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STANDARDIZATION AND DETERIORATION OF RENNIN.*

BY L. D. HAVENHILL.

Early this spring while removing some drugs from a storage cabinet, I uncovered some samples of rennin on which considerable work was done in 1912. Dr. Klein in his report on Digestive Ferments and Glandular Products (4) finds that the "N. F. method as an absolute means of estimating rennin activity is unreliable" and suggests the possibility of adopting a standard rennin for control purposes. It therefore occurred to me that a reëxamination of these would furnish useful information concerning their deterioration, and at the same time afford an opportunity to check upon the N. F. V method of assay.

When these samples were originally assayed, fresh, whole milk from a mixed herd and averaging 3.5% butter-fat by Babcock test was used. No serious discrepancies in the time of coagulation for the daily samples of milk were observed.

STANDARDIZATION OF MILK.

Before reassaying these samples, a series of daily assays on milk was made using a new sample of powdered rennin—Rennin No. 12, of Table III. The results of these assays are given in Table I.

TABLE I.—THE COAGULATING POWER OF THE SAME RENNIN SAMPLE[1] ON DIFFERENT SAMPLES OF MILK.[2]

Day of July '29.	Milk sample No.	Time of coagulation.	Coagulating power.[3]	Day of July '29.	Milk sample No.	Time of coagulation.	Coagulating power.[3]
6	1	10.7	23,300	17	12	10.75[6]	23,200
7	2	9.2	27,200	18	13	12.80[7]	19,500
8	3	11.1	22,500	19	15	11.06[8]	22,600
9	4	10.9	22,900	20	16	12.06[9]	20,700
10	5	10.1	24,700	23	17	11.8[10]	21,100
11	6	8.6	29,000	24	18	9.00	27,800
12	7	11.10	22,500				
13	9	11.3	22,100	Maximum		12.8	39,000
14	10	6.4[4]	39,000	Minimum		6.4	19,500
16	11	11.40[5]	21,900	Average		10.5	23,800

SUB-NOTES TO TABLE I.

[1] Sample of Powdered Rennin No. 12 received at the Laboratory, 4–15–29.

[2] Whole Jersey milk, drawn about five o'clock A.M. and tested about ten o'clock A.M. of the same day. The temperature of the milk when delivered from the dairy ranged from 15°–18° C. and a temperature of from 13° C. to 18° C. was maintained thereafter. Babcock test, averaging about 5% butter-fat.

[3] N. F. V method used. Determinations made in a water-bath electrically controlled to

* Scientific Section, A. PH. A., Rapid City meeting, 1929.

0.3° C. The milk was measured into the tubes and heated in a second water-bath at 60° C. until the temperature of 43° C. was reached and then transferred to the test bath and 1 cc. of the rennin solution at room temperature (26°–28° C.) added. Ten seconds used for adding the rennin solution, and fifteen seconds additional for mixing by stirring with a glass rod. Time noted at the beginning of the addition of the rennin and at the beginning of the coagulation. No deduction was made for the time required to add the rennin.

[4] Acidity—0.18%, calculated as lactic acid. [5] Acidity—0.15%. [6] Acidity—0.16%. [7] Acidity—0.146%. [8] Acidity—0.15%. [9] Acidity—0.148%. [10] Acidity—0.146%.

Albert Zimmerman, of York, Pennsylvania, in a communication to me, in 1911, stated that it was necessary to use standardized rennin in order to determine whether the milk used in testing the activity of rennin was fast or slow. He standardized the rennin for this purpose by testing it against twelve different specimens of milk as daily supplied from a reliable source and took the average.

Table II shows the power of Rennin No. 12 by the Zimmerman method of standardization.

TABLE II.—VALUES TAKEN FROM TABLE I FOR RENNIN NO. 12.

Series.	Milk samples average.	Average time (minutes).	Coagulating power.
1	1–13	10.4	24,000
2	2–15	10.4	24,000
3	3–16	10.6	23,600
4	4–17	10.7	23,400
5	5–18	10.5	23,800

Any one of these values for Rennin Sample No. 12 is regarded as within the limit of experimental error. It is, however, recognized that there may be a seasonal variation in the time of "set up" for milk, and this should be investigated. But since the assays in 1912 and 1929 were each made in the late spring and early summer, the possibility of seasonal variation in these results is a remote one.

OPTIMUM TEMPERATURE.

The assays in 1912 were made at 40° C. instead of 43° C. but otherwise in each case the technique of the N. F. V was observed.

The matter of temperature was given very careful consideration in 1912 and also again in 1929. Temperatures of 35° C., 37.8° C., 40° C. and 43° C. have been variously recommended for the assay of rennin. Doubtless each temperature has some point in its favor. It would seem logical that the optimum tem perature, *i. e.*, the temperature at which it acts most rapidly, would be the choice of all. This is stated to be 40.5° C. (11) and 41° C. by Harper F. Zoller (13). A curve plotted by me in 1912 located the optimum point at 43° C. and another curve plotted by my assistant in the same year, but using different milk and a different sample of rennin, showed the optimum temperature to lie between 40° and 43° C. The observations supporting the temperature of 43° are that the curve is very flat between 40° and 43°, and that the higher temperature is preferable because the addition of the rennin reduces slightly the temperature of the milk, and that the beginning of the "set up" is sharper and the total time shorter.

A replotting of the curve with Rennin 12 and milk samples 9, 12 and 13 located the optimum temperature between 38° and 42° C. and centering closely around

40° C. The temperature and age of the standard rennin solution and the temperature of the test and age of the milk are factors which must be reckoned with in determining this optimum temperature—too many to be controlled by a single pair of hands.

As a result of these optimum temperature experiments the following are deduced:

1. The acidity of milk drawn at 5:00 A.M. and refrigerated does not increase in acidity between the hours of 10:00 A.M. and 3:00 P.M. if kept at or below 15° C.

2. That this milk requires a longer time for coagulation at 3:00 P.M. than at 10:00 A.M.

3. That the difference in time for coagulation between 10:00 A.M. and 3:00 P.M. is greater when tested at 43° C. than when tested at 35° C.

4. That standard rennin solutions deteriorate more rapidly at 29° C. than at 25° C.

5. That the deterioration in the standard rennin solution is greater when determined by standard milk at 43° C. than at 35° C.

6. That the optimum temperature for different samples, of rennin and of milk, may vary within narrow limits, perhaps 3° C.

7. That the law of Segelcke-Stork (the product of the mass of rennin by the time required for the coagulation of the milk operated upon is a constant number), holds true at least within the limits of experimental error when the conditions do not differ too widely from those of the N. F. V.

8. The rennins are not completely soluble in water. Test portions of the liquid containing the flocculent matter coagulate a given specimen of milk more rapidly than corresponding portions of the clear liquid.

THE ANALYSIS OF COMMERCIAL RENNINS.

The results of the analysis, assay and reassay of the different samples of rennin are shown in Table III.

TABLE III.—TESTS ON VARIOUS SAMPLES OF RENNIN.

Sample No.	Starch, etc.	Ash %.	NaCl %.	Coagulating power in 1912.	Coagulating power in 1929.	Loss % in 17 years.
1	None	4.1	3.6	15400	6200	60
2	None			15200	2700	82
3	None	6.7	5.8	19500	7700	60.5
4	None			1:120	1:2.5	98
5	None	7.6	6.8	16100	5700	64.6
6	None	6.9	4.1	22000	Lost	
7	None	8.2	4.2	20000	250	99
8	None	11.5	9.6	15100	130	99
9	None	4.9	1.5	20800	2500	88
10	None	9.5	7.6	4600	Less than 300	
11	None	5.4	1.9	23600	7500	68
12	None	12.0	10.8		23800	

COMMENTS ON SAMPLES OF RENNIN.

The samples 1–11 were collected during the winter and spring of 1912, no particular care was taken to preserve them other than to keep the containers tightly stoppered. They were filed away in a storage cabinet some time in June 1913, where they remained until June 1929, protected from the light but subjected to the fluctuations of ordinary laboratory temperatures.

No. 1.—Powdered Rennet, claimed to be 1:30000. Received in Laboratory 5–13–12; 1-ounce sample in original amber-colored glass bottle. Bottle nearly full. Odor good. Sample shows no outward sign of deterioration.

No. 2.—Powdered Rennet, claimed to be 1:30000. Received in Laboratory 1–16–12. Sample transferred from original container to a 2-ounce flint-glass bottle. Bottle nearly empty. Odor good. Sample slightly darkened in color, otherwise in good physical condition.

No. 3.—Powdered Rennet, claimed to be 1:30000. Received in Laboratory in 1911. Sample transferred from original container to a 2-ounce flint-glass bottle. Bottle nearly full. Odor good. Sample shows no outward sign of deterioration.

No. 4.—Sample of liquid Rennet, claimed to be 1:120. Received in Laboratory in 1912. Kept in original 4-ounce flint glass container. Shows no outward sign of deterioration.

No. 5.—Powdered Rennin, claimed to be 1:15000. Received in Laboratory in May 1912; 1-ounce sample in original amber-colored glass bottle. Bottle two-thirds full. Odor good. Sample shows no outward sign of deterioration.

No. 6.—Powdered Rennin. Sample missing.

No. 7.—Powdered Rennin, claimed to be about 1:30000. Received in Laboratory in 1912; 1-ounce sample in original amber-colored glass bottle. Bottle about two-thirds full. Odor fair. Sample shows no outward sign of deterioration.

No. 8.—Soluble Rennet, powdered, claimed to be about 1:30000; 1-ounce sample transferred to a flint-glass bottle. Bottle full. Odor good. Sample shows no outward sign of deterioration.

No. 9.—Rennin, scaled rennin. Sample transferred to a $^1/_2$-ounce flint-glass container. Odor fair. Sample discolored and fused into a sticky mass. Physically unfit for use.

No. 10.—Rennin. Sample transferred from original container to a $^1/_2$-ounce flint-glass bottle. Bottle about one-half full. Odor fair. Sample very dark in color but otherwise in good condition.

No. 11.—Rennin. Scaled rennin, claimed to be 1:30000; 1-ounce sample in original amber-colored glass container. Bottle about one-half full. Odor not good. Discolored and fused into a sticky mass. Physically unfit for use.

No. 12.—Powdered Rennet, claimed to be 1:30000. Sample received, 4–15–29; 1-ounce sample kept in original amber-colored glass bottle. Odor good. Fine, white powder.

COMMENTS AND CONCLUSIONS.

1. It appears to be feasible to prepare a standard powdered rennin that when properly kept, in a well-stoppered amber-colored bottle in a cool place, will show but little deterioration even after a lapse of several years.

2. The activity of an unknown sample of rennin should be checked against a standard sample of rennin.

3. A rennin may be standardized with a fair degree of accuracy using the method proposed by Albert Zimmerman, which is very similar to the procedure suggested by Dr. J. D. Frederikson of Little Falls, New York.

4. A temperature of 40° C. is, on the whole, preferable to 43° C. for the assay.

5. Only powdered rennin containing sugar of milk and otherwise corresponding to the N. F. V requirements for purity should be recognized in the N. F. VI.

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ABSTRACT OF DISCUSSION.

F. O. Taylor remarked that the author had obtained some extraordinary results; results in the past have been at variance and also the methods; the author has contributed to a better understanding and explained the limitations of the test. He said that it is necessary to use a standard for rennin comparable to that employed by manufacturers for pepsin; to use a method without comparison, usually invites variation.

The author stated that Mr. Taylor's suggestion prompted him to go over the work again because of the variance in results; he believed that not only different samples must be collected, but from several dairies at various seasons of the year.

James C. Munch referred to the distribution of standards for rennin by the Bureau of Chemistry, which presented difficulties in the keeping of a series of standard cows, etc. He said the American Drug Manufacturers' Association had obtained definite standards for this work. The report of Mr. Havenhill shows that rennin may be stabilized for a long period of time.

REGISTERED PHARMACISTS IN HOSPITALS.

The following resolution was adopted at the Baltimore meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION:

> WHEREAS, Pharmaceutical Service to the sick in the Hospitals of the United States is an important branch of medical care and should be supervised by registered pharmacists, be it
>
> *Resolved* that the AMERICAN PHARMACEUTICAL ASSOCIATION urge upon the authorities in charge of hospital standardization, the advisability of placing Hospital Pharmacies under the supervision of Registered Pharmacists, and
>
> *Be It Further Resolved*, that the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION be requested to assign to the proper officers or committees of this ASSOCIATION, the task of preparing a suggested set of standards of adequate pharmaceutical service, equipment and personnel for Hospitals of various grades to the end that such suggestions may be available to Hospital Organizations if, and when, requested.

AUTOMATIC DRUG MACHINES PROHIBITED IN VICTORIA.

At the instance of the Pharmacy Board, the use of automatic drug selling machines has been prohibited. According to the *Australasian Journal of Pharmacy*, the Chief Secretary (Mr. T. Tunnecliffe, M. L. A.), concurs in the view that these machines have become a source of embarrassment to pharmacists. The Victorian Board has shown wisdom and foresight in securing the prohibition order before vested rights accrue. It is hoped now that similar action will be taken in New Zealand and the other Australian States.