of water is added. The gallium is reprecipitated as before, and the precipitate is filtered off upon the original filter paper, ignited, and weighed as Ga_2O_3. It was found that if the gallium is not reprecipitated when zinc is present a somewhat high result is obtained, but this is obviated by a single reprecipitation.

Expts. 9 and 10, Table II, are of special interest, because in the former the gallium was reprecipitated 3 times, and in the latter twice, the results showing that this precedure does not result in any loss of gallium.

TABLE II.

Quantitative Estimation of Gallium Precipitated by Ammonium Acid Sulfite.

Expt.	Amount $ZnCl_2$ taken. G.	Amount Ga_2O_3 taken. G.	Amount Ga_2O_3 found. G.	Error. G.
1	0	0.0202	0.0203	0.0001+
2	0	0.0236	0.0235	0.0001—
3	0.1	0.0202	0.0199	0.0003—
4	0.1	0.0202	0.0203	0.0001+
5	0.35	0.0151	0.0149	0.0002—
6	0.3	0.0202	0.0201	0.0001—
7	0.1	0.0236	0.0236	0.0000±
8	0.1	0.0236	0.0239	0.0003+
9	0	0.0472	0.0471	0.0001—
10	0	0.0236	0.0234	0.0002—

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE DEPARTMENT OF NUTRITION, OHIO AGRICULTURAL EXPERIMENT STATION.]

THE MODIFIED BENEDICT METHOD FOR THE ESTIMATION OF SULFUR IN FEEDS, FECES AND FOODS.[1]

BY J. O. HALVERSON.

Received July 21, 1919.

In the estimation of total sulfur in solid organic matter, the Wolf and Osterberg procedure[2] of adding fuming nitric acid in the preliminary oxidation and solution of the material (before final and complete oxidation with the Benedict reagent[3]) offers two great difficulties which prevent its general availability for those classes of substances for which the official peroxide method[4] is applicable. First, the use of large amounts (50 to 75 cc.) of fuming nitric acid in Kjeldahl flasks heated over a free flame is tedious, long, and effect solution with difficulty. Second, the

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[1] An abstract of the work included in this paper was presented at the Cleveland meeting of the American Chemical Society, Sept. 12, 1918.

[2] *Biochem. Z.*, **29**, 428 (1910).

[3] The Benedict reagent consists of 200 g. of crystallized copper nitrate, sulfur-free or of known sulfur content; 50 g. of potassium or sodium chlorate and distilled water to make 1000 cc.

[4] Report of the Committee on Editing Tentative and Official Methods of Analysis of the Association of Official Agricultural Chemists, **1915**, p. 32.

high values for blank determinations which are usually obtained when the amount of barium sulfate estimated on the basis of the ordinary charge is relatively small, offer another serious objection.

The length of this process, the constant care and attention required and the large quantities of reagents called for led the writer to investigate the possibility of adapting the Wolf and Osterberg procedure to the above mentioned classes of substances by simplifying the technique of rendering the charge soluble and completing the oxidation with the Benedict reagent without loss by spattering upon ignition; and by reducing to a minimum the values of blank runs on reagents.

The objection to the Wolf and Osterberg procedure on materials which are as difficult to render soluble as feeds and feces has been overcome and the proposed modified method has been in active use during the past year.

It has been shown that concd. nitric acid[1] alone with the Benedict reagent gives low results, probably due to the incomplete oxidation of all sulfur to sulfate. Wolf and Osterberg[2] therefore proposed the use of fuming nitric acid to complete the solution, followed by the Benedict reagent[3] to finish the oxidation. Twenty cc. of fuming nitric acid is added to the material in a 300-cc. round-bottom Kjeldahl flask. The flask is then heated over a small, *free* flame, and additional quantities of acid are added to effect *complete* solution. In practice, however, the charges are as a rule slowly heated over the free flame to dryness, with charring, and additional successive portions of fuming acid added with heating until approaching dryness no longer causes charring. The process requires careful and constant attention.

Our adaptation and modification of this tedious and difficult process consists in first softening and disintegrating the material in the flask with 10 to 15 cc. of hot water and allowing it to stand overnight at room temperature, or hot water or steam, the mouth of the flask being closed with a small glass funnel. After 10 hours of digestion, 20 cc. of a mixture[4] of fuming nitric acid and conc. nitric acid (1 : 4) is added, and the digestion at steam temperature is allowed to proceed continuously, with occasional shaking, for 24 hours.[5] Another portion of 10 or 15 cc. of mixed acid is then added and digestion is continued 15 hours longer until solution is almost complete. Small, resistant, protein-like residues may remain. When continued digestion appears to have no further effect, the solution is transferred in portions to a 60-cc. evaporating dish on the steam bath

[1] *Biochem. Z.*, **9**, 307 (1908).

[2] *Loc. cit.*

[3] S. Benedict, *J. Biol. Chem.*, **6**, 363 (1909).

[4] Conc. nitric acid containing $^1/_4$ its volume of fuming nitric acid—80 cc. of the latter to 320 cc. of conc. nitric acid.

[5] Or two days when steam is off at night.

and the Benedict reagent is added. The complete solution of feeds and feces with fuming nitric acid is not attempted. Almost complete solution is, however, effected with a diluted fuming nitric acid, and oxidation is then completed with the Benedict reagent. Occasionally it is necessary to add a third portion of 10 cc. of the weak fuming acid mixture.

This process does not require close or constant attention. After evaporation to complete dryness, the residues are gradually ignited. The blanks will sputter upon ignition, but this can be prevented by first adding 5 cc. of a 10% sucrose solution, followed by evaporation on the steam bath to complete dryness before heating. Sputtering upon ignition is due to a large excess of the Benedict reagent, in comparison with the amount of organic matter present. On the other hand, too much organic matter in proportion will cause a hard, dry crust to form without fusion. The approximate proportions are given later.

The cause of high blank determinations was found to be due to sulfate in the copper nitrate. The red fuming nitric acid contained none. Various samples of copper nitrate were then tested. One sample from a chemical manufacturer gave a blank of 0.0044 g. barium sulfate per 20 cc. of the Benedict reagent. Almost sulfur-free Benedict reagent was made from Kahlbaum's cupric oxide, pea size, and from Kahlbaum's cupric oxide wire rods, "Zur Analyse."[1] This suggested that sulfur-free copper nitrate[2] may be made from pure sheet copper.

The modified Benedict method as developed by us was checked by the modified official peroxide method of Krieble and Mangum.[3] The latter was first checked by the official peroxide method[4] (see Table I).

The method of Krieble and Mangum was chosen because with it results were more uniform and the limits of variation were narrower. It allows considerable exactness of detail; constant amounts of sodium carbonate and sodium peroxide are weighed out for each determination; the acid used in precipitation of the barium sulfate is 1.6 to 2.1 times the strength recommended by Folin.[5] After precipitation and standing in the cold for two days, the solutions are evaporated to dryness on the steam bath, and the residue is then taken up with 300 cc. of water and 2 cc. of conc. hydrochloric acid. This gives a granular precipitate.

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[1] They contained 0.0009 and 0.0007 g. $BaSO_4$, respectively, per 20 cc. of Benedict reagent; J. T. Baker Chemical Co.'s conc. nitric acid was used.

[2] Later it was found that Baker and Adamson, Easton, Pa., furnish a sulfate-free grade of copper nitrate giving a blank of 0.0008 g. $BaSO_4$ per 20 cc. of the Benedict reagent.

[3] V. K. Krieble and A. W. Mangum Method, personal communication. Also THIS JOURNAL, 41, 1317 (1919).

[4] U. S. Dept. Agr., Bur. of Chem., *Revised Bull.* 107, 23.

[5] Otto Folin, *J. Biochem.*, 1, 147 (1906).

TABLE I.

Results by the Official Peroxide and the Modified Peroxide Methods.

Sample.	Quantity. G.	$BaSO_4$. Less Blank.[1] G.	Sulfur.	
			G.	%.
	Official Method			
Milk 1403	23.85	0.0525	0.0072	0.030
	23.90	0.0516	0.0071	0.030
	24.28	0.0465	0.0064	0.026
	24.45	0.0490	0.0067	0.028
Av.	...	....	....	0.028
	Modified Method			
	25.02	0.0518	0.0071	0.029
	24.90	0.0513	0.0071	0.028
	23.89	0.0469	0.0064	0.027
Av.	...	....	....	0.028
	Modified Method			
1404	...	0.0623	0.0086	0.035
	...	0.0614	0.0084	0.035
	...	0.0619	0.0085	0.033
Av.	...	....	....	0.034
	Modified Method			
1405	...	0.0526	0.0072	0.028
	...	0.0524	0.0072	0.030
	...	0.0500	0.0069	0.028
Av.	...	....	....	0.029

The tendency of the sodium peroxide method to give high results is shown by Wolf and Osterberg[2] who, determining sulfur in 50 to 100 mg. of cystin, found 26.89% which is 0.19% more than formula requires. Furthermore, two samples of feces averaged 0.031 and 0.009% higher than by their modified Benedict method. Schreiber[3] also noted this tendency. One factor causing this in feeds and feces may be silica; this is indicated in Table II, which shows the effect of the precipitation of partly dehydrated silica.

Removal of the silica in feeds and feces gave results in percentages agreeing closely with those by the modified Benedict method to the third decimal place, with an average of 0.003% difference for the modified Benedict method (Table III). The peroxide method without silica removal gave a difference of 0.023% higher. This relation held for feeds (Table III), but this close agreement must not be taken as an indication of the absolute accuracy of the method. The Benedict method checked with an average difference of 3.67%.

The tendency of the peroxide method to give high results due to other causes than the presence of silica is shown by Allen and Johnston,[4] who

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[1] Blank, official method, 0.0019 g.; modified method, 0.0035 g.

[2] Wolf and Osterberg, *Loc. cit.*

[3] Herman Schreiber, THIS JOURNAL, **32**, 977 (1910).

[4] Allen and Johnston, *Ibid.*, **32**, 597 (1910).

TABLE II.

Comparison of the Modified Peroxide Method with the Modified Benedict Method, Both Without and With Silica Removed.

	Mod. peroxide method.			Mod. peroxide method with SiO_2 removed, compared			
				with the same method without SiO_2 removed.		with the mod. Benedict method.	
Sample.	Without SiO_2 removed.[1] %.	With SiO_2 removed.[2] %.	Mod. Benedict method.[3] %.	Difference.	%.	Difference.	%.
Swine Feces 1502	0.131	0.105	0.105	0.026	....	0.000	...
	...	0.112	0.110	...	....	0.002	...
	0.124	0.104	0.113	0.020	....	0.009	...
	...	...	0.110	...	....	...	...
Av.	0.127	0.107	0.110	0.020	18.7	0.003	2.8
Feces 1503	0.126	0.111	0.108	0.015	....	0.003	...
	0.140	0.104	0.111	0.036	....	0.007	...
	0.132	0.108	0.105	0.024	....	0.003	...
	...	...	0.104	...	....	...	...
Av.	0.133	0.108	0.107	0.025	23.1	0.001	0.9

worked on pure solutions, also by Wolf and Osterberg[4] and by Schreiber.[1] In the modified peroxide method the solution of 350 cc. (of oxidizing reagent) contained 24 g. NaCl, which with a higher acidity causes a high occlusion as shown by Allen and Johnston. The extent of occlusion of the barium sulfate, however, is determined by running with each set of analyses 3 entirely similar determinations of known amounts of pure potassium sulfate solution approximately equivalent in barium sulfate content to the quantity obtained from the analyses of the organic material itself. The actual sulfate content of an equivalent quantity of the potassium sulfate solution is found by direct precipitation. The difference obtained between these two sets of results gives the values for both occlusion and the sulfate in the reagents. When occlusion is negligible (as indicated by the small quantity of the barium sulfate obtained) the method showed that there was little sulfate present in the reagents. The amount present[5] is, perhaps, due to adsorption and varies with each lot; the average was 0.0046 g. The modified Benedict method gave a blank of 0.0011 g. which was but slightly higher than that found for the determination of sulfur in urine. The fuming nitric acid gave a blank of 0.0005 g. for the usual amount taken.

Loss of Barium Sulfate in Filtration and Washing.

Any perceptible precipitate in the filtrate and wash water is, of course, recovered at once. Our experience indicates, however, that slight losses

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[1] Less Blank, 0.0070 g. $BaSO_4$.

[2] Less Blank, 0.0006 g. $BaSO_4$.

[3] Less Blank, 0.0001 g. $BaSO_4$.

[4] *Loc. cit.*

[5] Blank as determined by Krieble and Mangum, personal communication.

not readily apparent are always experienced,[1] in filtering and washing less than 0.1 g. of barium sulfate precipitate caused by passage of the finer particles of precipitate through the pores of the filter paper, even when paper of such fine texture as Whatman's No. 44 or S. & S. 590 is used with a gravity washing system. These losses were very evident when the filtrates and wash water of an entire set of determinations—all of which looked to be free from precipitates[2]—were combined and allowed to stand covered for 2 to 3 days, for a fine precipitate of

TABLE III.

Comparison of the Modified Benedict Method with the Modified Sodium Peroxide Method, with Silica Removed.

	Quantity.		$BaSO_4$.		% sulfur.		Comparison of Benedict and peroxide method.	
Material and sample No.	Peroxide method. G.	Benedict method. G.	Peroxide method[3] without silica. G. av.	Benedict method.[4] G. av.	Peroxide method.	Benedict method.	Difference.	%.
Ground Corn								
1517......	2.7476	2.7476	0.0245	0.0257	0.123	0.129	+0.006	4.9
Linseed Oil Meal								
1518......	2.7476	2.7476	0.0802	0.0833	0.401	0.417	0.016	4.0
Wheat Middlings								
1519......	2.7476	2.7476	0 0389	0.0403	0.195	0.202	0.007	3.6
General Av..................			....	....	...	...	0.010	4.1
Feces (Swine)								
1502......	4.99	3.43	0.0388[5]	0.0274[6]	0.107	0.110	0.003	2.7
1503......	3.47	3.44	0.0273	0.0268	0.108	0.107	—0.001	—0.9
1504......	4.03	3.45	0.0299	0.0269	0.102	0.107	0.005	4.9
1505......	4.07	3.97	0.0301	0.2096	0.102	0.102	0.000	0.0
1506	4.14	3.10	0.0330	0.0230	0.110	0.102	—0.008	—7.3
1507......	3.74	3.57	0.0309[7]	0.0302[8]	0.114	0.116	0.002	1.8
1508......	4.18	3.23	0.0344	0.0264	0.113	0.112	—0.001	—0.9
1509......	3.52	3.35	0.0282	0.0247	0.110	0.101	—0.009	—8.2
1510......	4.28	3.45	0.0321	0.0261	0.104	0.104	0.000	0.0
1511......	3.58	3.51	0.0262	0.0271	0.101	0.106	+0.005	+5.0
General Av..................			....	....	...	...	0.003	3.17

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[1] Excluding dust, etc., this recovery from a large number of determinations averaged 0.0012 g. per determination. A number of more recent results by E. B. Wells, using more care and precautions, reduced this to 0.0003 g. per determination.

[2] The combined filtrates and wash water held up to the direct sunlight showed very fine particles held in suspension.

[3] G. $BaSO_4$ average of 3 determinations, less blank, 0.0040 g. per determination.

[4] Plus loss in filtrate exceeding blank, 0.0006 g. per determination.

[5] Samples 1502–1506 inclusive, less blank, 0.0006 g. per determination.

[6] Samples 1502, 1503, less blank, 0.0001 g.; Samples 1503–6, less blank, 0.0008 g.; Samples 1502–1506 inclusive, g. $BaSO_4$ average of 4 determinations.

[7] Plus loss in filtrate exceeding blank, 0.0013 g. per determination.

[8] Less blank, 0.0018 g.

barium sulfate settled out. The supernatant liquid was siphoned off, the precipitate filtered and washed, and weighed.

The usual laboratory practice is to discard filtrates and wash water immediately. Recovery of additional precipitate as indicated above shows that the loss may be greater than is here determined, unless unusual care is taken to reduce this loss to a minimum by slow filtering and gentle washing. Furthermore, allowing the precipitated sulfate to stand at room temperature for two days,[1] appears to aid filtration by permitting the particles to grow. The formation of a granular barium sulfate precipitate is also assisted by adding the barium chloride hot to the boiling sulfate solution, slowly or by drops. The precipitated solutions are then heated on the steam bath for 3 hours after which they are allowed to stand in the cold for two days. Neglect of this precaution will render filtration difficult.

Allen and Johnston[2] have shown, contrary to the statements of some chemists, that the presence of potassium chloride does not increase the solubility of barium sulfate. In the modified Benedict method, however, approximately 1 g. of potassium chloride is used per determination.

TABLE IV.

Uniformity of Results for Swine Feces Shown by the Modified Benedict Method.

Sample No.	Quantity. G.	$BaSO_4$ less blank.[3] G.	%.
1512	2.94	0.0263	0.123
	3.20	0.0281	0.121
	3.73	0.0324	0.119
Av.	....	....	0.121
1513	2.89	0.0258	0.123
	3.04	0.0285	0.129
	3.37	0.0313	0.128
Av.	....	....	0.127
1514	4.81	0.0325	0.093
	4.58	0.0310	0.093
	4.52	0.0312	0.095
Av.	....	....	0.094
1515	4.14	0.0355	0.118
	3.08	0.0256	0.114
	3.97	0.0336	0.116
Av.	....	....	0.116
1516	2.86	0.0241	0.116
	4.28	0.0364	0.117
	2.79	0.0238	0.117
Av.	....	....	0.117

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[1] Allen and Johnston recommend 18 hours. They state that losses from volatility and occlusion are higher when the precipitate is filtered immediately.

[2] THIS JOURNAL, 32, 606 (1910).

[3] Plus loss in filtrate exceeding blank by 0.0011 g. per determination.

On the other hand, the official peroxide method uses 4 or more g. of sodium chloride per 100 cc. of solution, while the modified peroxide method contained about 13.7 g. per 100 cc.

Comparison of results obtained by the modified Benedict method with those by the modified peroxide method, shows that complete solution of feeds and feces is not necessary, provided that enough Benedict reagent is present to assure complete oxidation. It is, however, desirable to have as complete solution as possible. Table III gives the average results by the modified Benedict method of triplicate determinations obtained on feeds and feces in comparison with those by the peroxide method when silica is removed. The percentages varied in the third decimal place for the modified Benedict method, while in the modified peroxide method a number of results varied in the second place.

Table IV shows the consistency of results obtained by the proposed method. In the hands of another chemist in this laboratory, the method has given as uniform results as are here indicated, but with lower blanks on reagents and less loss in the filtrate.

The Modified Benedict Method.

About 3.44 g. of feces, or 2.7476 g. of feeds[1] is weighed out by difference, placed on half a filter paper, rolled up, and is then put into a 300 cc. Kjeldahl flask. Ten to 15 cc. of hot water is added, the flask rotated occasionally (preferably in the steam bath) to allow any hard lumps to disintegrate and 20 cc. of a mixture of fuming and concentrated nitric acids (1 : 4) is added. The flask is stoppered with a small funnel and allowed to stand for half a day. It is then placed in the steam bath and allowed to digest *slowly* for about 4 days with occasional rotation. At the end of that time another 10 cc. of the acid mixture is added. Digestion is continued until the solution is clear,[2] when it is transferred in portions to a 60-cc. evaporating dish and treated with 20 cc. of the Benedict reagent[3] during evaporation to *thorough dryness* on the steam bath.

Blanks should be run on the reagents. To prevent spattering in heating the blanks, add 5 cc. of a 10% sucrose solution and evaporate to thorough dryness before ignition. The blanks are given the same treatment as outlined above. They should be low in barium sulfate content.

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[1] With this quantity the g. of barium sulfate multiplied by 5 gives the per cent. of sulfur direct.

[2] More resistant residues in feces and feeds may remain; these will not interfere if not present in too great quantity. Small quantities of resistant residues will be taken care of by the Benedict reagent.

[3] Kahlbaum's crystallized copper nitrate was used of which the sulfur content was small. Baker and Adamson's crystallized copper nitrate was found equally low in sulfate.

Procedure in the Benedict Method.

The partly oxidized material in the porcelain dish is evaporated to *dryness*[1] on the steam bath and later baked *slowly* on a gas hot-plate[2] or sand bath. The blackened residue is finally heated until the mass has fused, The heat is then gradually lowered, allowing the fused mass to solidify slowly. The dish when cooled is $^1/_3$ filled with approximately 20 cc. of hot dil. hydrochloric acid (1 : 4) from a wash bottle, and the sides carefully washed down. The dish is then covered with a watch glass and set on the steam bath for 15 minutes.

The perfectly clear solution is then filtered into a 250-cc. beaker, made up to a volume of at least 100 cc., heated to boiling on a hot-plate, and 10 cc. of a boiling 5% barium chloride solution added drop by drop from a calcium chloride tube, the addition consuming about 4 minutes. The covered solution is then placed on the steam bath and allowed to digest for 2 to 3 hours, after which it is allowed to stand in the cold for at least 48 hours, thereby producing a granular precipitate which is readily filtered on No. 590 S. & S. or No. 44 Whatman's filter paper.

By the removal of silica[3] in the sodium peroxide method, this substance was prevented from interfering with the accuracy of the results. In the Benedict method no flocculent silica has been observed in the solution after the barium sulfate has settled. As this solution is not made strongly alkaline or acid, one would not expect silicic acid to be even partly dehydrated or precipitated out.

Advantage of the Modified Benedict Method.

For feeds and feces, results are more uniform[4] than by other methods and the blanks on reagents are consistently low. Occlusion and adsorption appear to be less. There is a complete absence of partly dehydrated silicic acid in the solution after dissolving the fused mass in dilute hydrochloric acid such as is frequently observed in the peroxide method.

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[1] Evaporation to thorough dryness on the steam bath prevents spattering later, on the hot-plate. Beginning ignition of feces on the hot-plate should be gradual. Too much Benedict reagent will cause spattering; too little is indicated, upon ignition, by a hard, dry crust. Too long intense ignition with a large excess of the Benedict reagent will cause excessive etching of the evaporating dishes.

[2] In the baking and ignition processes by this method no evidence of sulfur contamination was obtained when natural gas was used. With coal or water gas, however, the sulfur contamination would be serious unless first removed by passing the gas through an absorbing solution.

[3] Whether any silica comes from the glass container by the action of the strong sodium hydroxide solution has not been determined. Occasional silica in the blanks tends to indicate that this may be one source.

[4] The limits of variation for results on 10 samples of feces by the peroxide method average 1.65 times greater than for the modified Benedict method (Table III). This relation also holds for feeds.

Conclusion.

When silica is present the peroxide method has a tendency to give high results. With removal of the flocculent silica which partly precipitated out, results checked the Benedict method in percentage to the third decimal place. Previously the peroxide method checked to the second decimal place.

The technique of the modified Benedict method has been adapted to facilitate the estimation of sulfur in feeds and feces without requiring undue attention and care. The method of solution of the sample by the Benedict method gives results which check the peroxide method when silica is removed, within an average of 3.17 and 4.17%, respectively, for feces and feeds.

The source of a high value for the blank determination due to sulfate present in the reagents and the need of procuring reagents of sufficient purity for the Benedict method have been pointed out. Not undue quantities of reagents are required for the small amounts of barium sulfate recovered. The modified method is short; the processes are few and require little attention.

The writer acknowledges his obligation to Dr. V. K. Krieble and A. W. Mangum for advice given in the use of their modified sodium peroxide method.

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THE MOLECULAR MECHANISM OF COLLOIDAL BEHAVIOR.

II. THE SWELLING OF FIBRIN IN ALKALIES.

BY RICHARD C. TOLMAN AND RUSSELL S. BRACEWELL.

Received July 25, 1919.

In a previous article,[1] Tolman and Stearn have studied the swelling of fibrin in acid solutions, and correlated the swelling with the amount of acid adsorbed from the solution. On the basis of their results, they advanced the theory that a piece of fibrin covered with water is a fibrous, spongelike structure with many minute pores or pockets which are themselves full of water. The addition of acid is followed by the adsorption of hydrogen ions to form a double layer on the surface of these pockets with a consequent increase in their size owing to electrostatic repulsion. The addition of a neutral salt to the solution, or the addition of further acid after the limit of adsorption has been reached, is followed by a decrease in swelling, since further ions added arrange themselves, in accordance with well known electrical principles, in such a way as to neutralize the original electrostatic repulsion. The addition of a neutral salt to the solution also leads to further adsorption of acid owing to the fact that the neutralization of the electrostatic forces makes it easier for further hydrogen ions to attach themselves to the walls of the pockets.

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[1] THIS JOURNAL, 40, 264 (1918).