, there was a pre-mortal decline in glomerular filtration.

Both animals exhibited a marked decline in sodium clearance during the later experimental periods. This decline did not appear to be related to an increase in the level of adrenal activity as adjudged by the indices employed. A somewhat closer temporal relation appeared to obtain between changes in potassium clearance and the occurrence of adrenal activation: terminally, as adrenal hyperactivity became manifest, there was a pronounced increase in potassium clearance. If the renal clearance of potassium is expressed as a percentage of the glomerular filtration rate, it appears that at the time eosinopenia began, some influence came into play which resulted in a decreased tubular reabsorption or an increased tubular secretion of potassium. Limitations in present-day investigative techniques (38) unfortunately preclude the drawing of similar inferences with respect to the tubular handling of sodium.

Renal clearance rates for total ketones, acetone and beta hydroxybutyric acid are summarized in Tables VI–A and B. Both animals exhibited a moderate decline in the clearances of all of these substances terminally. Neither acetoacetic nor beta hydroxybutyric acid appeared to behave as a threshold substance, a result in disagreement with that reported by Martin and Wick (39).

II. *Insulin Sensitivity Experiments*

The results of nine tests of insulin sensitivity in control experiments upon the same animal em-

TABLE IV—*Continued*

Summary of balance data presented in Table III

Calcium (mEq.)			Magnesium (mEq.)			Phosphorus (mg.)				
Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Theoretical P balance based on calcium	P balance minus P based on calcium
A. Brownie										
4.6	8.4	− 3.8	10.8	8.3	+2.5	1100	1045	+ 55	− 34	+ 89
4.6	8.4	− 3.8	10.8	8.3	+2.5	1100	1045	+ 55	− 34	+ 89
4.6	8.4	− 3.8	10.8	8.3	+2.5	1100	1045	+ 55	− 34	+ 89
4.6	8.4	− 3.8	10.8	8.3	+2.5	1100	1045	+ 55	− 34	+ 89
4.6	8.4	− 3.8	10.8	8.3	+2.5	1100	1045	+ 55	− 34	+ 89
0	8.4	− 8.4	0	7.9	−7.9	0	417	−417	− 75	−342
0	8.4	− 8.4	0	7.1	−7.1	0	542	−542	− 75	−467
0	6.1	− 6.1	0	7.2	−7.2	0	609	−609	− 55	−554
0	6.3	− 6.3	0	7.1	−7.1	0	685	−685	− 57	−628
0	8.1	− 8.1	0	6.5	−6.5	0	855	−855	− 73	−782
0	7.2	− 7.2	0	6.3	−6.3	0	607	−607	− 65	−542
B. Blackie										
29.7	20.7	+ 9.0	9.0	4.7	+4.3	789	695	+ 94	+ 81	+ 13
29.7	20.6	+ 9.1	9.0	4.6	+4.4	789	630	+159	+ 82	+ 77
29.7	11.6	+18.1	9.0	3.5	+5.5	789	751	+ 38	+162	−124
29.7	11.7	+18.0	9.0	3.6	+5.4	789	806	− 17	+161	−178
29.7	11.6	+18.1	9.0	4.5	+4.5	789	777	+ 12	+162	−150
21.4	11.5	+ 9.9	6.5	4.1	+2.4	568	464	+104	+ 89	− 15
3.3	12.7	− 9.4	1.0	4.2	−3.2	87	572	−485	− 84	−401
1.2	13.4	−12.2	0.4	8.0	−7.6	33	738	−705	−109	−596
C. Frisky										
15.9	7.6	+ 8.3	7.0	6.4	+0.6	653	544	+109	+ 74	+ 35
15.9	7.8	+ 8.1	7.0	7.2	−0.2	653	690	− 37	+ 73	−110
15.9	5.1	+10.8	7.0	5.8	+1.2	653	821	−171	+ 97	−268
15.9	5.5	+10.4	7.0	5.9	+1.1	653	717	− 64	+ 93	−157
12.2	5.0	+ 7.2	5.4	5.9	−0.5	503	691	−188	+ 65	−253
0.6	5.9	− 5.3	0.3	6.7	−6.4	25	652	−627	− 48	−579
7.7	11.8	− 4.1	3.4	7.7	−4.3	319	878	−559	− 37	−522

ployed for the subsequent insulin deprivation experiments are summarized in Table VII. The tests were performed at intervals over a period of six months. The absolute fall in blood sugar tends to increase with increasing initial values, and the fall expressed as a percentage of the initial value also increases with increasing initial values. These findings accord well with those reported by Radoslav (40), Hemmingsen and Marks (41) and Klatskin (42). Inasmuch as the fasting blood sugars of all but one of the insulin deprivation experiments (that continued for 60 hours) fall within the range of blood sugars obtained in the initial eight control experiments, the mean and standard deviation of the index of insulin sensitivity in these eight have been computed, excluding the ninth experiment.

The results of a series of five experiments on the same animal at the conclusion of progressively longer periods of insulin deprivation are summarized in Table VIII. The observations were not extended beyond deprivation periods of 60 hours since by that time the condition of the animal had become critical and it was feared that further prolongation would result in its death.

No alteration in insulin sensitivity could be detected until deprivation had been continued for 36 hours, but thereafter sensitivity abruptly declined. There was a close temporal relation between the occurrence of eosinopenia and of insulin insensitivity. It seems probable that the sharp rise in blood sugar which occurred between the 48th and 60th hours after insulin withdrawal enhanced insulin sensitivity appreciably. Therefore, the slight further decline in sensitivity which was observed after 60 hours of deprivation in all likelihood represents but a portion of the true decline. Correction for the increased level of blood sugar by ex-

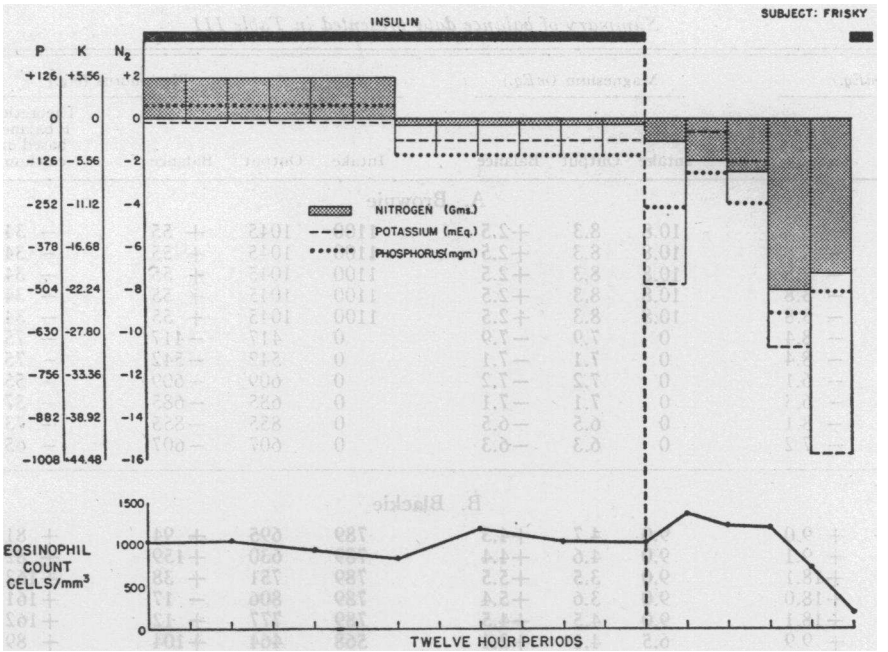

FIG. 3–C. METABOLIC BALANCES OF NITROGEN, POTASSIUM, AND PHOSPHORUS AS RELATED TO THE EOSINOPHIL COUNT BEFORE AND AFTER THE WITHDRAWAL OF INSULIN FROM A DEPANCREATIZED DOG

TABLE V

Renal clearances of creatinine, sodium and potassium as related to the eosinophil count before and after the withdrawal of insulin from depancreatized dogs

Period		Creatinine clearance (GFR)	Sodium clearance	Potassium clearance	$\frac{\text{K clearance} \times 100}{\text{GFR}}$	Eosinophil count at beginning of period
		cc./min.	*cc./min.*	*cc./min.*		*Cells/mm.*³
			A. Blackie			
C	I	67	0.56	7	11	981
	II	60	0.50	7	12	1075
E	I	80	0.77	13	16	1050
	II	81	0.74	12	15	1350
	III	65	0.32	9	15	1725
	IV	72	0.29	8	11	1659
	V	57	0.09	5	8	1741
	VI	45	0.05	8	17	1564
			B. Frisky			
C	I	57	0.46	6	10	1033
	II	73	0.56	7	10	843
E	I	83	0.51	13	15	1012
	II	86	0.54	7	8	1360
	III	85	0.50	6	8	1215
	IV	68	0.12	9	13	1190
	V	72	0.09	15	21	712

TABLE VI

Renal clearances of total ketones, acetoacetic and beta hydroxybutyric acids as related to the creatinine clearance before and after the withdrawal of insulin from depancreatized dogs

Period		Creatinine clearance (GFR)	Total ketone clearance	Acetoacetic acid clearance	Beta hydroxybutyric acid clearance
		cc./min.	*cc./min.*	*cc./min.*	*cc./min.*
		A. Blackie			
C	I	67	0.7	1.6	0.6
	II	60	1.2	1.1	1.3
E	I	80	2.3	2.8	2.1
	II	81	3.5	2.9	3.7
	III	65	2.1	3.4	1.8
	IV	72	2.0	3.0	1.5
	V	57	1.4	1.4	1.3
	VI	45	1.1	1.3	1.0
		B. Frisky			
C	I	57	0.6	0.3	0.9
	II	73	0.6	0.4	0.7
E	I	83	0.4	1.0	0.3
	II	86	1.8	3.4	1.1
	III	85	2.7	4.7	1.3
	IV	68	4.7	5.4	4.0
	V	72	3.9	4.4	3.2

trapolation from the control data is felt to be unwarranted owing to the width of the gap between the highest fasting blood sugar observed during the control studies (246 mg. per cent) and that after 60 hours' insulin deprivation (570 mg. per cent). Despite this difficulty, however, the inference that an additional reduction in insulin sensitivity of uncertain extent occurred terminally seems justified.

DISCUSSION

Of the various components of the metabolic response to the stress of insulin deprivation, a number appear to bear a close temporal relation to the onset of adrenal activation. It is of course recognized that the indices of adrenal cortical activity employed are indirect, and that neither the sensitivity nor the specificity of these indices is fully established. However, within these limitations it would seem that there is a close relation in time between an increase in the level of adrenocortical activity and the following metabolic events: (*1*) An acceleration in the rate of catabolism of protoplasm, manifested by an increased negativity of the nitrogen, potassium, and phosphorus balances; (*2*) a loss of potassium in excess of nitrogen, presumably from the cells of the body as a whole; and (*3*) a decrease in sensitivity to injected insulin. A sudden increase in lipemia and in some, but not all instances, of ketonemia coincided with evidences of increased adrenal activity. However, the levels of both blood lipoids and ketones had begun to rise before eosinopenia became manifest. Any apparent temporal relation between adrenal acti-

TABLE VII

Control observations on the response of the fasting blood sugar of a depancreatized dog (Frisky) to the intravenous injection of 10 units of crystalline insulin*

No. of test	Eosinophil count	Fasting blood sugar	Maximum absolute fall in blood sugar	Maximum per cent fall in blood sugar	Insulin sensitivity 0–90 min.
	no./mm.³	*mg. %*	*mg. %*		*% min.*
1	962	246	168	68	3481
2	819	224	154	64	3369
3	—	206	151	73	3878
4	756	196	123	63	3386
5	837	190	109	54	3089
6	794	179	111	62	2966
7	—	176	98	55	3147
8	900	165	94	53	3165
9	819	103	35	33	1969
Mean of tests 1–8	845	198	—	—	3310
S.D. of tests 1–8	75	27	—	—	287

* 12 hours after the animal's previous feeding and injection of crystalline insulin.

TABLE VIII

The response of the fasting blood sugar of a depancreatized dog (Frisky) to the intravenous injection of 10 units of crystalline insulin together with measurements of the eosinophil count, total leukocyte count, plasma CO_2 content, blood ketone levels, and the serum chylomicron index and lipokrit at the conclusion of progressively longer periods of insulin deprivation

Duration of insulin deprivation (*hrs.*)	0 (Control)	12	24	36	48	60
Fasting blood sugar (*mg. %*)	140	198	229	195	185	570
Insulin sensitivity 0–90 min. (*% min.*)	3,312	3,294	3,294	3,299	1,918	1,838
Eosinophil count:						
Absolute (*no./mm.³*)	666	762	769	850	475	25
Change from initial count of individual experiment (%)	—	−4	+19	−1	−34	−92
Total leukocyte count:						
Absolute (*no./mm.³*)	9,410	8,500	10,750	10,000	7,800	26,240
Change from initial count of individual experiment (%)	—	−11	+26	−10	−26	+355
Plasma CO_2 content (*mEq./L.*)	22.8	18.0	16.8	17.8	14.0	10.2
Blood ketones:						
Total (*mM/L.*)	0.1	0.1	0.2	1.0	1.2	4.9
Acetoacetic acid (*mM/L.*)	0.0	—	0.1	0.3	0.3	2.4
Beta hydroxybutyric acid (*mM/L.*)	0.0	0.1	0.2	0.8	0.8	2.5
Chylomicron index	23	186	31	414	>1000*	>1000*
Lipokrit (*mg. %*)	1,080	1,104	1,568	1,760	2,680	1,804

* Too dense to photograph for accurate counting.

vation and these metabolic events must, therefore, be regarded as equivocal.

In view of the purely descriptive character of these initial studies, detailed discussion of the results will be reserved to the second of the two papers. A brief digression concerning the possible role of acidosis in these studies is, however, required. As Guest, Mackler, and Knowles (43, 44) have pointed out, acidosis apparently favors, if it does not actually induce, a loss of intracellular constituents such as phosphorus and potassium, and a decrease in sensitivity to injected insulin. Notwithstanding, it seems unlikely that acidosis *per se* figured prominently in the terminal losses of phosphorus and potassium noted in the present experiments. Were these losses due primarily to acidosis, phosphorus as well as potassium might have been expected to have been lost in excess of nitrogen. Rapoport and Guest (45) have, moreover, shown that a pH of less than 7.3 is requisite for the decomposition of phosphoric esters and liberation of inorganic phosphorus from red blood cells. Only one of our animals (Brownie) displayed a pH below this level at any time, and that during the final experimental period.

The mildness of the acidosis which developed during these experiments would also seem to militate against its having exerted a significant effect upon insulin sensitivity. Mackler, Lichtenstein, and Guest (46) have observed that moderate insulin resistance appears in dogs when acidosis is induced by the administration of ammonium chloride. However, the level of the serum CO_2 content at which resistance was observed (6.0 mEq. per liter) is considerably below that attained in the present experiments. Moreover, the possibility that adrenal activation, or other factors, may have contributed to Mackler's results was not excluded.

SUMMARY

1. The temporal relation between adrenal hyperactivity and other metabolic phenomena has been investigated in depancreatized dogs during the evolution of experimental diabetic acidosis by comparison of serial eosinophil counts and urinary corticosteroid determinations with (a) estimations of blood and urine chemical constituents, (b) balance measurements, and (c) changes in insulin sensitivity.

2. Eosinopenia and an increase in the rate of corticosteroid excretion were found to be comparatively late features of ketoacidosis induced by the omission of insulin.

3. A close temporal relation was found to obtain between increased adrenal activity, as judged by these indices, and the following metabolic events: (a) An acceleration in the rate of catabolism of protoplasm, manifested by an increased negativity of the nitrogen, potassium, and phos-

phorus balances; (b) a loss of potassium in excess of nitrogen, presumably from the cells of the body as a whole; and (c) a decrease in sensitivity to injected insulin.

4. An increase in lipemia and, in some instances, of ketonemia was observed to occur in association with evidences of adrenal activation. However, inasmuch as the blood levels of both lipoids and ketones had begun to rise before eosinopenia became manifest, the temporal relation between adrenal activation and these metabolic events must be regarded as equivocal.

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REFERENCES

1. Selye, H., The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol., 1946, **6**, 117.
2. Engel, F. L., A consideration of the roles of the adrenal cortex and stress in the regulation of protein metabolism. Recent Progress in Hormone Research, New York, Academic Press, Inc., 1951, **6**, 277.
3. McCormick, N. A., Macleod, J. J. R., Noble, E. C., and O'Brien, K., The influence of the nutritional condition of the animal on the hypoglycemia produced by insulin. J. Physiol., 1923, **57**, 234.
4. London, S., The insulin tolerance test in controlled and uncontrolled diabetes mellitus. Quart. Bull., Sea View Hosp., 1946, **8**, 228.
5. Butler, A. M., and Tuthill, E., An application of the uranyl zinc acetate method for determination of sodium in biological material. J. Biol. Chem., 1931, **93**, 171.
6. Wilson, D. W., and Ball, E. G., A study of the estimation of chloride in blood and serum. J. Biol. Chem., 1928, **79**, 221.
7. Fiske, C. H., and Litarczek, G., Determination of potassium in urine *in* supplement on Urine, Laboratory Manual of Biological Chemistry, cited by Folin, O., ed. 5, New York and London, D. Appleton Century Co., 1934, p. 234.
8. Fiske, C. H., and Logan, M. A., The determination of calcium by alkalimetric titration. II. The precipitation of calcium in the presence of magnesium, phosphate, and sulfate, with applications to the analysis of urine. J. Biol. Chem., 1931, **93**, 211.
9. Fiske, C. H., and Subbarrow, Y., The colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66**, 375.
10. Briggs, A. P., Some applications of colorimetric phosphate method. J. Biol. Chem., 1924, **59**, 255.
11. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. Vol. II. Methods. Baltimore, Williams and Wilkins, 1932.
12. Folin, O., The micro method for the determination of blood sugar. New England J. Med., 1932, **206**, 727.
13. Folin, O., Determination of non-protein nitrogen *in* supplement on Blood, Laboratory Manual of Biological Chemistry, ed. 5, New York and London, D. Appleton-Century Co., 1934, p. 265.
14. Lowry, O. H., and Hunter, H. H., The determination of serum protein concentration with a gradient tube. J. Biol. Chem., 1945, **159**, 465.
15. Van Slyke, D. D., and Neill, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. J. Biol. Chem., 1924, **61**, 523.
16. Hastings, A. B., and Sendroy, J., Jr., Studies of acidosis. II. The colorimetric determination of blood pH at body temperature without buffer standards. J. Biol. Chem., 1924, **61**, 695.
17. Herrman, L. G., Ames, A., and Tapke, R. J., Observations upon the lipokrit method for the determination of the lipoid content of the blood. J. Lab. & Clin. Med., 1934, **19**, 411.
18. Point, W. W., Unpublished data. For brief description of method see Jones, C. M., Benson, J. A., Jr., and Roque, A. L., Whipple's disease. New England J. Med., 1953, **248**, 665.
19. Bonsnes, R. W., and Taussky, H. H., On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 1945, **158**, 581.
20. Hare, R. S., Endogenous creatinine in serum and urine. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 148.
21. Nanavutty, S. H., Van Slyke's method for determination of acetone bodies applied to small volumes of blood and urine. Biochem. J., 1932, **26**, 1391.
22. Behre, J. A., A modified salicylaldehyde method for the determination of acetone bodies in blood and urine. J. Biol. Chem., 1940, **136**, 25.
23. Randolph, T. G., Blood studies in allergy. I. The direct counting chamber determination of eosinophils by propylene glycol aqueous stains. J. Allergy, 1944, **15**, 89.
24. Talbot, N. B., Saltzman, A., Wixom, R. L., and Wolfe, J. K., A colorimetric assay of urinary corticosteroid-like substances. J. Biol. Chem., 1945, **160**, 535.
25. Hedon, E., La survie indéfinie du chien dépancréaté traité par l'insuline et les effets de l'interruption du

traitement. J. de physiol. et de path. gén., 1927, **25**, 1.

26. Barner, K., Untersuchungen komatoser und präkomatöser Zustände bei Diabetes mit der biologischen Leukocytenkurve. Ztschr. f. Klin. Med., 1927, **105**, 102.
27. Allan, F. N., Diabetic acidosis and leukocytosis. Am. J. Med. Sc., 1927, n.s. **174**, 506.
28. Long, C. N. H., Personal communication.
29. Guest, G. M., Personal communication.
30. McArthur, J. W., Sprague, R. G., and Mason, H. L., The urinary excretion of corticosteroids in diabetic acidosis. J. Clin. Endocrinol., 1950, **10**, 307.
31. Marks, L. J., and Leaf, A., The relationship of the renal excretion of adrenal corticoids to variations in renal hemodynamics. J. Clin. Invest., 1953, **32**, 813.
32. Atchley, D. W., Loeb, R. F., Richards, W. D., Benedict, E. M., and Driscoll, M. E., On diabetic acidosis; detailed study of electrolyte balances following the withdrawal and reestablishment of insulin therapy. J. Clin. Invest., 1933, **12**, 297.
33. Van Slyke, D. D., Studies of acidosis. VII. The determination of B-hydroxybutyric acid, acetoacetic acid, and acetone in urine. J. Biol. Chem., 1917, **32**, 455.
34. Dixon, C. F., Comfort, M. W., Lichtman, A. L., and Benson, R. E., Total pancreatectomy for carcinoma of the pancreas in a diabetic person. Metabolic studies. Arch. Surg., 1946, **52**, 619.
35. Greenberg, L. A., and Lester, D., A micromethod for the determination of acetone and ketone bodies. J. Biol. Chem., 1944, **154**, 177.
36. Darrow, D. C., Harrison, H. E., and Taffel, M., Tissue electrolytes in adrenal insufficiency. J. Biol. Chem., 1939, **130**, 487.
37. Ryberg, C., On the formation of ammonia in the kidneys during acidosis. Acta physiol. Scandinav., 1948, **15**, 114.
38. Berliner, R. W., Renal excretion of water, sodium, chloride, potassium, calcium, and magnesium. Am. J. Med., 1950, **9**, 541.
39. Martin, H. E., and Wick, A. N., Quantitative relationships between blood and urine ketone levels in diabetic ketosis. J. Clin. Invest., 1943, **22**, 235.
40. Radoslav, C. S., Uber die Wirkung des Insulins auf den Blutzucker beim Menschen. Wien Arch. f. inn. Med., 1924, **8**, 395.
41. Hemmingsen, A. M., and Marks, H. P., The correlation between the blood-sugar fall and the initial blood sugar in rabbits injected with insulin. Quart. J. Pharm. & Pharmacol., 1932, **5**, 245.
42. Klatskin, G., The response of diabetics to a standard test dose of insulin. J. Clin. Invest., 1938, **17**, 745.
43. Guest, G. M., Mackler, B., and Knowles, H. C., Jr., Effects of acidosis on insulin action and on carbohydrate and mineral metabolism. Diabetes, 1952, **1**, 276.
44. Knowles, H. C., Jr., and Guest, G. M., Tissue electrolytes in alloxan-diabetic rats with ketoacidosis. Proc. Soc. Exper. Biol. & Med., 1952, **79**, 552.
45. Rapoport, S., and Guest, G. M., The decomposition of diphosphoglycerate in acidified blood: its relationship to reactions of the glycolytic cycle. J. Biol. Chem., 1939, **129**, 781.
46. Mackler, B., Lichtenstein, H., and Guest, G. M., Effects of ammonium chloride acidosis on the action of insulin in dogs. Am. J. Physiol., 1951, **166**, 191.