little importance, but, as no reference as to the resin content of the bark seems easily accessable in literature, this crude effort at establishing same, may prove of some service to future investigators, who have sufficient time and material to go into the subject thoroughly.

COLLEGE OF PHARMACY, COLUMBIA UNIVERSITY, April, 1912.

EFFECTS OF SODIUM CHLORIDE, SUGAR OF MILK, CANE SUGAR, DIFFERENT KINDS OF MILK, ETC., ON THE ASSAY OF RENNIN.*

L. H. BERNEGAU AND GEORGE E. E'WE.

Out of ten lots of Rennin received and assayed by us during the past twelve months, only three came up to labeled strength in milk coagulating power, namely 1:30,000. One sample assayed less than 1:10,000; one sample assayed only 1:13,000; one 1:15,000; two 1:20,000; one 1:23,000 and one 1:28,000, or 93 per cent. of required strength.

The following method is used by us for the assay of Rennin:

Dissolve 0.1 gm. rennin in water to make 100 cc., by gentle inversion of the bottle containing the rennin and water for about half an hour. Avoid any vigorous shaking which tends to lower the milk coagulating power. (This fact was illustrated in a previous paper by L. H. Bernegau). Take some so-called pepsin bottles and place into each 75 cc. of fresh unpasteurized milk, warm to 40-43° C. and add 2½, 3, 4, 5, and 7½ cc. respectively of the rennin solution. Keep at the same temperature in a water bath and remove each bottle at the end of *exactly* 7½ minutes and note whether or not the milk is coagulated.

2½ cc. indicate a milk coagulating power of..... 1:30,000
3 cc. indicate a milk coagulating power of..... 1:25,000
4 cc. indicate a milk coagulating power of..... 1:18,000
5 cc. indicate a milk coagulating power of..... 1:15,000
7½ cc. indicate a milk coagulating power of..... 1:10,000
etc.

Limit of accuracy of above method.

We made many experiments to find out the difference between duplicates made with the same rennin solution and the same milk by the above method.

Experiment No. 1 showed a difference between duplicates of 4.7%
Experiment No. 2 showed a difference between duplicates of 6.0%
Experiment No. 3 showed a difference between duplicates of 4.7%
Experiment No. 4 showed a difference between duplicates of 4.6%
Experiment No. 5 showed a difference between duplicates of 5.2%
Experiment No. 6 showed a difference between duplicates of 3.1%

Average, 4.7%

} Of total activity of the Rennin.

Numerous other experiments gave exact duplicates. As the limit of accuracy between duplicates is about 5 per cent. the figures given in the following tables

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are within 2.5 per cent. of the exact figures in this sense. When, for example, there is an increase in activity of 26 per cent., this figure may really be 23.5 per cent.

The following experiments were carried out with two samples; No. 1 an European sample and No. 2 a domestic sample. Both were found to contain chlorides and to reduce Fehling's solution. Both came up to standard at the time they were received, but source and standard has nothing to do with our comparative experiments. We only found it interesting to experiment with samples which we knew did not come from the same source. In our tables, therefore, we will use only No. 1 and No. 2, representing the sources mentioned above.

Effect of admixture of Sodium Chloride with the Rennin.

Rennin.	Rennin: NaCl	Increase in Activity.
No. 1	1:0	0 (Dupl.)
	1:14	10% (Dupl.)
	1:7	26% (Dupl.)
	1:3	13% (Dupl.)
No. 2	1:0	0 (Dupl.)
	1:7	16% (Dupl.)
	1:3	0 (Dupl.)

Conclusions: There seems to be an optimum proportion of NaCl which will give highest milk coagulating power to rennin; above or below this optimum proportion the NaCl apparently lowers its ability to increase the activity of the rennin. This optimum proportion lies around 1:7. Rennin-sodium chloride tablets made in this proportion should be therefore most efficient in milk coagulating power. Blanks run with plain NaCl without the rennin the proportion used in the above experiments had no coagulating effect on the milk after four hours, so that the NaCl has no appreciable effect when used alone.

Effect of admixture of Milk Sugar with the Rennin.

Rennin.	Rennin: Milk Sugar.	Increase in Activity.
No. 1	1:0	0 (Dupl.)
	1:14	3 % (Dupl.)
	1:7	17 % (Dupl.)
	1:3	9 % Aver of 3 assays.
No. 2	1:0	0 (Dupl.)
	1:7	5.6% (Dupl.)
	1:3	0 (Triplicates.)

Conclusions: Milk sugar also increases the milk coagulating power of Rennin, but not to so great an extent as sodium chloride. There is also an optimum proportion of rennin to milk sugar; this proportion being, like that of sodium chloride, near 1:7.

Effect of admixture of Cane Sugar with the Rennin.

Rennin.	Rennin: Cane Sugar.	Decrease in Activity.
No. 2	1:14	16 % Aver. of Dupl.
	1:7	8.6% (Dupl.)
	1:3	4.4% (Dupl.)
	1:0	0 (Dupl.)

Conclusions: Cane sugar mixed with rennin decreases the activity of the rennin apparently in almost direct proportion (within the limits of the above experiments) to the proportion of the cane sugar in the rennin-cane sugar mixture.

Effect of Cane Sugar dissolved in the Milk.

Rennin.	% Cane Sugar in Milk.	Decrease in Activity.
No. 1.....	2.5%	7 %
	5 %	7 %
No. 2.....	2.5%	4.4%
	5 %	16 %

Conclusions: The cane sugar used in sweetening milk when making junket evidently reduces activity of the rennin employed.

Effect of different lots of Unpasteurized Milk.

Milk bo't on morning of 2-7-12.	Rennin No. 1.	Assayed 1:36,400 Average of Duplicates.	Difference 35%
Milk bo't on morning of 2-8-12.		Assayed 1:26,800 Average of Triplicates.	
Milk bo't on morning of 2-7-12.	Rennin No. 2.	Assayed 1:25,000 Duplicates.	33%
Milk bo't on morning of 2-8-12.		Assayed 1:18,800 Duplicates.	

Conclusions: Different lots of milk may exert a great influence on the assay of rennin.

Effect of treatment of Pasteurized and Unpasteurized Milk.

Milk.	Assay w/ same rennin.	Difference.
Pasteurized	1:25,000	12.5%
Unpasteurized	1:28,125	

Two other samples of rennin gave results between 10-20 per cent. higher with unpasteurized milk than with pasteurized milk. A sample of unpasteurized milk gave 4.7 per cent. (aver. of four experiments) higher after being aged 24 hours at about 5° C.

Conclusions: Unpasteurized milk gives higher results in the assay of rennin than pasteurized milk and the age of the milk also has some effect on the assay.

Rennin Tabletss Four lots of old tablets in which sodium chloride was used as a diluent, were tested recently by us to find out if an appreciable deterioration took place. Rennin certainly acts queerly on getting older,—some lots seem to die at a very young age and become inert, while others seem to grow in strength, the older they get. All four lots were tested on the same day (2-6-12) and the same milk was used. All tests were carried out at the same temperature, namely 40-43° C. The results were as follows::

No. 1. One tablet coagulated 1 qt. milk in 10 minutes. (Tested on 9-29-10; 1 tablet required 14 min.) Increase.

No. 2. One tablet coagulated 1 qt. milk in 19 minutes. (Tested on 2-14-11; 1 tablet required 12 min.) Decrease.

No. 3. One tablet coagulated 1 qt. milk in 30 minutes. (Tested on 5-2-11; 1 tablet required 11 min.) Decrease.

No. 4. One tablet coagulated 1 qt. milk in 9 minutes.

This last lot is about seven years old and as seen from this table is today the best of all of them. Unfortunately, we were unable to find the record of the first assay, seven years ago.

As rennin and its preparations are undoubtedly of interest to any pharmacist and physician, we hope that others will take up this subject and report upon it.

ANALYTIC LABORATORY OF THE H. K. MULFORD CO.