Chemical Blood Studies. IX—The Fractional Determination of the Acetone Bodies in Blood and Urine of Sheep Suffering from Domsiekte.

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I. INTRODUCTION.

DUE to the fact that acetonaemia is being encountered more frequently lately in veterinary practice and, in South Africa is manifested in Domsiekte (Pregnancy Disease) in sheep, the occasion arose to study the acetone bodies in the blood and urine of sheep suffering from Domsiekte.

Since, as far as is known, no attempt has been made as yet to separate the acetone bodies in Domsiekte into their different components, this aspect was also considered. Determinations were done on both blood and urine of the acetone, plus the acetoacetic acid fraction by the Messinger technique, and of β-hydroxybutyric acid by the same technique, after oxidation by the Shaffer method, using certain modifications as will be noted later.

II. EXISTING METHODS.

A. *Removal of Proteins, Glucose and Other Interfering Substances.*

Before the acetone bodies can be estimated in both blood and urine, the proteins in the former and the interfering substances, such as glucose, paired glucuronic acids, aldehydes, hydrogen sulphide and volatile acids, such as lactic and formic, in both have to be removed.

To achieve this, when using blood, the following procedures are adopted: —

Marriot (1913c) used a modification of the Seegen procedure of sodium acetate precipitation for removal of the proteins and subsequent treatment of the distillate with basic lead acetate and ammonium hydroxide; van Slyke and Fitz (1917) and Barnes (1937) used a solution of mercuric sulphate which removes both proteins and other interfering substances; Behre and Benedict (1926) employed the Folin-Wu method of sodium tungstate and sulphuric acid for removal of the proteins, followed by the van Slyke (1917) procedure, consisting of copper sulphate and calcium hydroxide when β-hydroxybutyric acid is also to be determined.

Kennaway (1918) used lead acetate and ammonia, as recommended by Shaffer (1908) and as used by Kennaway (1914) for urine, for the removal of both the proteins and the interfering substances; Hubbard (1921c) used colloidal iron and basic lead acetate, followed by sodium hydroxide to precipitate the lead and to remove not only proteins but also other interfering substances. It may be here mentioned that van Slyke and Fitz (1917) stated in a footnote that colloidal ferric hydroxide when added to blood gave a beautiful protein-free filtrate, but that the precipitate absorbed about one-third of the β-hydroxybutyric acid present.

Widmark (1919) describes a micro-method for estimation of acetone in blood in which the blood is distilled with one per cent. phosphoric acid and the acetone evolved passed directly into the alkaline iodine solution of the Messinger method.

For the removal of the glucose and interfering substances in urine Shaffer (1908) recommended the use of basic lead acetate and ammonium hydroxide. This procedure has also been adopted by Shaffer and Marriot (1913) and Kennaway (1914). Van Slyke (1917) employed copper sulphate and calcium hydroxide and this latter procedure was also used by Behre and Benedict (1926) and van Slyke (1929). Hubbard (1921B) combined these two procedures to use lead acetate and copper sulphate, followed by sodium hydroxide, instead of ammounia.

B. *Purification of Acetone after Removal of Interfering Substances.*

In the blood and urine, after the proteins, glucose and certain of the interfering substances have been preliminarily removed, and the preformed acetone plus that derived from the aceto-acetic acid has been distilled over, usually from a sulphuric acid medium, a further purification is essential before the actual estimation can be carried out, if the Messinger procedure is adopted.

Shaffer (1908) added sodium hydroxide and hydrogen peroxide to the first distillate to hold back the volatile acids and to oxidise the aldehydes to the corresponding acids in the final distillation. This method has been adopted by Shaffer and Marriot (1913), Marriot (1913) and others. Folin and Denis (1914), used sodium peroxide instead of hydrogen peroxide for this purpose and this latter procedure has been enlarged upon by Hubbard (1921), who accomplished further purification by redistilling first from a solution of acid plus potassium permanganate and then from a sodium peroxide solution.
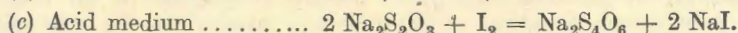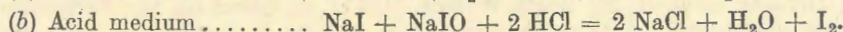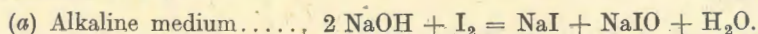
C. *Final Estimation of the Acetone Bodies.*

When the preformed acetone, plus that derived from aceto-acetic acid has been distilled over as acetone, and also the β-hydroxybutyric acid oxidised to acetone (see D, determination of β-hydroxybutyric acid) and purified as necessary, the final estimation can be carried out.

The Messinger (1888) titration method has been mostly used for this estimation. This method depends on the formation of iodoform from acetone in an alkaline iodine solution, the excess iodine from the known amount added, is determined, after acidification, with a standard sodium thiosulphate solution :—

$$CH_3COCH_3 + 3I_2 + 4\ NaOH = CHI_3 + CH_3COONa + NaI + 3\ H_2O$$

Excess Iodine :—

(*a*) Alkaline medium......, $2\ NaOH + I_2 = NaI + NaIO + H_2O$.

(*b*) Acid medium........ $NaI + NaIO + 2\ HCl = 2\ NaCl + H_2O + I_2$.

(*c*) Acid medium .... $2\ Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2\ NaI$.

Decinormal solutions of iodine and thiosulphate are usually used and from the first equation since—

$$1\ c.c.\ \frac{N}{10}\ I_2 = 1\ c.c.\ \frac{N}{10}\ CH_3COCH_3.$$

$$1\ c.c.\ \frac{N}{10}\ I_2 = \left\{\frac{58\cdot048 \times 1000}{6 \times 10 \times 1000}\right\}\ mgm.$$

$$= 0\cdot9675\ mgm.\ acetone.$$

The method has been proved by Marriot (1913a) and others to be accurate even when using dilute solutions of acetone. The method has been employed by Folin (1907), Stuart-Hart (1908), Shaffer (1908), Shaffer and Marriot (1913), Marriot (1913), Hurtley (1916), Widmark (1919), Hubbard (1920 and 1921) and others.
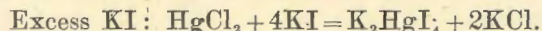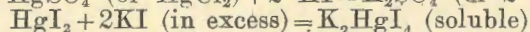
Another method is that proposed in 1898 by Denigés, depending on the formation of a compound of acetone and mercuric sulphate. The compound, which was crystalline could be weighed or the mercury content could be estimated by titration with silver nitrate and potassium cyanide. This method was adopted by van Slyke (1917) and van Slyke and Fitz (1917) using the titration method of Personne (1863), the precipitate being dissolved in hydrochloric acid, excess potassium iodide solution added, and the excess iodide estimated by titration with mercuric chloride.

The compound formed between the acetone and mercuric sulphate may be one of the following :—

$$3\ HgSO_4 \cdot 5HgO \cdot 2(CH_3)_2CO\ or$$

$$2\ HgSO_4 \cdot 3HgO(CH_3)_2CO,$$
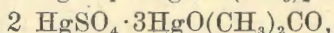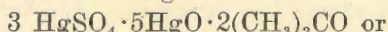
both of which contain about 77 per cent. mercury.

The reactions involved in the titrations are :—

$$HgSO_4\ (or\ HgCl_2) + 2\ KI = K_2SO_4\ (or\ 2\ KCl) + HgI_2\ (insoluble)$$

$$HgI_2 + 2KI\ (in\ excess) = K_2HgI_4\ (soluble)$$

Excess KI : $HgCl_2 + 4KI = K_2HgI_4 + 2KCl$.
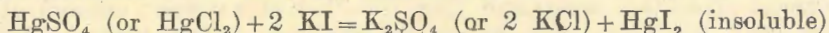
Barnes (1937), after dissolving the acetone-mercury compound in acid, distilled the acetone with heat into an alkaline iodine solution and titrated the excess with thiosulphate (Messinger).

A colorimetric method based on the reaction between acetone and salicylic aldehyde with the formation of a coloured product, dihydroxydibenzene acetone was introduced by Engfeldt (1915), based on the qualitative method of Frommer (1905) : —

$$2C_6H_4(OH)CHO + CH_3COCH_3 = [C_6H_4(OH)CHCH]_2CO + 2H_2O.$$
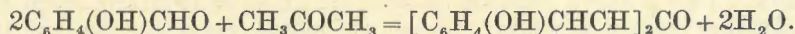
Csonka (1916) did not find the method applicable to small quantities of acetone, but Behre and Benedict (1926), however, overcame this drawback by substituting an alkaline solution for the alcoholic solution of the aldehyde employed by Csonka. The method has also been used by Urbach (1931) and Neuweiler (1933).

A further method, by Scott-Wilson (1911), is based on the formation of a double compound of acetone and basic mercuric cyanide in an alkaline medium, with subsequent titration of the mercury present in the precipitate with sulphocyanate, after decomposition of the precipitate with nitric acid and permanganate. Scott-Wilson gives the reaction for the precipitation as : —

$$CH_3COCH_3 + 2\ Hg(CN)_2 + 3\ HgO = HgCOC_2(HgCN)_4 + 3\ H_2O$$
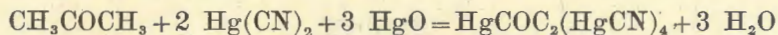
This method has been adopted by Marriot (1913b) for the determination of very small amounts of acetone by measuring the precipitate with a nephelometer, and by Folin and Denis (1914) who adopted it for using in a Duboscq colorimeter.

D. *The Determination of β-Hydroxybutyric Acid.*

The history as regards the discovery and the subsequent investigation into the properties and determination of β-hydroxybutyric acid has been discussed and enlarged upon by Shaffer (1908), Hurtley (1916) and Hubbard (1921a).

The next method was based upon the optical activity of the laevorotatry greater than that theoretically needed to neutralize the inorganic acids found in the urine, as β-hydroxybutyric acid, was necessarily inaccurate as it merely expressed the amount of organic acidity (Stadelmann, 1883).

The next method was based upon the optical activity of the laevorotatory acid or its salts after fermentation of the urine (Kulz, 1884). The results were, however, almost of no value and extraction of the acid by ether was first used by Wolpe (1886), and improved by Magnus Levy (1901), Bergell, and Black (1908). Black dehydrated the evaporated urine with plaster of Paris and employed a continuous ether extraction apparatus, finally determining the strength in the polariscope. Hurtley (1916) employing the method discarded the addition of ammonium sulphate as recommended by Magnus Levy (1901) and Geelmuyden (1906), as it was of no real advantage.

A further method was that of Darmstädter (1903), who evaporated the alkalinised (Na_2CO_3) urine and then distilled at constant concentration of 50 per cent. sulphuric acid to convert the β-hydroxybutyric acid into α-crotonic acid, which was extracted with ether and after removal of volatile acids was estimated by titration.

The inadequacy of these methods led Shaffer (1908), to propose the oxidation of the β-hydroxybutric acid with potassium dichromate and sulphuric acid to acetone and carbon dioxide, with the usual Messinger estimation of the former.

This method appears to be the most reliable and has certainly been used extensively. The oxidation is, however, not quantitative, and although it has been improved and enlarged upon by Shaffer and Marriot (1913), Marriot (1913), Folin and Denis (1914), van Slyke (1917) and Hubbard (1921) and improvements brought on as regards time employed and its adaptability, it is apparent that at its best a yield of 90 per cent. can be considered as the optimum.

III. Experimental.

A. *Is Lead Acetate Treatment Compulsory?*

Since in Domsiekte we find a condition of hypoglycaemia, in direct contrast to that seen in diabetes, coupled with hyperacetonaemia in both, and since we failed to find any glucose in the urine in Domsiekte, the question arose whether it was necessary to remove preliminarily the remaining interfering substances.

Shaffer (1908) in formulating his method for the oxidation of β-hydroxybutyric acid with chromic acid noted that the chief danger in not applying the lead acetate treatment lay in the conjugated glucuronic acids, which on oxidation with the chromic acid also gave rise to acetone.

Spaeth-Kaiser (1936) mentions that the paired glucuronic acids are formed chiefly, both as a result of incomplete sugar oxidation and in marked respiratory disturbances as are manifested in diabetes mellitus. Since these primary causes are not seen in Domsiekte, it appeared natural to assume that the excretion of glucuronic acids in Domsiekte was at a minimum.

The acetone bodies in Domsiekte urine were thus determined separately on the same sample, treating one aliquot with basic lead acetate and ammonia and omittng it on a second portion. The acetone plus acetoacetic acid was determined first after acidification with sulphuric acid and the β-hydroxybutyric acid then oxidised with chromic acid. The distillates in all cases wery subsequently redistilled after the addition of caustic soda and hydrogen peroxide, and Messinger procedure carried out on the final distillates. Results were as follows; data expressed as c.c. decinormal iodine on the aliquot urine used.

Urine Sample.	Aliquot.	Treatment.	Acetone plus Acetoacetic.	β-Hydroxy-butyric Acid.
A......................	10 c.c.	Without lead...........	15·70	12·85
		With lead.............	15·69	12·88
B......................	20 c.c.	Without lead...........	13·25	6·50
		With lead.............	13·05	6·63
C......................	100 c.c.	Without lead...........	4·45	—
		With lead.............	4·44	—

The Kennaway (1918) procedure of basic lead acetate and ammonia as applied to blood was checked against the Folin-Wu system of sodium tungstate and sulphuric acid for the removal of the proteins with subsequent redistillation of all the distillates with caustic soda and hydrogen peroxide. Results were as follows, data expressed as c.c. decinormal iodine per 25 c.c. blood, the aliquot used:

	Treatment.	Acetone plus Acetoacetic.	β-Hydroxy-butyric Acid.
Blood A...............................	Without lead...........	4·90	5·90
	With lead.............	4·70	5·20
Blood B...............................	Without lead...........	6·70	8·95
	With lead.............	6·60	8·85
Blood C...............................	Without lead...........	11·90	13·90
	With lead.............	11·80	14·00

The values obtained were thus quite accurate enough to warrant the omission of the lead acetate treatment in determining the acetone bodies in the blood and urine of sheep suffering from Domsiekte.

In this connection it may be noted that Folin and Denis (1914), using the turbidity method also eliminated the "rather tedious" preliminary treatment with basic lead acetate and ammonia. They found that all the oxidation products with chromic acid which pass into the final distillate and which react with the Scott-Wilson acetone reagent, are destroyed or removed by the second distillation with the alkaline peroxide mixture. They also state that in urines containing no sugar, as in children, and in that obtained from fasting persons the determination of β-hydroxybutyric acid requires no second distillation, but that the addition of sodium peroxide to the first distillate suffices to remove all the disturbing effects of the normal urinary constituents.

B. *Methods Used.*

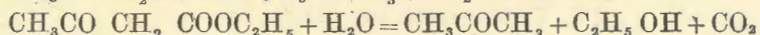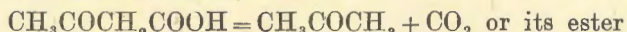The methods used may be summarized as follows:

1. *Determination of Preformed Acetone plus Acetone from Acetoacetic Acid.*

The blood proteins were removed by the Folin-Wu system of tungstic acid precipitation, using the strengths as modified by Graf (1933), namely 11 per cent. sodium tungstate ($Na_2WO_42H_2O$) and 0·725 normal sulphuric acid. The distilled water, tungstate oxalated blood and acid (in sequence mentioned) were used in proportions of 7 : 1 : 1 : 1 and after thorough mixing were filtered through Schleicher and Schüll's folded filter paper No. 588, no difficulty being encountered in obtaining clear filtrates.

From 100-250 c.c. filtrate for blood and from 5-100 c.c. of urine were used for an analysis. The Rothera's Reaction, consisting of a mixture of powered ammonium sulphate and a one per cent. sodium nitroprusside which is added to a small aliquot of urine, followed by the addition of ammonium hydroxide—a permanganate colour indicating a positive case— was employed to give an indication of the severity of acetonuria, which indirectly gave also an indication of the degree of acetonaemia.

The aliquot used was diluted to about 300 c.c. with water, 10 c.c. 1:1 sulphuric acid added and distillation carried out for at least 40 minutes, but avoiding a longer period to prevent splitting off of acetone from the β-hydroxybutyric acid (van Slyke 1917). The receiver was previously charged with water, and delivery tube allowed to dip below the surface. With a medium flame from 200 to 250 c.c. distillate was collected, water being added from a dropping funnel to distilling flask to keep the volume up to 150 c.c.

The distillate which contained the preformed acetone, plus that derived from the acetoacetic acid,

$$CH_3COCH_2COOH = CH_3COCH_3 + CO_2 \text{ or its ester}$$
$$CH_3CO\ CH_2\ COOC_2H_5 + H_2O = CH_3COCH_3 + C_2H_5\ OH + CO_2$$

was redistilled after the addition of 5 c.c. of about 30 per cent. caustic soda solution and a few c.c. of a 6 per cent. hydrogen peroxide, distillation being continued for about 30 minutes with same precautions as given above as regards the receiver, to give approximately 150 c.c. distillate.

The acetone was estimated on this final distillate by the Messinger technique as follows: To the distillate was added 5 c.c. 40 per cent. sodium hydroxide solution, and after mixing, 20 c.c. standard iodine solution accompanied with further mixing. After standing for 15 minutes with occassional shaking, 10 c.c. 25 per cent. hydrochloric acid was added, and the excess iodine thus liberated was titrated with a standard thiosulphate solution, using starch as indicator.

The difference in c.c. between the volume of iodine solution added and the thiosulphate solution used (standards being both exactly decinormal) multiplied by 0·9675 gives the acetone content in mgm.

2. Determination of β-Hydroxybutyric Acid.

The β-hydroxybutyric acid was estimated on the same sample as that used for estimation of acetone as described above.

To the residue in the distilling flask was added 25 c.c. of a solution containing 2 per cent. potassium dichromate and 35 per cent. sulphuric acid as recommended by Folin and Denis (1914). The volume of flask was made up to about 300 c.c. and distillation carried out for about 45 minutes, with the same precautions as given above for acetone. The β-hydroxybutyric acid was hereby oxidised to acetone.

$$CH_3CHOHCH_2COOH + O = CH_3COCH_3 + CO_2 + H_2O.$$
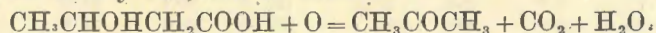
The 25 c.c. dichromate reagent proved to be ample for all blood filtrates, but in the urine samples dilute potassium dichromate had to be added occasionaly through the dropping funnel when solution tended to assume a too predominantly green colour. The volume was not allowed to sink below 150 c.c.

The distillate thus obtained was redistilled and the estimation carried out as for acetone determination.

3. Determination of Total Acetone Bodies.

In a few cases in both blood and urine, the time available did not allow a separation of the acetone bodies into the fractions, and a "total acetone" determination had to be done.

As in the above determinations 10 c.c. 1 : 1 sulphuric acid was added to the aliquot of the blood filtrate or to the urine sample and the contents of flask boiled for about 10 minutes, with the receiver attached, before the chromic acid was added. This was done to enable the acetoacetic acid to resolve into acetone and carbon dioxide as otherwise low results were obtained. Distillation was hereafter carried on for a further 45 minutes and after redistillation as above, the final estimate was carried out, using Messinger technique.

For all determinations apparatus with glass ground joints were used throughout.

IV. Discussion and Results.

Some of the values obtained by this method are tabulated below. Two sheep, in the last month of pregnancy, are considered, both as normal and pathological for both blood and urine. All values are expressed in terms of acetone as mgm., in blood, per 100 c.c., and in urine, either as per 100 c.c., or as the total output of urine for the 24 hours period over which collection was carried out. The urine was always collected for the 24 hours period following immediately after the time of bleeding.

Abbreviations used:

Acet. for acetone,

A.A. for acetoacetic acid,

H.B.A. for β-hydroxybutyric acid.

Particulars regarding sheep:— Sheep A was placed on veld hay diet on 12 April and she aborted on 28 April. For sheep B these dates are 8 March and 25 March respectively.

No.	Date.	Blood.			Urine.					
		Acet. + A.A.	H.B.A.	Total Acetone Bodies.	Acet. + A.A.			H.B.A.		Total Acetone Bodies per 24 hours.
					c.c. Urine.	Per 100 c.c.	Per 24 hour.	Per 100 c.c.	Per 24 hour.	
A.	1940									
	12/4	1·45	2·90	4·4	910	—	—	—	—	39·6
	15/4	3·09	4·25	7·4	295	47·4	139·8	8·1	23·9	163·7
	18/4	6·58	6·96	13·6	640	140·2	897·3	10·6	67·8	965·1
	22/4	11·99	10·44	22·4	560	243·7	1,364·7	15·5	86·8	1,451·5
	25/4	7·35	16·25	23·7	450	301·7	1,357·7	68·7	309·2	1,666·9
	29/4	1·93	3·87	5·8	—	—	—	—	—	—
B.	8/3	0·4	1·3	1·7	445	2·6	11·6	3·7	16·5	28·1
	18/3	14·8	13·2	28·0	665	318·1	2,115·4	31·9	212·1	2,327·5
	20/3	12·3	11·9	24·5	370	365·5	1,352·4	51·3	189·8	1,542·2
	23/3	—	—	37·9	610	318·1	1,940·4	39·7	242·2	2,182·6
	26/3	—	—	4·3	66	—	0·7	—	1·0	1·7

The rest of the data obtained from sheep, both normal and suffering from Domsiekte, by this method are published in the article Domsiekte, or Pregnancy Disease in Sheep, II, by Groenewald, Graf, Bekker, Malan and Clarke (1941).

V. SUMMARY.

Methods for the determination of the acetone bodies in both blood and urine in sheep suffering from Domsiekte are given. The preliminary treatment with basic lead acetate and ammonia, has been found to be of no advantage in this disease and has been omitted.

VI. REFERENCES.

BARNES, R. H. (1937). A Method for Determination of Blood Acetone Bodies. *Proc. Soc. Expt. Med. and Biol.*, Vol. 36, pp. 352-353.

BEHRE, J. A., AND BENEDICT, S. R. (1926). A Colorimetric Method for the Determination of Acetone Bodies in Blood and Urine. *Jl. Biol. Chem.*, Vol. 70, pp. 487-494.

BLACK, O. F. (1908). The Detection and Quantitative Determination of β-Hydroxybutyric Acid in Urine. *Jl. Biol. Chem.*, Vol. 5, pp. 207-210.

CSONKA, F. A. (1916). A Colorimetric Method for the Determination of Acetone in Urine. *Jl. Biol. Chem.*, Vol. 27, pp. 209-212.

ENGFELDT, N. O. (1915). *Berl. Klin. Woch.*, Vol. 52, pp. 458 and 796. Cited by Behre and Benedict (1926).

FROMMER, V. (1905). *Berl. Klin. Woch.*, Vol. 42, p. 1008. Cited by Behre and Benedict (1926).

FOLIN, O. (1907). On the Separate Determination of Acetone and Diacetic Acid in Diabetic Urines. *Jl. Biol. Chem.*, Vol .3, pp. 177-182.

FOLIN, O. (1934). Laboratory Manual of Biological Chemistry, 5th Edition.

FOLIN, O., AND DENIS, W. (1914). Turbidity Methods for Determination of Acetone, Acetoacetic Acid and β-Oxybutyric Acid in Urine. *Jl. Biol. Chem.*, Vol. 18, pp. 263-271.

GRAF, H. (1933). Chemical Blood Studies I. *Onderstepoort J.*, Vol. 1, pp. 269-278.

GROENEWALD, J. W., GRAF, H., BEKKER, P. M., MALAN, J. R., AND CLARKE, R. (1941). Domsiekte or Pregnancy Disease in Sheep II. *Onderstepoort J.*, Vol. 17, pp. 245-296.

HUBBARD, R. S. (1920). The Determination of Minute Amounts of Acetone by Titration. *Jl. Biol. Chem.*, Vol. 43, pp. 43-56.

HUBBARD, R. S. (1921a). A Note on the Determination of β-Hydroxybutric Acid. *Jl. Biol. Chem.*, Vol. 49, pp. 351-355.

HUBBARD, R. S. (1921b). The Determination of Acetone Bodies in Urine. *Jl. Biol. Chem.*, Vol. 49, pp. 357-374.

HUBBARD, R. S. (1921c). The Determination of Acetone Bodies in Blood. *Jl. Biol. Chem.*, Vol. 49, pp. 375-384.

HURTLEY, W. H. (1916). The Four Carbon Atom Acids of Diabetic Urine. *Quart. Jl. Med.*, Vol. 9, pp. 301-405.

KENNAWAY, E. L. (1918). The Acetone Bodies of the Blood in Diabetes. *Biochem. Jl.*, Vol. 12, pp. 120-130.

MARRIOT, W. M. (1913a). The Determination of Acetone. *Jl. Biol. Chem.*, Vol. 16, pp. 281-288.

MARRIOT, W. M. (1913b). Nephelometric Determination of Minute Quantities of Acetone. *Jl. Biol. Chem.*, Vol. 16, pp. 289-291.

MARRIOT, W. M. (1913c). The Determination of β-Oxybutyric Acid in Blood and Tissues. *Jl. Biol. Chem.*, Vol. 16, pp. 293-298.

MESSINGER, W. (1888). *Ber. d. Chem. Ges.*, Vol. 21, p. 1888, cited by Stuart-Hart (1908).

NEUWEILER, W. (1933). Quantitative Bestimmung des Acetonkörper im Blut mittels des Stufenphotometers. *Klin Wochenschr.*, Bd. 12, pp. 869-870.

SCOTT-WILSON, H. (1911). A Method for Estimating Acetone in Animal Liquids. *Jl. Physiol.*, Vol. 42, pp. 444-470.

SHAFFER, P. A. (1908). The Determination of β-Hydroxybutyric acid. *Jl. Biol. Chem.*, Vol. 5, pp. 211-223.

SHAFFER, P. A., AND MARRIOT, W. M. (1913). The Determination of Oxybutyric Acid. *Jl. Biol. Chem.*, Vol. 16, pp. 265-280.

SPAETH-KAISER (1936). Chemische und Mikroskopische Untersuchung des Harns. 6e Auflage.

STUART-HART, T. (1908). On the Quantitative Determination of Acetone in Urine. *Jl. Biol. Chem.*, Vol. 4, pp. 477-481.

URBACH, C. (1931). Quantitative Bestimmung des Acetons im Harn mittels des Stufenphotometers. *Biochem. Zeitschr.*, Bd. 236, pp. 164-173.

VAN SLYKE, D. D. (1917). Studies of Acidosis. VII: The Determination of β-Hydroxybutyric Acid, Acetoacetic Acids and Acetone in Urine. *Jl. Biol. Chem.*, Vol. 32, pp. 455-493.

VAN SLYKE, D. D. (1929). The Determination of Acetone Bodies in Blood and Urine. *Jl. Biol. Chem.*, Vol. 83, pp. 415-423.

VAN SLYKE, D. D., AND FITZ, R. (1917). Studies of Acidosis. VIII: The Determination of β-Hydroxybutyric Acid, Acetoacetic Acid and Acetone in Blood. *Jl. Biol. Chem.*, Vol. 32, pp. 495-497.

WIDMARK, E. M. P. (1919). Studies in the Acetone Concentration in Blood, Urine and Alveolar Air. I: A Micromethod for Estimation of Acetone in Blood, Based on the Iodoform Method. *Biochem. Jnl.*, Vol. 13, pp. 430-445.