Substance tested.	Impurity tested for.	Delicacy of U. S. P. test.	Delicacy of suggested test.
Acid Phosphoric, diluted	Hypophosphorous	0.007%	
	Nitric	0.03%	
	Phosphorous	over 1%	0.02%
	Sulphuric	0.015%	
Sulphuric Acid	Lead	0.0003–0.0004%	
	Nitric	0.003%	
Tartaric Acid	Oxalic		0.8%
	Sulphuric	0.025%	

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THE CHEMISTRY OF MILK CURD MODIFICATION IN INFANT FEEDING.*

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INTRODUCTION.

The specific intent of this article is an endeavor to explain in chemical terms the action of certain substances commonly used in artificial infant feeding to modify the character of milk curds. While medical literature has given this subject due consideration from a practical and clinical standpoint, the writer has been able to find but little theoretical explanation for the action of these substances. The data presented in this paper are based on experiments *in vitro;* the deductions and recommendations are purely theoretical and are not presented as clinical facts. For years physicians have been adding certain substances to milk to modify the character of the curds and their experience has unmistakably shown that these substances have a beneficial action. However, this knowledge has been almost wholly empirical and it is thought that the presentation of certain theoretical facts concerning the action of these substances will help to place their use on a more logical and satisfactory basis. The suggestions regarding the use of certain substances are based only on their action as milk curd modifiers. For instance, under alkalies where certain quantities of lime water are suggested, this is done with absolute disregard of the controversy as to the necessity or advisability of the addition of more calcium to the infant's food or to its effect on fat metabolism. In this connection, as Brennemann remarks, "The ultimate test of any therapeutic measure in infant feeding is the baby himself, the living, clinical, digesting baby that often laughs at our theories and weeps over our science."

THE OBJECT OF MODIFICATION.

The object of modifying the character of the milk curds is due to the great difference in the physical condition of these curds as obtained from human milk and cows' milk. The curd from human milk is flocculent and each individual curd is very small and in the aggregate resembles extremely fine cottage cheese in a watery condition. When cows' milk is coagulated under the same conditions it forms in three or four large curds. In human milk, where the curd

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is flocculent, the gastric juice can permeate all through the curd and can form hydrochloric acid-casein, in which form the casein is digestible, and, due to the antiseptic action of the gastric juice, it will be able to arrest bacterial activity and lactic acid fermentation. It is axiomatic that the more surface exposed to the gastric juice the more rapid and thorough will be the performance of its proteolytic functions.

When, through some misfortune, it becomes necessary to deprive an infant of its mother's milk, it is then necessary to feed the infant artificially; the most commonly used and logical substitute being cows' milk; and, because of the great differences, particularly in the curds of the two milks, it becomes necessary to so modify the cows' milk that the infant can digest it properly. Nature intended an infant to obtain its nutrition from milk the same as a calf, but it certainly expected it to obtain it in a different physical condition, as is evidenced by the differences in the curds formed under identical conditions and the more diverse difference in the anatomy of the stomachs of the two offspring.

CHEMISTRY OF MILK COAGULATION.

One of the most disputed questions in milk chemistry is whether the caseins from the two milks are identical. This question has arisen from the difference in the physical properties of the curds from the two milks. At the present time the weight of scientific opinion seems to indicate that they are identical and that the difference in the curd formation is due to other concomitant factors, such as the concentration of the caseinogen, acidity of the precipitation medium, the ratio of caseinogen to lactalbumin, the ratio of calcium to citric acid in the milk, etc. The casein from human milk when properly purified has the same nitrogen, phosphorus and sulphur content; the same degree of valency; gives the same series of salts with bases; has the same molecular weight; and is acted upon in the same manner as the casein from cows' milk.[1]

Considerable confusion exists as to the difference in the terminology of casein products used by various writers and it becomes almost imperative that each writer give a key to his articles. The product precipitated from milk by acids is termed *casein;* this term is also used in a collective or general sense. The mother substance of the curd exists in milk in colloidal suspension and is called *caseinogen.* The rennin transformation product is termed *paracasein.* The curd produced by the combined action of calcium and magnesium ions on paracasein (the normal milk curd) is termed *calcium paracaseinate.* While the term magnesium paracaseinate would be equally applicable here, it seems that the literature speaks only of the calcium salt, so that term will be used throughout this article. Rennin is a proteolytic enzyme found in the stomachs of human beings and some animals, and its only function appears to be that of curdling milk. Rennin from the calf's stomach is termed *chymosin,* while that from the human stomach is termed *parachymosin.* These two substances are very similar in action but not identical. All rennin referred to in this article will be *chymosin.*

The coagulation of milk in the stomach is practically identical to its coagulation in the test tube. The time noted for the following changes that take place are those of test-tube observation, but are approximately correct for the stomach. Cows' milk appears to coagulate in the stomach in from three to five minutes.

When milk is taken into the stomach and comes in contact with the gastric juice containing the enzyme rennin, the milk immediately begins to undergo a change, the rapidity of which is dependent upon the temperature and concentration of the rennin and caseinogen. This change is not macroscopically noticeable. In about four or five minutes the viscosity of the milk begins to increase to a noticeable extent, and in a few seconds it will be so viscous that it will not flow; in another few seconds, small curds begin to appear. In an additional few seconds, these small curds begin to cohere to form several large curds. In from three to five minutes these curds start to expel the whey by contraction. In this condition the curds are semi-rubbery and occupy a large percent of the original volume of the milk. The expulsion of the whey probably eliminates the bulk of the gastric juice which might happen to be enclosed when the curds first form. This is the end of coagulation and digestion proper then commences.

The coagulation of milk is accomplished by two distinct factors. The first is the proteolytic action of rennin, that is, the conversion of caseinogen to paracasein. Caseinogen exists in milk in colloidal suspension as submicrons at the rate of from three to six billions per cubic centimeter, and appears to be combined with calcium phosphate.[2] According to Van Slyke & Bosworth,[3] caseinogen is an octobasic acid and appears to exist in milk as calcium caseinogenate (caseinogen Ca_4). Neutral dicalcium phosphate also appears to be colloidally suspended. In whatever condition caseinogen exists in milk it is changed by rennin to paracasein, which substance is insoluble in the presence of sufficient calcium and magnesium ions. The velocity of this rennin activity is probably not affected by any ordinary change in the degree of acidity of the medium.[4] Paracasein in the presence of sufficient calcium or magnesium ions flocculates into curds. When the calcium and magnesium ions are diminished beyond a certain extent the paracasein remains in solution or suspension. The change of caseinogen to paracasein is independent of the presence or absence of calcium or magnesium ions. Experiment No. 1, which is essentially Hammarsten's[5] famous experiment, will clearly demonstrate the function and independent action of rennin and calcium and magnesium ions.

Experiment No. 1.—Two equal sized samples of cows' milk are placed in flasks in a water-bath and heated to 38.5 C., which temperature is maintained throughout the experiment. To one flask is added a certain amount of ammonium oxalate solution, which precipitates all the soluble calcium as insoluble calcium oxalate. Then to each flask is added an equal amount of fresh rennin solution. In about five minutes the flask holding the unaltered milk will be coagulated. Allow the other flask to remain in the bath for an additional thirty minutes to demonstrate that no coagulation will take place, then boil this milk to destroy the rennin, and add a few drops of a solution of calcium chloride; immediately a precipitate of calcium paracaseinate is produced, showing that the presence of calcium is essential for the coagulation of milk. From this experiment it will be seen that rennin is not a coagulating enzyme, but that it is a proteolytic enzyme and that the calcium is the substance that produces the coagulation. This same effect can be produced by a solution of magnesium chloride.

If the same number of paracasein particles and calcium and magnesium ions are confined in a space smaller than that of the original space and all particles in motion, it is evident that the chances of a union between the particles is greater in any given interval; and if the space be increased over the original, the chances of a union will be less in the same interval. In other words, this is the principle of the law of mass action—that the speed of a reaction is proportional to the concentration of the active masses. This is a very important fact in milk curd modification. It has been shown by Brennemann[6] that nascent calcium paracaseinate (true milk curds) has a very powerful coalescing property but this property rapidly disappears. This means that if a certain number of colloidal particles of calcium paracaseinate are formed rapidly there will be a large number of collisions while they are comparatively nascent and having powerful coalescing properties and under these conditions large aggregates are possible. If this same number of colloidal particles are slowly liberated in the same volume, it is evident that collisions will not take place so often, but when they do take place, it will be when one or more of the particles or aggregates has lost to a great extent its coalescing power and by deduction from the natural frequency of coincidence, no large aggregates are possible. Therefore, anything added to milk which delays the formation of calcium paracaseinate is a milk curd modifier because of the rapidly fleeting power of the curds to coalesce. It may be stated that the specific action of coagulation of calcium or magnesium ions on paracasein is due to the ion giving the colloidal particle temporary cohesive properties.

The following procedures are in use for modifying milk curds and are listed, not in the order of their importance, but in the most logical sequence of their presentation:

1. Dilution with water.
2. By the use of gruels and carbohydrates.
3. By boiling the milk.
4. By the use of citrates.
5. By the use of alkalies.

DILUTION WITH WATER.

The theory of the action by simple aqueous dilution is based on the concentration of the active masses—the more dilute, the more slowly are the colloidal particles formed and, as explained before, they then have less coalescing power and hence numerous small curds are the result. The effect on the velocity of coagulation by dilution is given in Experiment No. 2.

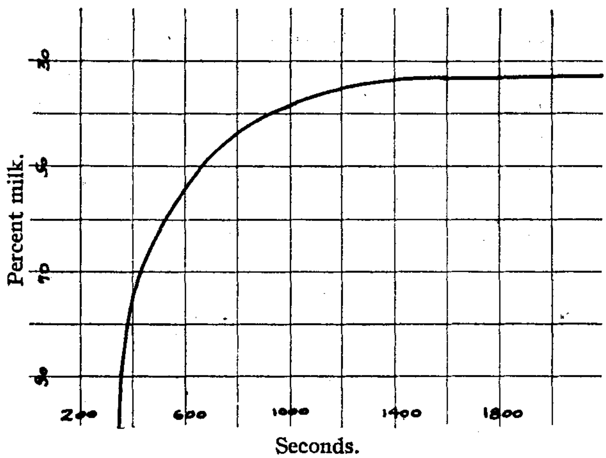
Experiment No. 2.—

1. 100% milk coagulated in 345 seconds.
2. 75% milk coagulated in 380 seconds.
3. 50% milk coagulated in 655 seconds.
4. 33% milk coagulated in 2100 seconds.
5. 25% milk uncoagulated after 2 hours.
6. 12$^1/_2$% milk uncoagulated after 2 hours.

Tests No. 1 and No. 2 gave normal coagulations. While No. 3 coagulated, it was not quite complete, the whey being slightly milky. Small flakes of calcium paracaseinate appeared in test No. 4 in about thirty-five minutes, but after 2 hours only about 10% seemed to be precipitated. The addition of 1 mil of about a five percent solution of calcium chloride to tests Nos. 4, 5 and 6 (200 mils each) produced, immediately, beautiful floccules of calcium paracaseinate.

This data is plotted in Graph No. 1. Here it will be noted that relatively little delay is occasioned unless the milk occupies about fifty percent or less of the volume of the mixture. Obviously this type of modification cannot be practiced without limit, because of the capacity of the infant's stomach. This method produces a curd of very desirable properties. The time noted here for coagulation may not be the same as in the stomach, but the ratio of time to effect is the same.

Graph No. 1.—Dilution of milk with water.



Brennemann[7] has studied the coagulation of milk, plain and modified by various substances, in the stomach, and his contribution is very comprehensive and conclusive in experimental detail and results. He obtained the coöperation of a young adult who could empty the stomach of its contents by "digital irritation of the fauces." This meant delivery of the stomach contents in their actual condition, something that is not true by the use of emetics or the stomach pump. Brennemann's numerous experiments covered practically everything used for milk curd modification and it is most interesting to note that in no case except lactose, where his conclusions were drawn from inference and not experiments, did his conclusions differ from the writer's, wherein all experiments were performed in "test tubes" and mechanical stomachs.[8] Brennemann's conclusion regarding diluted milk is, "The size of the curds varies inversely as the dilution, or directly as the amount of casein."

GRUELS AND CARBOHYDRATES.

The substances from which gruels are commonly made are wheat flour, barley flour, rolled oats and, occasionally, rice and arrowroot. In these gruels the starch is present in the gelatinous or colloidal state—the only condition in which it is capable of modifying the curd. Its action is entirely mechanical, that of interference as a protective colloid, and its effectiveness is proportional to the amount of gelatinized starch present.

In some cases dextrinized gruels are used; these are prepared by adding some form of liquid diastase to the gruels to convert the starch to dextrins and maltose. In this condition the "starch" is more readily assimilated but unfortunately it has lost its effectiveness and is unable to perform the function intended of it.[9]

One manufacturer of an infant food states that when milk is modified with his product the curd formed occludes about thirty percent of the food and that this being rather soluble it is rapidly removed from the curd structure, leaving it porous. This statement has not been affirmed or denied. Brennemann's experiments on carbohydrates were allowed to remain in the stomach only from thirty to sixty minutes and his conclusions are, "Starch concoctions very radically influence the coagulation of milk in the stomach. Carbohydrates, dextrose, milk sugar, cane sugar and maltose have no appreciable influence."

Sugars have practically no effect on the rapidity of curd formation. Table

No. 1 shows the rapidity and physical condition of curd formation in milk to which various sugars have been added.

It is interesting to note that neither dextrose nor maltose have any appreciable effect on the character of the curd, while sucrose has a decided detrimental effect in that it makes the curd semi-rubbery and comparatively difficult to disintegrate. Lactose, the sugar obtained from cows' milk, has a very decided beneficial action. From this, it would seem, all other conditions being equal (purity, etc.), that lactose is the best sugar to use in increasing the carbohydrate content of cows' milk.

The velocity of the coagulation as influenced by sucrose and lactose is practically identical, whereas the character of the curds is very different. This illustrates the point that the *rapidity* of coagulation is not the *only* factor affecting the character of the curd. Each substance has its specific action on the character

TABLE No. 1.—SUGARS.

Part A.—Rapidity of Coagulation of Cows' Milk as Influenced by Sugars.

No.	Substance.	Control.	Grammes.	Seconds to coagulate.			Average.	Percent.
1	Sucrose	343	5.00	380	374	365	373	108.7
2	Lactose	388	5.00	422	415	413	416.7	107.4
3	Dextrose	390	5.00	407	397	405	403	103.3
4	Maltose[1]	355	5.00	302	295	300	299	84.2

Part B.—Effect on the Physical Condition of the Curd by the Sugars from A.

No.	Substance.	Macroscopical condition of the coagulum.
	Controls	Fairly smooth and fairly easily disintegrated.
1	Sucrose	Smooth and semi-rubbery.
2	Lactose	Semi-flocculent and very easily disintegrated.
3	Dextrose	Fairly smooth and fairly easily disintegrated.
4	Maltose	Smooth, but not so easily disintegrated.

NOTES.—Control test is plain cows' milk; the number under "Control" is the number of seconds to coagulate the control test, or 100%.

Each test consisted of 200 mils of cows' milk to which 5 mils of 0.5% rennin solution was added. Rennin 1–30000.

Temperature 37–39° C.

Each test bottle was given a slight inverted motion to simulate in a manner the peristaltic movements of the stomach.

[1] The apparent accelerating action of the maltose is probably due to calcium salts; most samples contain calcium, but before this could be substantiated the sample was accidentally destroyed. This matter will be cleared up at some future date.

of curd formation independent of all other actions. However, it may be stated that, *all other conditions being identical, the character of the curd is dependent upon the rapidity of its formation.* "The solidity of a curd produced by rennet is inversely proportional to the length of time occupied by the coagulation process."[10]

BOILING.

Boiling milk decidedly affects its coagulation; the curds form slowly and are very flocculent and easily digested. It has been shown[11] that rennin coagulation in milk previously heated to 70° C. and cooled, is not delayed; but if the milk is heated beyond this temperature, delay in coagulation occurs somewhat in proportion to the degree of heat and the length of exposure. This has been attributed to milk enzyme destruction which happens to commence at about 70° C., but from experiments conducted, the writer is led to believe that milk enzymes have nothing to do with coagulation. The writer noticed that the formation of scum on milk commences at about 70° C. Certain factors increase the amount of scum formation, such as the degree of heat, length of exposure and movement of the liquid which presents new surfaces to the air, etc. Milk scum is rich in calcium salts, so that it would seem that the retarding effect on the coagulation of milk by boiling is due to the removal of calcium in the scum. The following experiment will demonstrate these facts.

Experiment No. 3.—

1. 200 mils milk, 5 mils rennin solution, 38.5° C.
   Coagulated in 380 seconds.
   Curd normal.
2. 200 mils milk, boiled for 10 minutes and rapidly cooled; strained through cheesecloth; 5 mils rennin solution, 38.5° C.
   Coagulated in 1720 seconds.
   Finely flocculent.
3. 200 mils milk, heated as above with about 0.10 gramme calcium chloride in solution added after cooling; 5 mils rennin, 38.5° C.
   Coagulated in 255 seconds.
   Semi-rubbery.

This removal of calcium salts by the scum causes a slower coagulation due to the decrease in the concentration of calcium and if approximately this amount of calcium is added, coagulation takes place normally.

It is evident from these facts that *correctly* pasteurized milk will coagulate in the same time as raw milk.

Boiling the milk reduces its potential acidity from eighteen degrees to fourteen degrees. This is due principally to the loss of dissolved carbon dioxide.

CITRATES.

Citrates are employed very extensively in England for modifying curd formation but their use is rather limited in America. The effect is all that could be desired of a milk curd modifier, and there can be no objection to their use on the grounds of introducing a foreign substance to the milk, as citrates are normal constituents of both human and cows' milk.

Jerome Alexander[12] explains the action of sodium citrate as a protective colloid and states that "when going into solution actually exhibits actively moving ultramicrons in the ultramicroscope, a fact which indicates its colloidal condition."

Another theory that has been advanced is that the citrate reacts with the hydrochloric acid of the gastric juice to form sodium chloride and citric acid which is a comparatively weak acid in relation to hydrochloric. Since the effect of citrates are observable in test-tube experiments where neither gastric juice nor hydrochloric acid is present, obviously this is not the explanation for their action.

The theory that is proposed by Van Slyke and Bosworth[13] is: "The addition of sodium citrate to normal milk increased the amount of soluble calcium in the milk, this increase resulting from a reaction between the calcium caseinate of the milk and sodium citrate, by which is formed sodium caseinate (or calcium sodium caseinate) and calcium citrate. The reaction is reversible."

According to their theory, it is the calcium paracaseinate which is insoluble or when it is formed the milk coagulates. Calcium paracaseinate (Ca_2 paracasein) is insoluble and is the normal milk curd. Sodium paracaseinate (Na_4 paracasein) and calcium-sodium paracaseinate ($CaNa_2$ paracasein) are soluble by reason of the sodium they contain. When the amount of sodium becomes less than that present in calcium-sodium paracaseinate, the paracasein compound becomes insoluble and coagulation becomes possible.

If this theory were correct, we would expect a citrate to entirely prevent coagulation if it retarded it at all, because the sodium which renders the compound soluble should be active for any reasonable length of time. Experiments show that this is not the case. Also, if it is the sodium of the sodium citrate which renders the paracasein compound soluble, why would not any sodium salt have the same effect?

Table No. 2 shows the effect on the rapidity of coagulation of milk by the use of citrates, acetates, tartrates and chlorides of sodium and potassium, and it will be seen here that the citrates are the only salts that have any marked influence on the rate of coagulation. Any marked difference in the degree of ionization of two calcium salts would cause the equilibrium to be established wherein

TABLE NO. 2.—ALKALI SALTS OF ORGANIC ACIDS AND CHLORIDES.

Part A.—Rapidity of Coagulation of Cows' Milk as Influenced by Acetates, Chlorides, Citrates and Tartrates.

No.	Substance.	Control.	Grammes.	Seconds to coagulate.			Average.	Percent.
1	Potassium Citrate	254	0.100	389	370	375	378	148.8
2	Sodium Citrate	305	0.100	478	460	480	472.7	155
3	Potassium Acetate	390	0.100	395	390	397	394	101
4	KNa Tartrate	372	0.100	380	380	390	383.3	103
5	Sodium Chloride	270	1.00	315	315	315	315	116.7
6	Potassium Chloride	345	1.00	370	370	365	368.3	106.7
7	Calcium Chloride	280	1.00	50	52	58	53.3	19
8	Magnesium Chloride	282	1.00	101	119	103	107.7	38.1
9	Ammonium Chloride	285	1.00	265	273	283	273.7	96

Part B.—Effect on the Physical Condition of the Curd by Acetates, Chlorides, Citrates and Tartrates.

No.	Substance.	Macroscopical condition of the coagulum.
	Controls	Fairly smooth and fairly easily disintegrated.
1	Potassium Citrate	Fairly smooth and fairly easily disintegrated.
2	Sodium Citrate	Slightly flocculent and easily disintegrated.
3	Potassium Acetate	Coarse and rubbery.
4	KNa Tartrate	Fairly smooth and fairly easily disintegrated.
5	Sodium Chloride	Fairly smooth and semi-rubbery.
6	Potassium Chloride	Smooth and fairly easily disintegrated.
7	Calcium Chloride	Smooth and fairly easily disintegrated.
8	Magnesium Chloride	Smooth and rubbery.
9	Ammonium Chloride	Smooth and semi-rubbery.

NOTES:—

For general notes Table No. 1.

Calcium chloride refers to $CaCl_2.2H_2O$. Magnesium chloride, $MgCl_2.6H_2O$.

Whey expulsion from the potassium chloride tests was slow.

the one case there would be more available calcium than in the other. The equilibrium of a reaction is always shifted towards the formation of the slightly ionized compound.[14] If the calcium chloride formed by the equilibrium after the addition of sodium chloride is ionized to a greater extent than the calcium citrate formed by the rearrangement after the addition of sodium citrate, then less sodium would be available for combination with the caseinogen. It does not seem reasonable, however, that this theory alone would explain the marked difference in the action of these two salts.

The writer's theory of the action of citrates is based on the fact that solutions of calcium citrate are but slightly ionized[15] and that if sodium or potassium citrate is added it combines with the bulk of the available calcium to form the slightly ionized calcium citrate; this means a diminution of calcium-ions by fixation, and the concentration of one of the active masses is thereby reduced and hence coagulation takes place more slowly. This demonstrates that it is the calcium-ion that is active in coagulation and that molecular calcium such as calcium citrate has but little effect on coagulation. As far as availability for coagulation is concerned it might as well be precipitated out of solution. This theory explains every known condition of the action of citrates in experimental work and is in harmony with the theoretical action of all other modifying agents.

By examination of Table No. 2 it is noted that sodium citrate has slightly greater retarding power than the potassium salt. The sodium salt contains more citrate radical per gramme than the potassium salt, but the ratio is greater than their retarding action on coagulation. Potassium acetate has practically no retarding influence on coagulation yet the curd is coarse and rubbery; here again is the specific action probably of the acetate radical. The tartrate has apparently no action and the chlorides of potassium and sodium have little retarding effect, although sodium chloride had a slight detrimental effect on the character of the curd. Both calcium and magnesium chlorides greatly accelerated the coagulation. The calcium chloride acted about twice as fast as the magnesium chloride, but the curd produced was not very much different from the control milk curd, while the magnesium chloride curd had very undesirable properties. This would indicate that the specific action of calcium salts is far better than that of magnesium salts on the physical condition of the curds.

Citrates slightly reduce the potential acidity of the milk; this is due to the increased solubility of calcium phosphate in citrate solutions or to the formation of sodium phosphate which hydrolyzes alkaline.[4]

The addition of two grains of sodium citrate to the ounce of milk, which is the proportion usually recommended, will prevent practically all coagulation of milk in the stomach.

Brennemann's conclusions in regard to the action of citrates are that "sodium citrate has a very marked influence on casein coagulation, both delaying and altering it."[7]

ALKALIES.

Of all the methods used for curd modification, alkalies have by far been given preference. In this connection, Brennemann has aptly stated that "It is gratifying to note that a procedure that has enjoyed so many years of confidence should seem to have a rational as well as an empirical basis."[7]

About the only statement explaining the action of alkalies that the writer can find in medical literature is that they reduce the acidity of the milk. This is certainly true, but it is an indirect statement and not an explanation. All alkalies act by precipitating calcium salts[16] and when precipitated they are not capable of reacting with the paracasein and we have a reduction of potential calcium ionization or available calcium. The alkalies generally employed are sodium and potassium bicarbonates, milk of magnesia and lime water.

Sodium Bicarbonate.—This substance, although formerly used extensively, has of late years fallen into disuse. For the quantitative effect of sodium bicarbonate and other alkalies see Table No. 3. It will be noted that on the same alkalinity basis sodium bicarbonate has less retarding effect than any of the

Experiment No. 4.—

1. 200 mils milk, 5 mils rennin solution at 38° C.
   Coagulated in 450 seconds.
   Curd—normal.
2. 200 mils milk, saturated with carbon dioxide by passing the gas in a steady stream through the milk; chilled by ice for 20 minutes just previous to coagulation; 5 mils rennin solution at 38° C. Coagulated in 90 seconds.
   Curd—small and rubbery.

Further verification of this action can be noted by just neutralizing a weak solution of calcium hydroxide (sucrose) with phosphoric acid; phenolphthalein as indicator; chilling this moderately and passing a stream of carbon dioxide through this magma; filtering and passing a stream of carbon dioxide-free air through the filtrate; in a few minutes the filtrate will become cloudy due to the precipitation of calcium phosphate by the removal of the loosely held carbonic acid.

TABLE No. 3.—HYDROXIDES AND BICARBONATES.

Part A.—Rapidity of Coagulation of Cows' Milk as Influenced by Hydroxides and Bicarbonates.

No.	N. O. 100 V. S.	Control.	Mils.	Seconds to coagulate.			Average.	Percent.
1	Sodium Hydroxide	378	7.46	920	928	925	924.3	244.5
2	Potassium Hydroxide	376	7.46	975	965	950	963.3	256.2
3	Ammonium Hydroxide	380	7.46	903	865	880	882.7	232.3
4	Magnesium Hydroxide[2]	367	7.46	640	625	630	631.7	172.1
5	Calcium Hydroxide	291	16.43[1]	448	443	440	443.7	152.4
6	Sodium Bicarbonate	345	7.46	425	418	423	422	122.3
7	Potassium Bicarbonate	400	7.46	502	515	502	506.3	126.6

[1] Equivalent to 7.46 mils N. O. 100 V. S.

[2] Magnesium hydroxide in suspension.

Solutions standardized with methyl-orange as indicator.

Part B.—Effect on the Physical Condition of the Curd by Hydroxides and Bicarbonates.

No.	Substance.	Macroscopical condition of the coagulum.
	Controls	Fairly smooth but not so easily disintegrated.
1	Sodium Hydroxide	Semi-flocculent and very easily disintegrated.
2	Potassium Hydroxide	Semi-flocculent and very easily disintegrated.
3	Ammonium Hydroxide	Semi-flocculent and very easily disintegrated.
4	Magnesium Hydroxide	Fairly smooth and fairly easily disintegrated.
5	Calcium Hydroxide	Fairly smooth and fairly easily disintegrated.
6	Sodium Bicarbonate	Smooth and fairly easily disintegrated.
7	Potassium Bicarbonate	Fairly smooth but not so easily disintegrated.

NOTES:

For general notes see Table No. 1.

other alkalies. This is due to the action of the dissolved carbonic acid, liberated from the bicarbonate by the acid phosphates of the milk, on the secondary calcium phosphate probably forming a very unstable double salt of calcium (soluble) ($Ca.H_2PO_4.HCO_3$) analogous to the formation of calcium bicarbonate. This means an increase of available calcium. Experiment No. 4 will demonstrate this action.

Both Brennemann[7] and Southworth[17] have noted the excessive liberation of gas in the form of eructions from milk modified with bicarbonates; this is due partly to the dissolved carbon dioxide and partly to the unstable compound above referred to, breaking down by heat and liberating carbonic acid.

The effect on the potential acidity of milk by sodium bicarbonate is as follows:

100 mils milk—18.5° acidity and 0.100 $NaHCO_3$	13.1° acidity
100 mils milk—18.5° acidity and 0.100 $NaHCO_3$—after reaching 38° C.	13.1° acidity
100 mils milk—18.5° acidity and 0.100 $NaHCO_3$ 20 minutes at 38° C.	11.4° acidity

Phenolphthalein indicator.

This decrease of acidity by heat is due to the decomposition of the sodium bicarbonate with the formation of normal sodium carbonate. For this reason, if milk is to be pasteurized or sterilized and modified with sodium bicarbonate, the bicarbonate must be added after cooling, otherwise the mixture will be strongly alkaline.[18]

Potassium Bicarbonate.—Whatever applies to sodium bicarbonate applies equally to the potassium salt. From Table No. 3 it will be seen that the quantitative effects are practically identical. Potassium bicarbonate seems to be more stable in solution than the sodium salt, although both change alkalinity on standing or heating. In precipitating calcium salts (reducing the acidity of milk) bicarbonates act according to the following equation:

$$2NaHCO_3 + Ca(H_2PO_4)_2 = \underline{CaHPO_4} + Na_2HPO_4 + 2H_2CO_3$$

There are so many disadvantages in the use of bicarbonates that their employment should be discouraged and abandoned.

Sodium and Potassium Carbonates.—No data can be found where these salts have been used except in proprietary malt foods. On purely theoretical grounds, their use would be better than the corresponding bicarbonates because they will not increase in alkalinity on standing. If their use is thought desirable, it is recommended that nothing but sodium carbonate monohydrated be used, as this is the only salt the physician may expect to be dispensed in a uniform manner.

Hydroxides.—The commonly used hydroxides are lime water and milk of magnesia. Sodium and potassium hydroxides could just as well be used and even ammonia water. The main difficulty regarding the use of the three latter substances is due to the lack of uniformity in dispensing such unstable products. This practical point has been demonstrated by analyses of solutions purchased for this purpose. From the analyses, it is certain that if a physician desires to use either sodium or potassium hydroxide he must be assured the product dispensed is *assayed and adjusted to a definite standard,* preferably a one percent solution. Because of the apparent usefulness of these products, definite figures will be presented for those who desire to use them.

The use of alkalies has been on a very irrational basis. Most physicians add certain quantities of alkalies on a percentage basis of the total milk mixture regardless as to whether it is whole milk or diluted milk.[18,19] In some cases this method has resulted in the use of equal volumes of lime water and milk. While it is true that the modification of milk with alkalies, as has been practiced in the past, has been in most cases successful, their use on a definite basis would bring even happier results and would certainly clarify clinical data in this respect.

The use of alkalies has been based on the difference in acidity of cows' milk and human milk, cows' milk being about six times as acid as human milk. Human milk has three degrees acidity while cows' milk has on an average eighteen degrees acidity. A degree of acidity is equivalent to 1 mil of normal acid per litre. By the use of the following equations, a physician will be able to feed a milk mixture of any desired degree of acidity.

Sodium Hydroxide.—Sodium hydroxide neutralizes milk and precipitates calcium salts according to the equation

$$Ca(H_2PO_4)_2 + 2NaOH = \underline{CaHPO_4} + Na_2HPO_4 + 2H_2O.$$

1.92 minims of a 1% sodium hydroxide *w/v* solution will reduce the acidity of 1 fluidounce of milk 1 degree. If it is desired to reduce 16 fluidounces of milk to 3 degrees, multiply the number of minims that will reduce 1 fluidounce 1 degree by the number of degrees to be reduced and by the number of ounces of milk; thus—(15 degrees acidity to be reduced)

$$1.92 \times 15 \times 16 = 461 \text{ minims } 1\% \text{ NaOH solution required.}$$

In reducing the acidity of diluted milk mixtures, the above equation is not applicable because water added to milk will reduce the acidity of the mixture by simple dilution. Because this acidity reduction by dilution deviates from the mathematically calculated acidity, a correction factor must be introduced. These factors are derived from graph curves in an article by the writer in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, July 1919, and those required will be enumerated in this article as needed.

The equation for diluted milk mixtures is:

$$\left(\frac{m \times 18}{m + w}\right) \times \text{factor} - 3 = d$$

$d(m + w)\,1.92$ = minims 1% NaOH solution to add.

m = number of fluidounces of whole milk, top milk or cream in formula.

18 = degrees of acidity of whole milk, top milk or cream in formula.

w = number of ounces of water or gruels in formula.

Factors for Sodium Hydroxide.—

If the milk occupies about 75% of the volume of the mixture, the factor is......0.96

If the milk occupies about 60% of the volume of the mixture, the factor is......0.93

If the milk occupies about 50% of the volume of the mixture, the factor is......0.91

If the milk occupies about 33% of the volume of the mixture, the factor is......0.86

If the milk occupies about 25% of the volume of the mixture, the factor is......0.84

In all but clinical experimentation these factors can be disregarded.

3 = number of degrees of acidity of finished milk mixture desired; if six degrees acidity are desired, 6 is substituted for the 3, etc.

d = number of degrees of acidity to be reduced.

Example.—It is desired to reduce a mixture of $^1/_2$ pint of milk and $^1/_2$ pint of water to 5 degrees acidity.

$$\left(\frac{m \times 18}{m + w}\right) \times \text{factor} - 5 = d.$$

$$\left(\frac{8 \times 18}{8 + 8}\right) \times 0.91 - 5 = 3.19 \text{ degrees (practically 3.2)}.$$

$d(m + w)1.92$ = minims of 1% NaOH solution to be added.
$3.2 \times 16 \times 1.92$—98 minims of 1% NaOH solution to be added.

The acidity of milk of individual cows may vary somewhat from eighteen degrees, but the writer has titrated hundreds of samples of market herd milk and has not found any samples outside the limits of seventeen to nineteen degrees. The literature reports milks varying in acidity over a wide range but from the writer's experience he is led to believe this is due to a lack of uniformity in the methods used by the different chemists. Factors that do not affect an ordinary titration vitally affect a milk acidity titration. This matter was taken up in technical detail by the writer in a former article.[4]

Potassium Hydroxide.—All that applies to sodium hydroxide applies equally to potassium hydroxide. 2.69 minims of a 1% potassium hydroxide *w/v* solution will reduce the acidity of 1 fluidounce of milk 1 degree. Factors for potassium hydroxide are practically identical to those for the sodium hydroxide. All the other values of the equations are the same.

Ammonia Water.—No figures are given as it is thought desirable to use other hydroxides. Attention is called to the slightly less retarding effect of ammonia water as compared to sodium and potassium hydroxides (Table No. 3); this is due to the acid hydrolysis of the ammonium salts. See Table No. 2 and compare ammonium chloride to potassium and sodium chloride.

Lime Water.—This time-honored pharmaceutical equals, if not exceeds in popularity, milk of magnesia for milk curd modification. It seems paradoxical that if we desire to reduce the calcium-ions in milk this is actually accomplished by the addition of further calcium-ions in the form of lime water. However, it is not the calcium of lime water but the *hydroxyl* that functions here. This action is as follows:

$$Ca(H_2PO_4)_2 + Ca(OH)_2 = \underline{2CaHPO_4} + 2H_2O$$

or

$$Ca(H_2PO_4)_2 + 2Ca(OH)_2 = \underline{Ca_3(PO_4)_2} + 4H_2O$$

This shows that lime water acts as all other alkalies by precipitating calcium, which reduces the concentration of one of the active masses required for coagulation.

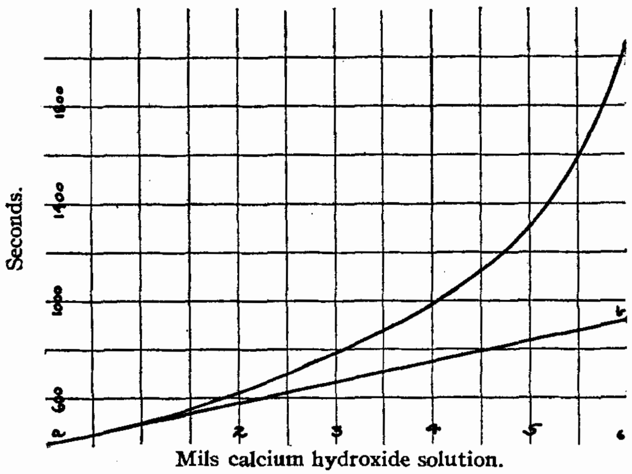
Since lime water contains only about $^1/_6$ of 1% calcium hydroxide, its proper use necessitates a rather large volume, and this is the chief objection to lime water. It is reduced in strength by the carbon dioxide in the air, but has little action on glass vessels. In this particular, it is far superior to sodium or potassium hydroxide solutions. Lime water is more uniform in composition and its action is more definite than any alkali used at the present time, milk of magnesia included. For the quantitative effect of lime water see Chart No. 3; this shows that about one-half ounce is equivalent to one and one-half grains of either sodium or potassium citrate.

A solution of calcium hydroxide was used assaying 1.357% $Ca(OH)_2$ *w/v*;* this was used to study the action of calcium hydroxide in a more concentrated

*Calcon.

form. For the effect on milk coagulation of progressively increasing amounts of this solution see Graph No. 2.

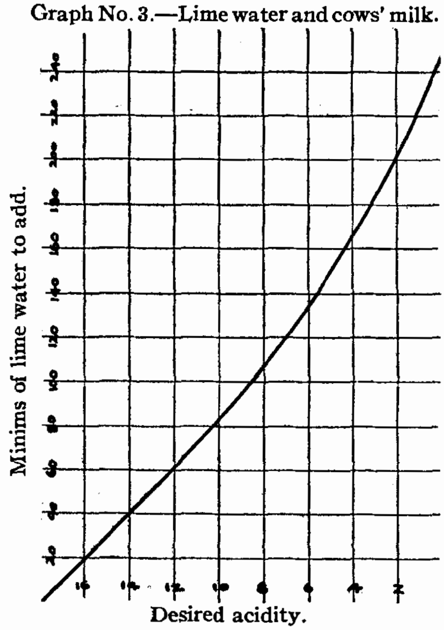
Graph No. 2.—Rate of coagulation.



Notes.—Milk: mixed herd. Specific gravity at 15 C 1.034. Fats, 3.9%. Acidity, 16.7 degrees
Rennin: 0.5% solution of 1–30000. Calcium hydroxide solution: Calcium hydroxide (sucrose) solution assaying 1.357% $Ca(OH)_2$; this minimizes aqueous dilution. *a–b:* Mathematical retardation.

Dr. T. Wood Clarke, of Rockefeller Institute, has contributed a very comprehensive paper on the effect of certain modifying agents on the gastric digestion of infants.[20] Dr. Clarke experimented on babies by feeding various mixtures and pumping the stomach after set intervals and analyzing the contents. His conclusions in regard to lime water are as follows: "Lime water itself appears not to act as is generally accepted in practical pediatrics, by reducing the acidity of the child's stomach. While unquestionably neutralizing a portion of the hydrochloric acid, the alkali stimulates a further secretion of gastric juice." This is certainly true of lime water *itself* (unaccompanied by milk) but it certainly is not true in regard to milk containing lime water. From five to ten percent of milk mixtures usually consist of lime water and there can be no free calcium hydroxide present in such mixtures to act on the gastric juice as such mixtures are still quite acid. Fifty-one percent of lime water (0.163% $Ca(OH)_2$) is required to neutralize, not alkalinize, fresh cows' milk. The stomach strives to maintain a certain normal degree of acidity during digestion and if we feed a food less acid than the contents of the stomach this momentarily reduces the acidity by simple dilution and then the stomach secretes more acid to maintain its normal acidity. Hawk[21] has shown in this manner that even water will stimulate the flow of gastric juice proportional to the amount of water ingested.

Graph No. 3.—Lime water and cows' milk.



Lime water does not delay the coagulation of milk quite as much as does milk of magnesia, but the character of the curds formed in each case seems to be identical. Brennemann[7] concludes in respect to lime water: "Subjectively they (the curds) are described as 'the softest yet' and the returned stomach contents were said to have a very sweet taste that was present in no other experiment. The curd of milk to which lime water has been added would thus seem, on account of its thin ribbon-like, porous character, to be a peculiarly favorable one for the action of the digestive juices." In this connection Helmick states that he believes the reason some physicians fail to obtain satisfactory results with lime water is that they do not use it in sufficient quantities.

Because of its bulkiness compared with its alkalinity, lime water reduces the acidity of the milk by both simple dilution and chemical action. Due to this double action one cannot calculate the amount of lime water required except by the use of experimental data plotted in the form of graph curves. Graph No. 3 gives the number of minims of lime water necessary to *add to* 1 fluidounce of undiluted whole milk to reduce the acidity of the *resultant mixture* to the desired degree of acidity.

Example.—16 ounces whole milk are desired to be reduced to 12° acidity. The chart shows that it requires 62.4 minims for 1 ounce; therefore, $62.4 \times 16 = 998$ minims of lime water to be added. This gives a total volume of 18 ounces of 12° acidity.

When lime water is used in diluted milk formulas, the equations given under sodium hydroxide for diluted milk are used by substituting the lime water values for the sodium hydroxide values. 15.0 minims of lime water (based on 0.163% $Ca(OH)_2$) will reduce the acidity of 1 fluidounce of cows' milk 1 degree.

Factors for Lime Water.—

If the milk occupies about 75% of the volume of the mixture, the factor is....0.96
If the milk occupies about 60% of the volume of the mixture, the factor is....0.94
If the milk occupies about 50% of the volume of the mixture, the factor is....0.92
If the milk occupies about 33% of the volume of the mixture, the factor is....0.77
If the milk occupies about 25% of the volume of the mixture, the factor is....0.71

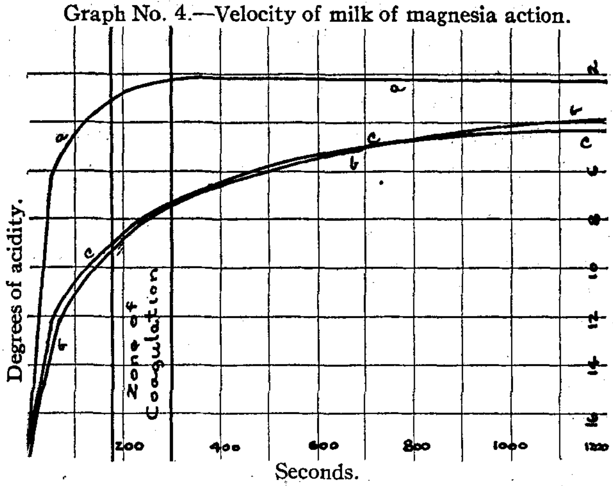
Milk of Magnesia.—For its quantitative action see Chart No. 3. The alkalinity of milk of magnesia is due to magnesium hydroxide in suspension. The fact that the active substance is in suspension and not in solution is the chief objection to this product. This explains its somewhat sluggish action. It is axiomatic that the more surface of the magnesium hydroxide exposed to the milk, the more quickly they will react with each other.[22] This is the reason that the more colloidal the magma is the more desirable it is for this purpose.

Although there is a standard for this product in the Pharmacopoeia, manufacturers seem to pay little attention to it. The Pharmacopoeia permits a variation of 6.5% to 7.5% $Mg(OH)_2$. Actual analyses of the most widely sold brands, and products from the most reliable pharmaceutical houses, vary from 5.22% to 9.79%.[23] It is evident, then, that a physician can never be certain of uniformity of milk of magnesia, not even by using only one brand, although the variation in strength in this case may not be as great as the above figures.

The ideal milk of magnesia for milk curd modification is a product containing a definite amount, between 1.5 to 2%, of magnesium hydroxide and so precipitated that the particles are almost colloidal in size. Such a product is a thick, translucent, viscous fluid which shows little water separation on long standing. The time will come when such a product as this will be demanded by the physicians and the public in preference to our present-day magmas.

Graph No. 4 shows the activity of such a product compared to a well-known proprietary brand and a standard pharmaceutical house product. It will be noted that this semi-colloidal product practically assumes maximum activity in about 300 seconds as compared with about 900 seconds for the other brands. As milk coagulates in the stomach in from three to five minutes, milk of magnesia should be made so as to exhibit as much alkalinity as possible in this interval. In these graph tests, all the magmas were mixed with the milk in the proportion to obtain three degrees acidity; this condition will probably not be reached in the case of the two market brands for about five hours, whereas the semi-colloidal magma has reached it in about 300 seconds. Where a day's feedings are mixed at one time this sluggish action is negligible except on the first feeding, because after that, sufficient time has elapsed for the reaction to take place. Quite a large number of mothers feed the infants on extemporaneously made mixtures, and in this case this condition becomes important. In this connection the writer does not want to be understood as condemning the present-day magmas as being inefficient—quite the contrary—they are efficient—but the point desired to be made is, that a magma made in a more colloidal condition would be more efficient and give better satisfaction generally.

Graph No. 4.—Velocity of milk of magnesia action.



Notes.—a, semi-colloidal magma; *b*, proprietary brand; *c*, pharmaceutical house product.
Zone of coagulation: Approximate interval of time for milk to coagulate in stomach under normal conditions.

It would seem from the wide variation in strength and its sluggish action that milk of magnesia could be much better substituted by one of the soluble alkalies such as potassium or sodium hydroxide or lime water. The only case where this would not be feasible is where the physician wants a laxative as well as an alkaline effect.

Milk of magnesia neutralizes milk as follows:

$$Ca(H_2PO_4)_2 + Mg(OH)_2 = \underline{CaHPO_4} + MgHPO_4 + 2H_2O$$

–or–

$$3Ca(H_2PO_4)_2 + 6Mg(OH)_2 = \underline{Ca_3(PO_4)_2} + 2Mg_3(PO_4)_2 + 12H_2O$$

The apparent weakness of lime water and milk of magnesia as compared with sodium and potassium hydroxides (Table No. 3) is due to the fact that a larger quantity of $\frac{N}{10}$ $Ca(OH)_2$ is required than of $\frac{N}{10}$ NaOH to neutralize milk. For the explanation of this phenomenon see reference No. 4.

For the quantitative effect in reducing milk acidity the equations under sodium hydroxide, both for whole and diluted milk, are correct for milk of magnesia after substituting the milk of magnesia values for the sodium hydroxide values.

0.236 grain milk of magnesia 7%* will reduce the acidity of 1 fluidounce of cows' milk 1 degree.

* By calculation the figures for any strength magma may be obtained.

Factors for Milk of Magnesia.—

If the milk occupies about 75% of the volume of the mixture, the factor is.....0.90
If the milk occupies about 60% of the volume of the mixture, the factor is.....0.84
If the milk occupies about 50% of the volume of the mixture, the factor is.....0.80
If the milk occupies about 33% of the volume of the mixture, the factor is.....0.77
If the milk occupies about 25% of the volume of the mixture, the factor is.....0.69

In clinical observations milk of magnesia must be weighed, as the error in measuring is quite considerable. This is impracticable for the mother at home; here, a weaker product such as the semi-colloidal magma would lessen the error from inequalities of measurement.

In connection with milk of magnesia, I cannot help but make a therapeutic trespass and call attention to the so-called calcium-magnesium balance in the body. It seems that the quantity of calcium and magnesium in the system is a constant and if we increase the consumption of one, we automatically diminish the other. In this connection, Benedict says that "magnesium forces calcium from the system and hinders the calcium retention necessary for bone building. This might be a highly undesirable effect from the repeated administration of milk of magnesia to infants."[24]

SUMMARY.

1. The object in modifying cows' milk is due to the great difference in the physical condition of the curds from it and human milk.

2. The caseins from the two milks probably are identical.

3. Milk is coagulated by the combined action of rennin and calcium-ions, each acting independent of the other.

4. Freshly formed curds have a powerful cohesive property, which property is rapidly discharged.

5. Diluting the milk with water modifies the curd by decreasing the concentration of the active masses.

6. Gruels or cereal decoctions act as protective colloids by virtue of the gelatinized starch present.

7. Sugars, except lactose, have little action on the character of the curd. Lactose seems to favorably modify it.

8. Boiling the milk profoundly alters the character of the curd by the removal of calcium salts by scum formation.

9. Citrates modify milk curd formation by fixation of calcium-ions.

10. All alkalies act by precipitating calcium salts; thereby reducing the concentration of one of the active masses.

11. Sodium or potassium hydroxides are desirable modifying agents but their purchase must be attended with care.

12. Lime water acts like all other alkalies by precipitating calcium. The curd from lime water has very desirable properties.

13. Milk of magnesia has practically the same effect on curd formation as lime water, but its action is slower.

No single method of modification produces the *ideal* curd. This can only be accomplished by a combination of dilution to the proper caseinogen concentration, the addition of a citrate to establish the correct calcium-citrate ratio, the adjustment of the mineral constituents, the addition of an alkali to reduce the acidity and properly stimulate gastric secretion, and by the addition of a protective colloid to establish the correct casein-lactalbumin ratio.

Before these combinations can be studied certain mechanical defects in artificial stomachs must be overcome and then the data obtained can be applied to clinical experimentation and then simplified for practical application. Until these matters are solved the modification of milk coagulation will remain as it is on an unscientific and unsatisfactory basis.

In conclusion the writer desires to express his appreciation to Dr. Arthur G. Helmick, of Columbus, Ohio, for many helpful suggestions and information regarding clinical experiences.

FEBRUARY 25, 1921,
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THE RELATIVE ACTIVITY OF DIFFERENT PARTS OF THE DIGITALIS PLANT.*

BY GEO. E. ÉWE.

The U. S. P. Ninth requires that official digitalis consist of "the carefully dried leaves of Digitalis Purpurea Linné (Fam. Scropulariaceae), without the presence or admixture of more than 2 percent of stems, flowers or other foreign matter. If made into the official tincture and assayed biologically, the minimum lethal dose should not be greater than 0.006 mil of tincture, or the equivalent in tincture of 0.0000005 Gm. of Oubain, for each gramme of body weight of frog."

Determinations of activity have proven that the separate parts of the plant individually answer the U. S. P. requirements for minimum lethal dose.

In order to ascertain the relative proportions of the different parts of the

* I am indebted to Dr. P. S. Pittenger and Arnold Quici for the careful tests of physiologic activity of the various tinctures mentioned in this paper.