ACETONE IN BREATH AND BLOOD

OSCAR B. CROFFORD, M.D., AND (BY INVITATION) ROBERT E. MALLARD, M.D., ROBERT E. WINTON, M.D., NANCY L. ROGERS, M.S., J. CRAIG JACKSON, B.A., AND ULRICH KELLER, M.D.

NASHVILLE

The "odor of decaying apples" on the breath of patients with severe diabetes was first r orded in 1798 by John Rollo in his classic monograph "Cases of the Diabetes Mellitus".[1] In 1857 Petters identified the odor producing substance as acetone[2] and in the Bradshawe lecture to the Royal College of Physicians of London in 1886 Dreschfeld stated that the odor of acetone on the breath was a characteristic feature of diabetic coma.[3, 4]

Quantitative methods for measuring acetone were developed in Germany in 1897 by Geelmuyden[5] and Nebelthau[6] and by 1898 Muller had described a technique suitable for measuring acetone in the breath of patients with diabetes.[7]

Significant methodological advances were made by Scott-Wilson,[8] Marriott[9] and by Folin and Denis[10, 11] and in 1920 Hubbard[12] and Widmark[13] successfully measured the concentration of acetone in the breath of normal humans. These chemical methods were insensitive and required tedious procedures for concentrating the sample prior to analysis. Nevertheless mar ' of these early papers made substantial contributions to our unders anding of acetone excretion in human breath.[14, 15]

In 1964 gas chromatography with flame ionization detection was applied to breath acetone measurements by Levey[16] and by Stewart and Boettner.[17] Breath could be analyzed directly and the rapidity, specificity, sensitivity and economy of the test meant that clinical application was no longer limited by technical or practical difficulties.

Since 1964 several groups of investigators have applied breath acetone measurements to clinical problems[18–25] but factors such as age, weight, sex, diet, exercise, stress, and diseases other than diabetes that might alter the breath acetone concentration have not been systematically studied. Furthermore standardized methodology has not been employed and the results have not been reported in a comprehensive form. This has made comparative interpretation of new data difficult and has

---

From the Department of Medicine, Venderbilt University School of Medicine, Nashville, Tennessee, 37232.

Supported by USPHS grants 5 P30 AM17026, 5 M01 RR-95 and a gift from the Justin and Valere Potter Foundation.

hampered the further use of breath acetone measurements in a clinical setting.

This report will be a summary of a comprehensive review of the literature supplemented by data collected in the Vanderbilt University Medical Center over the past five years. We well describe the methods that have been standardized and used throughout the study and report the frequency distributions of breath acetone measurements in healthy thin and healthy obese individuals and in a group of patients with miscellaneous illnesses. We will also suggest some uses of breath acetone measurements in weight reduction programs and in the management of diabetic outpatients. It is our hope that the study will provide the back-ground information, standardized methodology and control data that will facilitate the use of breath acetone analyses in other medical centers.

Methods and Normal Values

Analysis of Gaseous Samples. In all of our studies, the concentration of acetone in gaseous samples was determined with a Beckman GC-2A gas chromatograph with hydrogen flame ionization detector. The chromatographic column consisted of a 6-foot coil of stainless steel tubing, 1/4 inch O.D., packed with C-22 firebrick, flexol plasticizer 8N8, drierite, polyethylene glycol, and diisodecyl phthalate. The column temperature was maintained at 70°C, and helium was used as a carrier gas, flowing at a rate of 143 ml/min. Hydrogen and air entered the burner at flow rates of 48 ml/min and 240 ml/min., respectively.

Gaseous samples were introduced into the chromatograph through a stainless steel gas valve fitted with a Luer lock adapter, enabling a tight fit between the injection port and the 50 ml syringe containing the sample. To insure removal of all air and to prevent dilution of the sample, the gas valve was purged with 20–40 cc of the sample. Rotation of the valve handle then allowed a constant sample of 1 cc to be swept into the chromatographic column by the carrier gas, helium. The various components of the sample were differentially retarded by adsorption or absorption by the column fitting material. As the carrier gas continually flowed through the column, the individual components were eluted at different times. The effluent from the column was mixed with hydrogen and burned in filtered air. Ions produced in the flame were collected at an electrode above the flame, measuring the ion current. The ion current was amplified and indicated on the recorder.

Under the conditions above, acetone emerged from the column and was detected 2 min., 53 secs. after the sample had been injected in the chromatograph (Fig. 1).

Acetone in expired air was initially identified by comparing the

retention time of the presumed acetone peak to the retention times of acetone standards and a variety of other reagents (Table I). Next, the presumed acetone peak was shown to be increased in expired air samples obtained from patients with ketosis due to untreated diabetes mellitus or starvation. Finally, the identity of the acetone peak was confirmed by mass spectrometry. The other major components of expired air with retention times similar to acetone were 2-methyl butadiene (isoprene) and ethanol (Fig. 1). Under all circumstances, however,

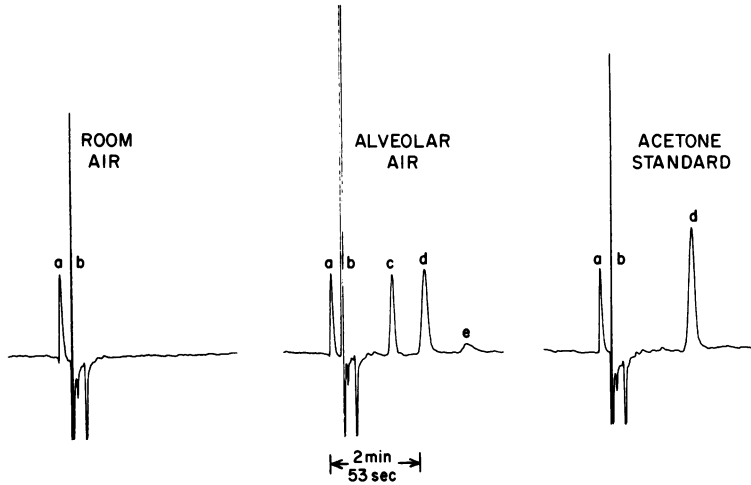

FIG. 1. Chromatograms from room air, alveolar air and actone standard run sequentially under the conditions described in the text. The identified peaks are: a – injection antifact, b – carbon dioxide (mostly), c – isoprene, d – acetone and e – ethanol. Acetone is not detectable in room air but elutes with a retention time of 2 minutes and 53 seconds when the acetone standard or alveolar are injected.

TABLE I

Retention Times of Various Compounds on the Gas Chromatographic Column

Compound	Retention Time (seconds)
Carbon monoxide	39
Acetaldehyde	75
Diethyl ether	97
Ethylene glycol	98
Isoprene	110
Formaldehyde	131
Acetone	*173*
Methanol	191
Ethanol	247
Isopropyl alcohol	300
Ethyl acetate	308
n-Heptane	330

acetone was well resolved and the signal noise ratio was favorable. The lower limit of detectability of acetone was approximately 1 nM and the gas chromatograph could be attenuated for higher levels of acetone.

Calibration and Standardization Procedures. The chromatograph was calibrated by diluting known microliter samples of the vapor over pure acetone into large volumes of air. The concentration of acetone in the standards was calculated from knowledge of the room temperature, vapor pressure of acetone at that temperature, and the ideal gas law. Calibration standards ranging from 8–550 nM were used to demonstrate a linear relationship between the height of the acetone peak and the concentration of acetone in the sample. In addition a linear relationship between the height and the area of the acetone peak was also demonstrated, but because of their utility, peak height measurements were used for routine determinations.

To facilitate daily standardization of the instrument, a large cylinder containing a compressed air-acetone mixture was obtained from a commercial source and standardized according to the procedure described above. The acetone concentration of this standard remains constant for about six months and can be used as a working standard to compensate for day to day variation in machine sensitivity.

Collection of Expired Air. The most satisfactory method for collecting the expired air sample proved to be the collection of an end-tidal air sample in a standard 50 cc glass syringe that could be sealed with a metal luer-lock cap. It provided a 30–50 cc breath sample with acetone concentrations that approximated those of alveolar air.

Each subject collected his own end tidal air sample after being instructed to "blow up the syringe with the last part of a big breath," and sealed the syringe with the Luer lock cap. As the anatomical dead space is usually less than 150 cc, few subjects failed to give an adequate sample, even those with a limited expiratory reserve volume. Samples of questionable adequacy were discarded and the collection repeated using the same syringe.

Glass syringes are relatively airtight and when sealed by the luer lock caps can be stored at room temperature for approximately six hours without significant loss of acetone. Samples collected in gas-tight syringes can be stored for at least 24 hours without loss of acetone but gas-tight syringes are too expensive for routine use. Disposable plastic syringes are unsuitable for this purpose since they contain organic components which contaminate the air sample and interfere with the gas chromatographic analysis. The simplicity of using a syringe as the collection device enabled samples to be taken outside the laboratory, stand for reasonable periods of time and be transported to the laboratory for analysis. Most importantly, all subjects including children and

acutely ill patients can be taught to give rapid, reproducible and accurate end-tidal expired air samples.

The reproducibility of this collection method was demonstrated by analyzing replicate samples collected from volunteers in whom the breath acetone concentration was assumed to be constant during the collection period. Five examples are given in Table II. As in these examples, the usual coefficient of variation of replicate analyses was approximately 4%.

Since other investigators[15, 22-24] had utilized rebreathing methods in achieving samples representative of alveolar air, a comparison of rebreathing and end expiratory methods was made. In addition, we studied the effect of pulmonary disease on the sampling procedure. The results obtained in 4 subjects, two normal individuals and two individuals with moderate to severe obstructive pulmonary disease, are shown in Table III. The end expiratory method employed was that described

TABLE II

Reproducibility of the End Expiratory Collection Method

Subject	Number of determinations	Breath acetone (nM)	Standard deviation	Coefficient of variation (%)
O.C.	10	39.4	1.1	2.7
J.C.	13	319.0	8.0	2.5
C.O.	10	45.3	2.4	5.2
M.M.	10	9.4	0.5	5.3
H.S.	10	30.4	1.3	4.4
Mean				4.0%

TABLE III

Comparison of Rebreathing and End Expiratory Collection Methods in Subjects With and Without Pulmonary Disease

Subject	Condition	End Expiratory			Rebreathing			Difference (End expiratory − rebreathing)
		Breath acetone* (nM)	S.D.	Coef. var. (%)	Breath acetone Mean (nM)	S.D.	Coef. var. (%)	
B.S-S.	Chronic obstructive pulm. disease	32.5	2.29	7.0	37.5	2.29	6.1	−5.0
H.W.	Chronic obstructive pulm. disease	12.5	.50	4.0	11.8	.42	3.6	+0.7
B.D.	No significant pulm. disease	49	.40	.8	47	1.1	2.3	+2.0
J.D.	No significant pulm. disease	62	4.3	6.9	64	3.2	5.0	−2.0
Mean of differences								−1.1†

* Mean of four repetitions

† *P* vs. zero = not significant

above. The rebreathing method was taken from Consolazio,[26] and was designed to deliver an alveolar air sample to approximate the mixed venous CO_2. Four samples using each method were obtained for all subjects. Since the acetone concentrations of the samples obtained by the two collection methods were approximately equal, we have assumed that the acetone concentration of the end expiratory samples approximate those of alveolar air.

Head Space Analysis. Van Stekelenburg[27, 28] has proposed an additional method for the direct measurement of blood acetone. This is the method of head space analysis and involves determination of the concentration of any volatile substance by analyzing the vapor phase which is in equilibrium with the liqiud phase. Knowledge of the vapor pressure of the volatile substance at any given temperature enables calculation of blood concentration from head space concentration.

The relationship of breath acetone to head space analysis of the subject's blood is shown in Table IV. A mean of 10 consecutive end expiratory air samples represents the breath acetone concentration. For head space analysis, 15 cc of venous blood was obtained, sealed in a container, and placed in a 37° water bath. The concentration of acetone in the head space over whole blood that was determined immediately after drawing the blood was approximately equal to the alveolar air acetone concentration. This is not surprising since the pulmonary alveolar-capillary membrane allows the acetone in alveolar air to reach equilibrium with the pulmonary arterial blood. Thus, sampling the alveolar air is, in effect, sampling the head space over pulmonary arterial blood.

A difficulty with head space analysis of acetone became apparent after serial samples of the head space were analyzed as the sealed container of whole blood remained in the 37° water bath. To site an example we noted that immediately after drawing the blood, the acetone concentration was 39 nM but after 150 minutes at 37° the head space acetone concentration was 73 nM. Similar observations have been made in all cases so studied and it is our belief that this represents conversion of acetoacetic acid to acetone. This conversion has been previously re-

TABLE IV

Comparison of Breath Acetone and Blood Head Space Analysis

Subject	Breath	Blood head space
	Mean acetone conc. (nM)	Mean acetone conc. (nM)
O.C.	39.4	38.9
J.C.	319.0	313.0
A.A.	69.5	68.5
R.K.	640.0	649.0
M.L.	14.6	13.9

ported to occur after heating serum to 90°. Thus, the utility of head space analysis in the determination of blood acetone does not appear to be as great as that of breath acetone. Besides necessitating an invasive sampling procedure by requiring blood, there is the possibility of a resultant falsely high acetone concentration unless the measurements are made immediately.

It is interesting that the head space method is highly satisfactory for the measurement of acetoacetate. Thus the blood is placed in a sealed container and the initial acetone level determined. The sample is then heated at 60° for one hour and the acetone concentration in the head space over the blood sample determined again. The difference represents the quantitative conversion of the acetoacetate to acetone and has proven to be the most sensitive and precise method for measuring acetoacetate that we have encountered. It is especially useful in performing hepatic AV-difference studies in dogs because a very sensitive and precise method is required.

Concentration of Acetone in Blood. In a carefully performed series of experiments, Haggard, et al. demonstrated that the acetone concentration in the liquid phase of a liquid:air mixture, in equilibrium at 38°, was 330 times that of the acetone concentration in the gaseous phase.[29] This value is in general agreement with previous studies[13, 30] and was reconfirmed in our laboratory by preparing standard solutions of acetone in water and measuring the acetone concentration in the gaseous phase by head space analysis when the mixture was in equilibrium at 38°. At low concentrations, this partition coefficient is independent of the solubility of acetone in the liquid phase and depends only upon the vapor pressure of acetone at 38°.[29]

Since the acetone in alveolar air is in equilibrium with pulmonary artery blood, one can derive the mixed venous blood acetone concentration by multiplying the breath acetone concentration by 330.

Breath Acetone Concentrations in Selected Populations. The healthy subjects had no history of diabetes mellitus or other metabolic disease. Their body weights were relatively constant and within ten pounds of the age, sex, frame and height corrected population averages. They were not participating in any weight modification program.

Breath samples were collected between 7 and 9 a.m. following an overnight fast of 9 to 16 hours. The frequency distribution of the results is shown in Fig. 2. It is apparent that the values were not normally distributed but were skewed in the direction of the higher values. The median, range and 95th percentile are given in Table V.

Additional studies indicated that in healthy subjects the breath acetone values obtained throughout the day did not differ significantly from the values obtained following an overnight fast (Table V). The samples

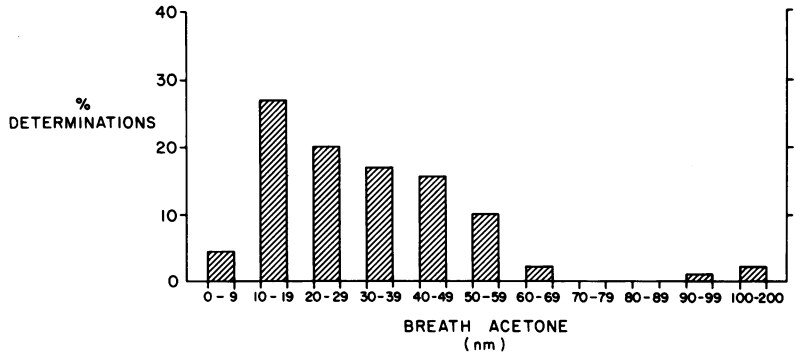

FIG. 2. Frequency distribution of breath acetone measurements in healthy subjects fasted overnight.

TABLE V

Distribution of Breath Acetone Measurements in Selected Populations

Population	Number of subjects	Number of determinations	Age range (yr.)	Breath acetone (nM)		
				Median	Range	95th percentile
Healthy subjects fasted overnight	89	89	21-50	28	5-194	63
Healthy subjects non-fasted	35	187	13-58	25	5-142	77
Diseased subjects fasted overnight†	30	30	12-54	23	6-101	60
Obese subjects fasted overnight*	31	31	22-65	16	5-38	36

* 9-16 hrs.

† Includes the following: Cushings syndrome, 4; Hirsutism, 2; Acromegaly, 1; Hypertension, 5; Hyperlipidemia, 6; Osteoporosis, 2; Total ileectomy, 1; Chronic renal failure, 6; Chronic liver disease, 2; Congenital heart disease, 1.

were collected without reference to the time elapsed since the last meal. Numerous measurements on subjects before and after meals have shown that if the breath acetone concentration is already normal for that particular individual, it does not decrease following a high carbohydrate meal.

Studies were also performed in a group of patients hospitalized with a variety of diseases other than diabetes (Table V). Again the results did not differ significantly from those obtained in healthy subjects.

The obese subjects were free of any known metabolic disease and were not attempting to lose weight at the time they were studied, though most had participated in weight reduction programs in the past. They all exceeded the age, sex, frame and height corrected population average by at least 20 percent. Although there was substantial overlap between the measurements made in the obese and non-obese groups, the values obtained in the obese group were significantly lower (Table V).

Some Clinical Applications

We have found breath acetone measurements to be useful as a motivational force in following patients on long-term weight reduction programs. Thus the patient can be instructed to restrict his caloric intake to such a level that the breath acetone concentration is maintained at about 500 nM. If a proper balance of carbohydrate, protein and fat is maintained one can then assure the patient that weight loss will be occurring at a rate of approximately one-half pound per week even though substantial time is required for this to be reflected in weight change. It is useful for the patient and the doctor to know that significant dietary indiscretion results in immediate normalization of the breath acetone value.

It is our opinion that breath acetone measurements are not particularly useful in following diabetics who are admitted to the hospital with ketoacidosis. Thus if the serum and urine acetoacetate levels are extremely high, the nitroprusside reaction is entirely satisfactory and no additional benefit is derived by using a gas chromatographic procedure.

We have found it very useful, however, to use breath acetone measurements to follow diabetic outpatients. For clinical purposes we classify diabetics with hyperglycemia as being in one of two categories depending upon the breath acetone concentration. The scheme is shown in Table VI. It is undoubtedly oversimplified but nevertheless useful. Patients with hyperglycemia and a normal breath acetone (less than 50 nM) are considered to have hyperglycemia of overeating and are treated with more vigorous dietary measures. Although it had been our practice in the past to treat hyperglycemic patients with "more insulin," the results were usually disappointing if overeating was the major problem. More insulin led to more overeating and to more obesity. More obesity led to more insulin resistance and, in most cases, the net result was that the hyperglycemia was not improved. This is generally referred to as the "diabetic clinic cycle." Our emphasis today is: "you don't treat overeat-

TABLE VI

Clinical Use of Breath Acetone Measurement

Serum Glucose (mg%)	Breath Acetone (nM)	Dx	Rx
Greatly elevated	Normal (10–50)	Overeating	Less food
Greatly elevated	High (>50)	Insulin insufficiency	More insulin
Not greatly elevated	High (>50)	Carbohydrate insufficiency	Continue diet if wt. loss is the objective or Reapportion diet adding more carbohydrate
Not greatly elevated	Normal (10–50)	Good metabolic control	Continue same Rx

ing with insulin but with better dietary management." Our general policy in well nourished adult diabetics is not to increase the insulin dose unless the breath acetone is elevated.

If the patient has hyperglycemia and an elevated breath acetone he is considered to be inadequately treated with insulin and the dose is adjusted appropriately.

Still another situation exists if the patient has an elevated breath acetone and a normal blood sugar. This results from an intake of dietary carbohydrate that is insufficient to meet the metabolic needs of the patient. If the patient is trying to lose weight, this is a very useful index of success and can be used to encourage the patient. In some instances, however, especially in children, the diet is malapportioned with the percentage of carbohydrate in the diet being too low. This can easily result if the child or his parents are unduly fearful that "starchy foods make his diabetes worse."

Although it is still too early to assess the long-term results of the program, we have found this systematic approach to the management of diabetic outpatients extremely useful in developing the skills of our entire health-care team.

In conclusion, it is our belief that breath acetone measurements have a number of potentially useful clinical applications and it is our hope that this report will stimulate the use of this procedure in other medical centers.

REFERENCES

1. ROLLO, JOHN: Cases of the Diabetes Mellitus; with the Results of the Trials of Certain Acids, and Other Substances, in the Cure of the Lues Venerea. Printed by T. Gillet for C. Dilly, in the Poultry, Second Edition, London, 1798.
2. PETTERS, W.: Untersuchungen uber die Honigharnruhr. *Vierteljahresschr. Praktische Heilkunde* 3: 81, 1857.
3. DRESCHFELD, J.: On Diabetic Coma. *British Med. J.* 2: 710, 1881.
4. DRESCHFELD, J.: The Bradshawe Lecture on Diabetic Coma. *British Med. J.* 2: 358–363, 1886.
5. GEELMUYDEN, H. C.: Ueber Aceton als Stoffwechselprodukt. *Z. Physiol. Chemic.* 23: 431–475, 1897.
6. NEBELTHAU, A.: Ein Beitrag zur Kenntnis der Acetonurie. *Centr. Inn. Med.* 18: 977–982, 1897.
7. MULLER, JOHANNES: Ueber die Ausscheidnngsstatten des Acetons und die Bestimmung Desselben in der Athemluft und den Hautausdunstunngen des Menschen. *Arch. Exp. Path. u. Pharmacol.* 40: 351–362, 1898.
8. SCOTT-WILSON, H.: A Method for Estimating Acetone in Animal Liquids. *J. Physiol.* 42: 444–470, 1911.
9. MARRIOTT, W. MCKIN: The Blood in Acidosis from the Quantitative Standpoint. *J. Biol. Chem.* 18: 507–517, 1914.
10. FOLIN, O., AND DENIS, W.: Turbidity Methods for the Determination of Acetone Acetoacetic Acid and β-Oxybutyric Acid in Urine. *J. Biol. Chem.* 18: 263–271, 1914.
11. FOLIN, O., AND DENIS, W.: On Starvation and Obesity, with Special Reference to

Acidosis. *J. Biol. Chem.* **21:** 183–192, 1915.
12. HUBBARD, R. S.: Determination of Acetone in Expired Air. *J. Biol. Chem.* **43:** 57–65, 1920.
13. WIDMARK, E. M. P.: Studies in the Acetone Concentration in Blood, Urine, and Alveolar Air. III: The Elimination of Acetone Through the Lungs. *Biochem. J.* **14:** 379–394, 1920.
14. HENDERSON, M. J., KARGER, B. A., AND WRENSHALL, G. A.: Acetone in the Breath. *Diabetes* **1:** 188–193 and 200, 1952.
15. GLAUBITT, D., AND RAUSCH-STROOMANN, J. G.: Eine Nove Methode zur Acetonbestimmung in der Atemluft. *Clin. Chim. Acta.* **4:** 165–169, 1959.
16. LEVEY, S., BALCHUM, O. J., MEDRANO, V., AND JUNG, R.: Studies of Metabolic Products in Expired Air. II. Acetone. *J. Lab. Clin. Med.* **63:** 574–584, 1964.
17. STEWART, R. D., AND BOETTNER, E. A.: Expired-air Acetone in Diabetes Mellitus. *N. England J. Med.* **270:** 1035–1038, 1964.
18. ROOTH, G., AND OSTENSON, S.: Acetone in Alveolar Air, and the Control of Diabetes. *Lancet* **2:** 1102–1105, 1966.
19. ROOTH, G.: Insulin Action Measured by Acetone Disappearance, Preliminary Report. *Acta Med. Scand.* **182:** 271–272, 1967.
20. ROOTH, G., AND TIBBLING, G.: Free Fatty Acids, Glycerol, and Alveolar Acetone in Obese Women During Phenformin Treatment. *Acta Med. Scand.* **184:** 263–267, 1968.
21. TASSOPOULOS, C. N., BARNETT, D., AND FRASER, T. R.: Breath-acetone and Blood-sugar Measurements in Diabetes. *Lancet* **1:** 1282–1286, 1969.
22. SULWAY, M. J., AND MALINS, J. M.: Acetone in Diabetic Ketoacidosis. *Lancet* **1:** 736–740, 1970.
23. SULWAY, M. J., TROTTER, E., TROTTER, M. D., AND MALINS, J. M.: Acetone in Uncontrolled Diabetes. *Postgrad. Med. J.* **47:** 382–387, 1971.
24. TROTTER, M. D., SULWAY, M. J., AND TROTTER, E.: The Rapid Determination of Acetone in Breath and Plasma. *Clin. Chim. Acta* **35:** 137–143, 1971.
25. ROOTH, G.: Clinical Value of Ketone Body Determination in Brittle Diabetes. *Acta Med. Scand.* **191:** 549–557, 1972.
26. CONSOLAZIO, C. F., JOHNSON, R. E., AND PECORA, L. J.: Physiological measurements of Metabolic Functions in Man. McGraw-Hill Book Company, New York, 1963, p. 209–211.
27. VAN STEKELENBURG, G. J., AND KOOREVAAR, G.: Head Space Analysis. *Clin. Chim. Acta* **34:** 305–310, 1971.
28. VAN STEKELENBURG, G. J., AND DEBRUYN, J. W.: A Simple Gas Chromatographic Determination of Acetone and β-Ketobutyric Acid in Blood Serum by Means of Head Space Gas Sampling. *Clin. Chim. Acta* **28:** 233–237, 1970.
29. HAGGARD, H. W., GREENBERG, L. A., AND TURNER, J. M.: The Physiological Principles Governing the Action of Acetone Together with Determination of Toxicity. *J. Industr. Hyg. Toxic* **26:** 133–151, 1944.
30. BRIGGS, A. A., AND SHAFFER, P. A.: The Excretion of Acetone from the Lungs. *J. Biol. Chem.* **48:** 413–428, 1921.

DISCUSSION

DR. FRANCIS D. W. LUKENS (Pittsburgh): I think most of us use weight to break up that clinical cycle you showed as a guide. I wondered, however, whether breath acetone has been particularly useful in your experience in handling patients with edema, in whom weight might be unreliable?

DR. CROFFORD: That is an excellent comment Dr. Lukens. Certainly, if you have long-term observations and weight measurements are adequately recorded, the same princi-

ple applies. Handling patients who have edema is another example of the potential use of the procedure. Still another is to establish a general rule that one should not increase the insulin dose if the breath acetone level is normal. One final point concerns the advantage of breath acetone compared to measures of acetone in the urine. Breath acetone measurements are 3 to 10 times more sensitive than the classical Acetest technique and are independent of urine volume and renal handling of acetoacetate. But the principle is the same as that of following the acetone-acetoacetate levels in the urine to decide whether the patient is insulin deficient.