

tion when shaken with 5 cc. of water and 3 drops of Nessler's reagent—a comparatively stringent requirement, for, while pure chloroform should, of course, give an absolute non-response with Nessler's reagent, since it is necessary to add alcohol for preservation, the test cannot be required wherein the reagent is used direct without dilution or other modification.

In order to determine the applicability of Nessler's reagent as a test for the presence of acetaldehyde in anaesthetic chloroform, the following experiments were performed, using 5 cc. of each sample and 5 cc. of Nessler's reagent U. S. P.:—

Sample and conditions.	Results.
1. Salicylid-chloroform, containing no detectable impurities; 0.25 per cent. pure ethyl alcohol.	Reagent assumed a faint opalescence after the mixture had been shaken and then allowed to stand 5 minutes.
2. Anaesthetic chloroform prepared from carbon tetrachloride and of full U. S. P. quality; 0.7 per cent. alcohol.	After 5 minutes, a turbidity was observed, but no coloration or precipitate.
3. A sample of anaesthetic chloroform prepared from acetone and complying in full with the requirements of the U. S. P.	Reagent assumed an orange opalescence after shaking and allowing to stand 5 minutes; after 10 minutes, the reagent became red colored.
4. An old sample of anaesthetic chloroform (48 years), which gave a marked reaction with François' reagent.	Reagent darkened immediately. After 5 minutes it possessed a dirty yellow color and a black precipitate was observed.
5. "Purified chloroform," of full U. S. P. grade; 0.9 cc. absolute alcohol in 100 cc.	After 5 minutes, the reagent possessed an opalescent orange color; it appeared turbid, but no precipitate settled out.
6. "Chloroform pure," of U. S. P. grade, and in addition conforming with the formalin-sulphuric acid test; 0.56 cc. absolute alcohol in 100 cc.	As in No. 5.
7. Anaesthetic chloroform prepared from chloral, and conforming with all requirements of the U. S. P. but the sulphuric acid test; 0.30 cc. absolute alcohol in 100 cc.	As in No. 5.
8. Anaesthetic chloroform conforming with all the requirements of the German Pharmacopoeia.	Red color at once; after 5 minutes, it became darker in color than No. 5.
9. Pure chloroform containing 1 part of acetaldehyde in 555.	Reagent assumed a brick-red color at once, but became black after several seconds; a heavy black precipitate after 5 minutes.
10. Pure chloroform containing an addition of 2 parts of acetaldehyde in 11,000.	Red color at once; a heavy brick-red precipitate after 5 minutes.
11. Ten cc. of pure chloroform containing an addition of 2 parts of acetaldehyde in 11,000; 10 cc. of water, and 5 drops of Nessler's reagent U. S. P.	Opalescent yellow color at once. After 5 minutes, the reagent possessed an opalescent orange-yellow color (turbid).
12. Ten cc. of No. 5; 10 cc. of water; and 5 drops of Nessler's reagent U. S. P.	No color at once, but a very faint yellow color after 5 minutes; the reagent was slightly turbid after agitation for 1 minute. The difference from the preceding was most marked.

From these general experiments, supplemented by

¹ Cf. Weigel, *Pharm. Centralt.*, 47, 400.

tests made on various other anaesthetic chloroforms, it was concluded that the test with Nessler's reagent is more delicate in this case than that with the reagent of Francois; and that while pure anaesthetic chloroform should behave as in Experiment 1, in our opinion it is sufficient to require that anaesthetic chloroform should comply with the following test: When 10 cc. of the sample are agitated with 10 cc. of water and 5 drops of Nessler's reagent U. S. P., and the mixture is then allowed to stand 5 minutes, there should result no precipitate and the reagent should assume no coloration, although it may become opalescent or slightly turbid.

(To be concluded in the August No.)

LABORATORY STUDIES OF RENNIN.¹

I. A Study of the Properties of this Important Ferment, when Prepared by Different Methods. II. The Acceleration of the Action of Rennin by Phosphoric Acid. III. The Variation in the Length of Time Required to Curdle Different Specimens of Milk.

By A. ZIMMERMANN.

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I. KINDS OF RENNIN.

Rennin Precipitated by Sodium Sulphate.—The rennets are digested to complete solution at 110° F. After separation of the fatty matter and clarification of the solution, this is precipitated by adding 3 volumes of a saturated solution of sodium sulphate, at 104° F.; the precipitate is collected, pressed and then dried at a temperature not exceeding 110° F. The yield is greater than that obtained by sodium chloride, the bulk being made up by albumose bodies. This makes it of a lower strength, ranging from 1 : 30,000 to 1 : 40,000 in 12 minutes.

Rennin Precipitated by Sodium Chloride.—The clarified solution of the rennets is saturated with sodium chloride at 104° F. The rennin is collected, pressed, then dried at a temperature not exceeding 110° F. The yield is much smaller than with sodium sulphate, giving a rennin of a much higher strength, from 1 : 150,000 to 1 : 200,000 in 12 minutes.

Rennin in Scales (Granular Rennin).—Prepared by scaling the clarified solution of the whole rennets at a temperature not exceeding 110° F. When perfectly dry, the scales are forced through a 16-mesh sieve. The strength of this rennin is from 1 : 30,000 to 1 : 40,000 in 12 minutes.

Commercial Rennin.—There are two different forms of rennin upon the market: (1) Rennin in powder form, prepared from the sodium chloride product, powdered and diluted with sugar of milk, testing 1 : 30,000 to 1 : 40,000 within 12 minutes. (2) Granular rennin, prepared from the scales, which also tests 1 : 30,000 to 1 : 40,000 within 12 minutes.

Difference between Precipitated and Scales Rennin.—The precipitated rennets are diluted with more or less albumose substance. They are acid in reaction, as they carry the greater part of the acid from the solution during the precipitation. The scaled rennin contains the soluble products, peptone and albumose

¹ Presented at A. C. S. meeting at Washington, D. C., December, 1911

substances, formed during the process of digestion of the rennets by aid of hydrochloric acid, which it also retains and which gives to it its acid reaction.

The rennin by sodium sulphate is no longer manufactured for use in the pharmaceutical preparations, the most important of these being the Essence of Pepsin, which is much used as a vehicle for bromides; the presence of sulphates would make it incompatible with strontium bromide. The complete separation of the sulphuric acid from rennin is not easily accomplished: by the use of calcium chloride, the reaction requires several days, during which time the rennin loses its activity; with barium chloride, there is formed a nearly colloidal barium sulphate, extremely difficult of separation.

The sodium chloride product is not so permanent as the granular rennin and the sodium sulphate product in a glycerine-water solution in the form of glycerol, which is a most excellent solvent for making permanent solutions of this class of substances.

The following tests made with a glycerol containing both rennin and pepsin, upon milk kept refrigerated for 17 to 18 hours after the milking, shows the permanency of the different forms of rennin, each giving a strength of 1 : 2500 in the time indicated:

	RENNIN AND PEPSIN GLYCEROL.					
Kind of rennin.	NaCl pptd.		Na ₂ SO ₄ pptd.		Granular.	
No. of milk.....	1	2	1	2	1	2
When prepared...	12 min.	12 min.	12 min.	12 min.	12 min.	12 min.
After 1 mo.....	30 "	25 "	12 "	12 "	12 "	12 "
After 2 mo.....	2.5 hrs.	2 hrs.	12 "	12 "	12 "	12 "
After 12 mo.....	3.5 "	3.25 hrs.	12 "	12 "	12 "	12 "
After 15 mo.....			12 "	12 "	12 "	12 "

The permanency of the rennin in solution seemed to be dependent upon phosphoric acid. This is contained in the rennets probably as a calcium salt, and during the digestion of these, for the preparation of rennin, the phosphoric acid is liberated by the hydrochloric acid used in the process. The granular scale and sodium sulphate product react for phosphoric acid, and in this they differ from the sodium chloride product, which appears to contain but very small quantities or none.

II. ACTION OF PHOSPHORIC ACID.

The addition of phosphoric acid in the proportion of 0.075 per cent. to milk, from individual cows or that mixed from the herd, has the effect of increasing the activity of the rennin very much. This applies to milk from 4 to 8 hours after the milking; as the milk gets older the rennin acts more rapidly, and with milk 2 days old preserved by refrigeration, the curd is formed within a few minutes without the need of any accelerant. The curd formed in milk from 4 to 8 hours old forms very gradually after the action of the rennin sets in and, when complete, is not nearly so firm as that formed in the presence of the phosphoric acid, which reduces the time of action to between $\frac{1}{10}$ and $\frac{1}{20}$.

Lactic, hydrochloric and oxalic acids also have an accelerating action upon rennin, but less than phosphoric acid. Lactic acid possesses about $\frac{1}{5}$, and the other acids about $\frac{1}{10}$ the activity of phosphoric acid. Lactic acid forms a firmer curd than hydrochloric or

oxalic acids, but not so perfect and firm a curd as is formed by the phosphoric acid:

COMPARATIVE ACTION OF RENNIN (R.) AND RENNIN ACCELERATED BY PHOSPHORIC ACID (R. P.).

Milk. Specimen.	4-5 hrs. old.		17-18 hrs. old. ¹		40-42 hrs. old. ¹	
	R. Min.	R. P. Min.	R. Min.	R. P. Min.	R. Min.	R. P. Min.
1.....	60	1.75	9.5	1	4.0	0
2.....	35	1.5	3.5	1	3.0	0
3.....	62	2.0	7.0	1	2.5	0
4.....	40	1.5	8.0	1	2.0	0
5.....	42	1.75	9.0	1	3.0	0
6.....	63	2.0	5.0	1	1.5	0

The glycerol solution, 12 months old, prepared with sodium chloride precipitated rennin, which requires 3 to 3.5 hours to curdle 18-hour milk, curdles this same milk in one minute when accelerated by phosphoric acid.

III. VARIABILITY OF MILKS.

The length of time required to curd by the same specimen of rennin seems to be influenced by the length of time the milk is kept after the milking, as already noted under "Action of Phosphoric Acid." There is a slight difference in the degree of acidity of the milk, that which curds the quickest being slightly more acid. Milk which curds very quickly with rennin can be modified to require a greater length of time to curd, by varying its degree of acidity by addition of an alkali. This is shown in the following table:

Milk. Cc.	N NaOH. Cc.	Curd formed in min.
75	0.0	0.5
75	0.6	0.5
75	1.2	13.0
75	1.5	None in 3 hrs.

Other investigators, Smeliansky² and Werncken,³ have noted the effect of acids (other than phosphoric) and alkalies upon the rennin coagulation of cows' milk, and these same investigators, with Van Dam⁴ and Gerber,⁵ have observed the influence of many other substances in this same direction.

The tendency of the milk to curd more quickly the longer it is kept after milking would appear to be of bacterial origin; but if this be the cause, how can we explain why a mixture of rennin and milk kept at 104° F. for several hours does not curd, while if the milk is kept at that temperature for several hours the addition of the same rennin causes very quick curdling? In the latter case the influence of bacterial growth is suggested, and in the first case we also have all the conditions to favor bacterial growth and hasten the curd forming, but such action is not perceptible.

Fuld and Wohlgemuth⁶ have found that cows' milk is more easily curdled after it has been frozen. According to Van Dam,⁷ when milk is heated the acidity increases. My observations in this direction have been with milk 18 to 20 hours after milking; when heated for 2½ hours at a temperature of 104° F., the

¹ Refrigerated.

² *Milchwirtsch. Zentr.*, **4**, 379-381; *Arch. Hyg.*, **59**, 187-215.

³ *Z. Biol.*, **52**, 47-77.

⁴ *Z. physiol. Chem.*, **58**, 295-330.

⁵ *Compt. rend.*, **66**, 552; **68**, 201-207; **145**, 577; **147**, 1320.

⁶ *Biochem. Z.*, **5**, 110-42.

⁷ *Milchwirtsch.*, **5**, 154-155.

acidity was raised from 30 to 40 per cent. in different specimens. With milk 2 hours after milking, kept at 104° F. for 2½ hours, there was no change in the acidity.

The results of these investigations would indicate that acidity to within a limited degree influences the more rapid curdling of the milk by the rennin, but whether the increased acidity is directly of bacterial origin or due to a change in the acidity of the phosphates, will have to be proved by further investigation.

Standardized Rennin to Control Milk Used for Testing the Activity of Rennin.—To control the variability of milk for the assay of rennin it was found necessary to prepare a standard rennin to show whether the milk was fast or slow. This rennin was standardized by testing its action upon not less than 12 different specimens of milk, as daily supplied from a reliable source, and the average taken. Thus, if the rennin is standardized to 1 : 30,000 in 8 minutes, its use as a control with the rennin to be assayed readily shows whether the milk is fast or slow, and allowance is made accordingly. The rennin should be added to the milk as soon as it has the temperature of 104° F., for a longer time before the rennin is added makes it curd more rapidly.

Permanency of Rennin Solutions.—Aqueous solutions of rennin of a density 1.010, containing 0.20 per cent. by volume hydrochloric acid, lost 30 per cent. of their activity in one day at a temperature of 70° F. More concentrated solutions of a density 1.090 per cent., containing 1.30 per cent. by volume hydrochloric acid, were more permanent. Elevation of temperature causes more rapid destruction. The denser the solution, the slower the destruction at an elevated temperature.

Essence of Pepsin.—The results here obtained would suggest the use of the granular scaled rennin and of phosphoric acid in place of lactic acid in the preparation of essence of pepsin. Investigations concerning the making of a permanent rennin preparation are now under way.

BROOKLYN, N. Y.

MICRO-CHEMICAL TESTS FOR THE IDENTIFICATION OF SOME OF THE ALKALOIDS.

By EARL B. PUTT.

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Though the use of certain precipitants for detecting alkaloids has been quite general for a long time, the characteristics of the precipitates so produced do not seem to have received the study which they deserve.

Most schemes of analysis employ these general precipitants to detect the presence of alkaloids and then rely upon various color tests to identify any particular alkaloid, without subjecting the first precipitate to any further examination. If the color tests which are, at present, so extensively applied were always satisfactory and distinctive, there would be little value attached to any other system of identification. But most analysts are familiar with the fact that, in order to secure these color reactions so generally relied upon,

the alkaloids being tested must be in a fairly high state of purity, since the original tests were applied to pure alkaloids: that, in "shaking out" alkaloids by means of immiscible solvents, there is always a considerable amount of foreign matter present in the alkaloidal residue which interferes with many color reactions.

Then again it must be recalled that colorations produced with metallic and many other substances are reactions of the reagent employed and not of the alkaloid. An example of this may be cited in the tests for morphine and codeine with selenous acid.

Finally, the amount of material required to perform a series of color tests on an alkaloidal residue is frequently many times greater than the entire available sample.

A survey of the standard tests for alkaloids indicates that cocaine is the only alkaloid commonly identified by micro-chemical means. The characteristics of its precipitates with such reagents as chromic acid, platinic chloride and potassium permanganate are familiar to all toxicologists and are relied upon for identifying that alkaloid.

While but few alkaloids can be precipitated as easily and in such peculiar forms as cocaine, yet it is safe to predict that a number of very satisfactory tests may be employed in a similar manner for the identification of other alkaloids. With this idea in mind, the writer has been engaged, during the past two years, in an investigation of the precipitates produced by about forty of the alkaloids with some of the common alkaloidal reagents.

The method of procedure differs slightly from that usually employed, in that solution of the alkaloid is made directly on a microscope slide and not in a test tube or flask. In making a microscopic examination of crystals, it is entirely unnecessary to make a solution in a test tube and then use but a few drops for testing. It is also important whether a precipitate is produced in a test tube, the crystals filtered off and then placed on a slide or whether precipitation is made directly on the slide and the precipitate viewed at once through the microscope. The significance of this point will be shown later. A moment's consideration ought to show also that it is just as easy to work with a milligram of alkaloid dissolved in a drop of solvent on a slide as to prepare a 1-100 solution and then use only a drop of the dilution. Moreover, the practical analyst does not have available the large amounts usually prescribed for many of the tests and if he identifies a given alkaloid with a 10 mg. sample that is his good fortune.

In every case the method of procedure in precipitating the alkaloids was the same and essentially as follows: Using a teasing needle or glass rod, transfer a fragment of the alkaloid to a perfectly clean glass slide and drop upon it, from a burette, one drop of $N/10$ HCl. By tapping the end of the slide with the finger, sufficient agitation of the liquid is produced to effect solution of the alkaloid. When all is dissolved, add from a dropping bottle a drop of the appropriate reagent and, without stirring or otherwise