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CLINICAL AND BIOCHEMICAL STUDIES ON THE BASIS OF KETONE BODIES IN CATTLE

III. COMPARISON BETWEEN THE VARIOUS SIMPLIFIED TESTS FOR QUANTITATIVE ESTIMATION OF KETONE BODIES IN URINE

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INTRODUCTION

For the purpose of diagnosing the bovine nutritional disorders especially in ketosis, the estimation of the values for ketone bodies in blood, urine and milk has been recognized to be very important. The authors already have reported in their previous papers[2,3], the results of estimation of the ketone bodies by the micro-diffusion method of THIN and ROBERTSON in blood and urine for the clinically healthy cows. Ordinarily, however, instead of the micro-diffusion method, some simplified tests such as Ross test, G·S test, Shino test No. 3 and others are being generally used for estimating the ketone bodies in the urine by veterinary practitioners, especially in field work. These tests are all considered to bemodified PATERSON's or ROTHERA's tests respectively. Ross test, G·S test and Shino test No. 3 are usually used clinically in this country, but the results obtained from their use are unreliable. Therefore, the authors have conducted experiments to judge the differentiation of sensitivity of several simplified tests applied in ordinary use. Furthermore, comparisons have been made of the results of the three tests with those obtained by the micro-diffusion method, which is recognized as the standard method for ketone bodies estimation. In the present report, the results of the investigations are described.

MATERIALS AND METHODS

Urine was used for investigations. The samples from 200 cows for determination of total ketone body and from 235 cows for estimation of acetone were employed respectively. Acetone and total ketone bodies were estimated at the same time for the same samples by the methods noted above, viz., micro-diffusion method as the standard one and ROSS

test, G·S* test and Shino test No. 3* as the simplified ones. The reagent of ROSS test consists of one part of sodium nitroprusside and 99 parts of ammonium sulfate. The constituents of G·S test and the patented Shino test No. 3 have not been published so they are uncertain but it may be thought that there are no great differences among these reagents.

The results of the investigations were analysed by statistical methods.

RESULTS OF THE INVESTIGATIONS

1. Comparison Among the Simplified Tests on the Basis of Total Ketone Bodies Estimated by Micro-diffusion Method

1) Comparison between ROSS test and micro-diffusion method: The values of ROSS test were assumed as follows: The negative reaction − (0 mg/100 ml), the appearance of violet at 30 minutes after addition of the reagent ± (5 mg/100 ml), at 10 minutes + (20 mg/100 ml), at 5 minutes ++ (30 mg/100 ml), immediately after the addition of the reagent +++ (50 mg/100 ml), and the colour extended all over the test-tube ++++ (70 mg/100 ml). For comparison between the three simplified tests, the standards of concentration of total ketone bodies estimated by micro-diffusion method were assumed as follows: 0 (0～2) mg/100 ml, 5 (3～7) mg/100 ml, 10 (8～15) mg/100 ml, 20 (16～25) mg/100 ml, 30 (26～40) mg/100 ml, 50 (41～75) mg/100 ml, 100 (76～150) mg/100 ml and 200 (151～250) mg/100 ml. In 200 cases, the values determined by ROSS test and micro-diffusion method were examined with the results

TABLE 1. *Comparison between Ross Test and Micro-Diffusion Method*

MIC. DIFF. METHOD (mg/100 ml)	ROSS TEST						
	− (0)	± (5)	+ (20)	++ (30)	+++ (50)	++++ (70)	Total
0 (0～ 2)	3 (100.0)	·	·	·	·	·	3
5 (3～ 7)	99 (98.0)	2 (2.0)	·	·	·	·	101
10 (8～15)	29 (56.8)	15 (29.5)	6 (11.7)	·	·	·	50
20 (16～25)	·	3 (27.3)	5 (45.5)	3 (27.3)	·	·	11
30 (26～40)	·	·	·	6 (100.0)	·	·	6
50 (41～75)	·	·	·	7 (77.8)	2 (22.2)	·	9
100 (76～150)	·	·	·	1 (9.1)	7 (63.6)	3 (27.3)	11
200 (151～250)	·	·	·	·	1 (11.0)	8 (89.0)	9
Total	131	20	11	11	10	11	200

* G·S test is offered from Gendai Pharmac. Co. Ltd., Tokyo, and * Shino test No. 3 Patent Nos. 211541 and 221576 is offered from Shino Test Laboratory, Ltd., Tokyo, respectively.

displayed in table 1. The values of ROSS test showed a lower tendency than the standard values by the micro-diffusion methods. If the values by ROSS test are called y and the values by micro-diffusion method are called x, the equation of the regression line derived is $y=0.3x+5$. That is, the gradient of the regression line is 1/3 of that of the micro-diffusion method. The sensitivity of the ROSS test is slight in comparison with the micro-diffusion method.

2) Comparisom between G·S test and micr-diffusion method: The values obtained by G·S test in comparison with colour of the reagent by means of the standarn colour-table at 5 minutes after the addition of the urine was as follows: 0 mg/100 ml, 5 mg/100 ml, 10 mg/100 ml, 20 mg/100 ml, 35 mg/100 ml, 50 mg/100 ml, 100 and 200 mg/100 ml. The values estimated by micro-diffusion method were classified on the basis of the standards as already

TABLE 2. *Comparison between G·S Test and Micro-Diffusion-Method*

MIC. DIFF. METHOD (mg/100 ml)	G·S TEST								
	0	5	10	20	35	50	100	200	Total
0 (0~ 2)	3 (100.0)	·	·	·	·	·	·	·	3
5 (3~ 7)	29 (28.7)	52 (51.5)	20 (19.8)	·	·	·	·	·	101
10 (8~15)	·	24 (48.0)	19 (38.0)	7 (14.0)	·	·	·	·	50
20 (16~25)	·	·	3 (27.3)	2 (18.1)	5 (45.5)	1 (9.1)	·	·	11
30 (26~40)	·	·	·	·	1 (16.7)	5 (83.3)	·	·	6
50 (41~75)	·	·	·	·	·	1 (11.0)	8 (89.0)	·	9
100 (76~150)	·	·	·	·	·	·	3 (27.3)	8 (72.7)	11
200 (151~250)	·	·	·	·	·	·	·	9 (100.0)	9
Total	32	76	42	9	6	7	11	17	200

described, and the results are shown in table 2. To generalize, the values of G·S test tend to be higher than the standard values. The equation derived to incorporate this tendency is $y=1.5x+7$. That is to say, the gradient of the regression line is 1.5 times that of the micro-diffusion method. In general, the values of G·S test seem to show the higher value of 1.5 times the standard values. Therefore, the sensitivity of this test is considered to be too strong.

3) Comparison between Shino test No. 3 and micro-diffusion method: The values of Shino test No. 3 semi-quantified by comparison with the colours at one minute after addition of 2 drops of urine on reagent by means of the standard colour-table were as follows: − (0 mg/100 ml), ± (5 mg/100 ml), + (10 mg/100ml), ++ (30 mg/100 ml) and +++ (more than 50 mg/100 ml). The results compared with the values estimated by micro-diffusion method,

TABLE 3. *Comparison between Shino Test No. 3 and Micro-Diffusion Method*

MIC. DIFF. METHOD (mg/100 ml)	SHINO TEST NO. 3					
	− (0)	± (5)	+ (10)	++ (30)	+++ (50)	Total
0 (0～ 2)	3 (100.0)	·	·	·	·	3
5 (3～ 7)	56 (55.4)	44 (43.6)	1 (0.9)	·	·	101
10 (8～15)	10 (20.0)	32 (64.0)	8 (16.0)	·	·	50
20 (16～25)	·	3 (27.3)	7 (63.6)	1 (9.1)	·	11
30 (26～40)	·	·	2 (33.4)	4 (66.6)	·	6
50 (41～75)	·	·	1 (11.0)	8 (89.0)	·	9
100 (76～155)	·	·	·	5 (45.5)	6 (54.5)	11
200 (151～250)	·	·	·	·	9 (100.0)	9
Total	69	79	19	18	15	200

are shown in table 3. The values obtained by Shino test No. 3 showed a tendency to be lower than the standard values. The equation derived to make clear that tendency is $y=0.5x+15$. That is, as the gradient of regression line is 1/2 in comparison with the gradient of that of the standard method, the values by Shino test No. 3 showed lower by half than the standard value.

4) Comparison among the simplified tests on the basis of the total ketone values estimated by the micro-diffusion method: The samples of urine of 200 cases were estimated by the micro-diffusion method, ROSS test, G·S test and Shino test No. 3 at the same time. In these cases, the number of appearance in each of the index by micro-diffusion method were regarded as the ideal expected value of the number of the appearance and comparisons were made among the expected value and the value of the appearances in each index of each simplified test. The results are displayed in table 4. In ROSS test, the value of appearance in 0 mg/100 ml showed about 40 times higher value than the expected value. But the values of the appearance above 5 mg/100 ml in the index, excepting the values of 30 and 50 mg/100 ml, showed very low values. In G·S test the number of the appearance in 0 mg/100 ml was 32, which was about 10 times the expected value, that is, G·S test yielded values most approximate to the standard value out of these 3 tests. The values of the appearances between 5～50 mg/100 ml were slightly lower than the expected values. In the index of 100 mg/100 ml, the value of the appearance entirely agreed with the expected values, but in the index of 200 mg/100 ml, it showed about twice as high a value as expected. The values of the appearances caused by Shino test No. 3 were situated generally between the values of appearances of ROSS and G·S tests. In consequence, the

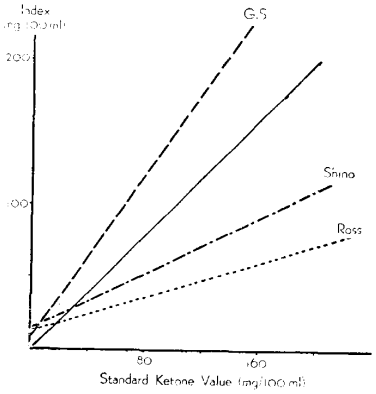
TABLE 4. *Comparison among the Simplified Tests*

METHOD	INDEX							
	0 (0~2)	5 (3~7)	10 (8~15)	20 (16~25)	30 (26~40)	50 (41~75)	100 (76~150)	200 (151~250)
micro-diff. method	3 (3)	101 (101)	50 (50)	11 (11)	6 (6)	9 (9)	11 (11)	9 (9)
G·S test	32 (3)	76 (101)	42 (50)	9 (11)	6 (6)	7 (9)	11 (11)	17 (9)
Shino test No. 3	69 (3)	79 (101)	19 (50)	18 (17)		15 (9)	· (11)	· (9)
ROSS test	131 (3)	20 (101)	11 (61)		17 (6)	21 (9)	· (11)	· (9)

( ) ····· Expected value.

G·S test showed generally the higher values with the exception of 0 mg/100 ml. On the other hand, Shino test No. 3 and ROSS test tended to show lower values than expected. But their compatibility with the expected value was worse than G·S test. Those mutual relationships are shown concretely by the regression line as displayed in chart 1; G·S test is $y=1.5x+7$, Shino test No. 3 is $y=0.5x+15$, and ROSS test is $y=0.3x+2.6$. If the coefficient of regression line is deemed as the proportion to the value of micro-diffusion method, Shino test No. 3 and ROSS test should show the 1/2 and 1/3 value of the standard value. For the estimation of total ketone bodies, the sensitivities of ROSS test and Shino test No. 3 are too dull, and the sensitivity of G·S test is too strong, but amongst these simplified tests, the G·S test seems to bear the closest resemblance to the standard value in practice.

CHART 1. *Regression Lines in Ketone*



2. Comparison Among the Simplified Tests on the Basis of Acetone Estimated by Micro-diffusion Method

1) Comparison between ROSS test and micro-diffusion method: The values estimated by ROSS test and micro-diffusion method were classified in accordance with the index as already mentioned, and the results as shown in table 5 were obtained. The values estimated by ROSS test in 235 cases tended to bear a close resemblance to the values estimated by the micro-diffusion method. If the values by ROSS test are called y, and the values by micro-diffusion method are called x, the equation derived is $y=0.7x+3.2$. The sensitivity of ROSS test is slightly duller than the standard method, because the gradient of the regression line is 0.7.

2) Comparison between G·S test and micro-diffusion method: The technique of the test and the standards of the determination were examined by the methods already described

TABLE 5. *Comparison between Ross Test and Micro-Diffusion Method*

MIC. DIFF. METHOD (mg/100ml)	ROSS TEST						Total
	− (0)	± (5)	+ (20)	++ (30)	+++ (50)	++++ (70)	
0 (0～ 2)	139 (88.8)	5 (3.2)	12 (7.6)	1 (6.4)	·	·	157
5 (3～ 7)	7 (29.2)	3 (12.5)	11 (45.8)	3 (12.5)	·	·	24
10 (8～15)	·	·	2 (18.2)	7 (63.6)	2 (18.2)	·	11
20 (16～25)	·	·	3 (33.3)	3 (33.3)	3 (33.3)	·	9
30 (26～40)	·	·	2 (14.3)	2 (14.3)	9 (64.2)	1 (7.2)	14
50 (41～75)	·	·	·	10 (71.5)	4 (28.5)	·	14
100 (76～150)	·	·	·	·	4 (66.7)	2 (33.3)	6
200 (151～250)	·	·	·	·	·	·	0
Total	146	8	30	26	22	3	235

TABLE 6. *Comparison between G·S Test and Micro-Diffusion Method*

MIC. DIFF. METHOD (mg/100 ml)	G·S TEST								Total
	0	5	10	20	35	50	100	200	
0 (0～ 2)	49 (31.2)	50 (31.8)	19 (12.1)	7 (4.5)	14 (8.9)	14 (8.9)	4 (2.5)	·	157
5 (3～ 7)	·	2 (8.3)	2 (8.3)	6 (25.0)	3 (12.5)	7 (29.2)	3 (12.5)	1 (4.2)	24
10 (8～15)	·	·	·	·	·	3 (27.3)	7 (63.6)	1 (9.1)	11
20 (16～25)	·	·	·	·	·	1 (11.1)	5 (55.6)	3 (33.3)	9
35 (26～40)	·	·	·	·	·	1 (7.2)	·	13 (92.8)	14
50 (41～75)	·	·	·	·	·	·	4 (28.5)	10 (71.5)	14
100 (76～150)	·	·	·	·	·	·	1 (16.7)	5 (83.3)	6
200 (151～250)	·	·	·	·	·	·	·	·	0
Total	49	52	21	13	17	26	24	33	235

in the considerations on total ketone. The results estimated in 235 cases are shown in table 6. The values obtained by the ROSS test showed generally a higher tendency than the values estimated by the micro-diffusion method. The equation of regression line derived to make clear the tendency is $y=2x+20$. Because the constant of the straight line is 20, the value by G·S test showed 20 mg/100 ml value in the case of 0 mg/100 ml. The values of G·S test should show a tendency to be double the standard value, because the gradient of the straight line is 2. Hence, it may be said that the sensitivity of G·S test is too strong. G·S test seems to be unsuitable for estimation of acetone.

3) Comparison between Shino test No. 3 and micro-diffusion method: The technique and the standards of the determination were the same as above described In comparison with the values by Shino test No. 3 and by the micro-diffusion method, the former values had a tendency to approximate to the standard values as shown in table 7. The regression line derived is $y=0.8x+2.4$. Values obtained by this test seem to bear a close resemblance to the values obtained in practice for the determination of acetone, because the gradient of the straight line is 0.8.

TABLE 7. *Comparison between Shino Test No. 3 and Micro-Diffusion Method*

MIC. DIFF. METHOD (mg/100 ml)	SHINO TEST NO. 3					Total
	− (0)	± (5)	+ (10	++ (30)	+++ (50)	
0 (0~ 2)	80 (51.0)	58 (36.9)	19 (12.1)	·	·	157
5 (3~ 7)	2 (8.3)	6 (25.0)	12 (50.0)	3 (12.5)	1 (4.2)	24
10 (8~15)	·	·	2 (18.2)	8 (72.7)	1 (9.1)	11
20 (16~25)	·	·	2 (22.3)	6 (66.7)	1 (11.0)	9
30 (26~40)	·	·	·	6 (42.8)	8 (57.2)	14
50 (41~75)	·	·	·	3 (21.4)	11 (78.6)	14
100 (76~150)	·	·	·	1 (16.6)	5 (83.4)	6
200 (151~250)	·	·	·	·	·	0
Total	82	64	35	27	27	235

4) Comparison among the values obtained by simplified tests on the basis of acetone values estimated by the micro-diffusion method: Each of the samples of 235 cases were estimated by the 4 methods, viz., micro-diffusion method, ROSS test, G·S test and Shino test No. 3. They were all used at the same time. If the number of the appearance in each of the standard values obtained by micro-diffusion method were regarded to be the ideal expected value of the appearance, the comparison among the expected values was as displayed in table 8. It will be noted in ROSS test, the values of the appearance in

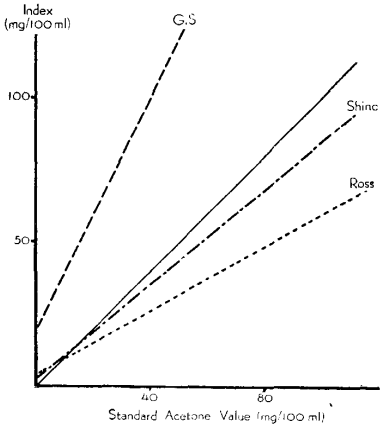
TABLE 8. *Comparison among the Simplified Tests*

METHOD	INDEX							
	0 (0~2)	5 (3~7)	10 (8~15)	20 (16~25)	30 (26~40)	50 (41~75)	100 (75~150)	200 (151~250)
Micro-diff. method	157 (157)	24 (24)	11 (11)	9 (9)	14 (14)	14 (14)	6 (6)	·
G·S test	49 (157)	52 (24)	21 (11)	13 (9)	17 (14)	26 (14)	24 (6)	33
Shino test No. 3	82 (157)	64 (24)	35 (11)	27 (23)		27 (14)	· (6)	·
ROSS test	146 (157)	8 (24)	30 (20)		26 (14)	25 (14)	· (6)	·

( ) ····· Expected value.

0 and 5 mg/100 ml of the standards were 7.6 and 62% lower, but in standard values of 10 to 50 mg/100 ml, they were 25 to 50% higher than the expected values respectively, and in 100 mg/100 ml, no cases appeared. The values of the appearance of G·S test were very much higher than the expected values with the exception of 0 mg/100 ml. The appearance of the values obtained by Shino test No. 3 had a tendency generally to approximate to the values of ROSS test, and they seemed to appear more on the higher side of the standards than do the values of the ROSS test. Therefore in general, Shino test No. 3 and ROSS test showed values approximate to the standard value. On the other hand, G·S test seem to be unsuitable for the determination of acetone, because it tended to yield very much higher values. The regression lines derived to make clear the mutual relationship among these simplified tests are shown in chart 2. The equations of regression lines of the 3 tests were respectively as follows: $y=0.8x+2.4$ in Shino test No. 3; $y=0.7x+3.2$ in ROSS test; $y=2x+20$ in G·S test, If the coefficients of regression lines are deemed as the proportion to the value of micro-diffusion method, they are slightly lower than the standard value, as the proportions of Shino test No. 3 and ROSS test are 4/5 and 2/3 of standard values respectively. The G·S test yielded very much higher values as reflected in its equation. Therefore, it is thought that the G·S test is unsuitable for the determination of acetone, but that Shino test No. 3 is the most useful as a simplified test among these 3 tests.

CHART 2. *Regression Lines in Acetone*



DISCUSSION

In the comparison among G·S test, Ross test and Shino test No. 3, it was found that the values obtained with Shino test No. 3 most closely resembled the

standard values. In the estimation and sensitivity, G·S test is believed to be too strong for the determination of acetone. NOGUCHI et al. and USHIMI et al. reported that Shino test No. 3 was the best among the simplified semi-quantitative tests for acetone as the present authors have confirmed in the present experiment. On the other hand, for the determination of total ketone bodies, the values estimated by G·S test bore closest resemblance to the standard value in practice. The sensitivities of the other two tests are so much lower that they are unsuitable for estimation of total ketone bodies. From the above described data, Shino test No. 3 and G·S test seem to be suitable for the determination of acetone and total ketone bodies, particularly the former test is regarded as the most useful for veterinary clinicians. They may be recommended in clinical practice as the best simplified tests.

SUMMARY

The values obtained by Ross test, G·S test and Shino test No. 3 as the simplified tests for estimation of the urine ketone bodies were compared with those obtained by the micro-diffusion method. The results thus obtained statistically may be summarized as follows.

1. The values of total ketone bodies obtained by G·S test showed 1.5 times in quantity those obtained by micro-diffusion method.
2. The values of total ketone bodies obtained by Shino test No. 3 and Ross test were 1/2 and 1/3 respectively of the values by micro-diffusion method.
3. So, when G·S test is used for estimating the urine total ketone bodies, the values approximated the standard value may be obtained.
4. In G·S test, the acetone values showed more than twice as high values as that estimated by micro-diffusion method.
5. The acetone values by Ross test and Shino test No. 3 showed 7/10 and 8/10 of the values by micro-diffusion method.
6. Therefore, Shino test No. 3 and Ross test, especially the former are considered to be suitable for determining the urine acetone.

The authors wish to express their cordial gratitude to the Gendai Pharm. Co. Ltd., and to Shino Test Laboratory for their kindness in willingly supplying reagents.

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