[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, U. S. DEPT. OF AGRICULTURE, NO. 55.]

DETERMINATION OF SULPHUR AND PHOSPHORIC ACID IN FOODS, FECES, AND URINE.[1]

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Received June 24, 1904.

THE determination of sulphur and phosphoric acid was undertaken in connection with the metabolism experiments conducted by Dr. Wiley and Dr. Bigelow, in which twelve men were fed a normal diet, which, however, included an added chemical quite often used in foods as a preservative. As is general in such experiments, the income of foods and the outgo of waste products were all subjected to chemical analysis, and so far as concerns the authors, this investigation was confined to the analysis of all foods, feces, and urine for sulphur and phosphoric acid.

During these experiments three different methods for the determination of sulphur and phosphoric acid have been given a severe test: Berthelot's calorimetric method;[2] Parr's calorimetric method;[3] and Osborne's sodium peroxide method.[4]

The sulphur and phosphoric acid were always determined in the same samples of foods and feces, so that a great saving of time was possible. With urine, however, it was found best to determine phosphoric acid by the uranium acetate method, whereas the sulphur was determined, as in the case of foods and feces, by the peroxide method.

In general, the method of procedure was as follows: A pellet of dried food or of feces, weighing about 2 grams, was burned in Atwater's[5] modification of the Berthelot calorimeter in the presence of 20 or 25 atmospheres of oxygen and in the presence of moisture. The residue, after combustion, was boiled in hydrochloric acid till all the phosphorus compounds were dissolved. This solution was added to the washings from the calorimeter, which contained most of the sulphur as sulphuric acid. The whole solution was boiled to considerably less than 200 cc., filtered into a 200 cc. flask up to the mark, a 50 cc. aliquot taken for the

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[1] Read at the Providence meeting of the American Chemical Society.

[2] *Compt. Rend.*, **114**, p. 317.

[3] This Journal, **22**, 646.

[4] *Ibid.*, **24**, 142 (1902).

[5] *Ibid.*, **24**, 1100 (1902).

determination of phosphoric acid and the remaining 150 cc. used for the determination of sulphur, using barium chloride as the precipitant.

With the Parr calorimeter only 1 to 1.25 grams of finely ground dried food or feces were used. This was placed in the Parr cartridge, 10 to 15 grams sodium peroxide added, the cartridge closed and thoroughly shaken. When satisfied that the substance and the sodium peroxide were intimately mixed, the cartridge was placed in water up to the narrow part and a red hot iron wire was introduced through the valve. Immediately combustion took place and after cooling, the contents of the cartridge were transferred to a beaker by means of hot water, the solution acidified with hydrochloric acid, the excess of peroxide boiled off, and, in the case of foods, the solution was filtered to 200 cc. and, as above described, 50 cc. taken for a phosphoric acid determination, while the remaining 150 cc. served for that of sulphur.

In the case of feces, however, a slight modification was used. After boiling off the peroxide the solution was made up to 300 cc. (filtering rarely necessary) and 50 cc. taken for the phosphoric acid determination. To the remaining 250 cc. in the flask ammonium hydroxide was added in order to precipitate the phosphates, the solution again made up to 300 cc and a 250 cc. aliquot filtered for the sulphur determination. It was found necessary to remove the phosphates, as otherwise some of them were very apt to become occluded with the barium sulphate.

The Osborne method is fully described in this Journal,[1] but in the hands of an inexperienced person is rather difficult and treacherous. We have used nickel crucibles of 100 cc. capacity. To 10 cc. water in the crucibles about 10 to 12 grams of sodium peroxide were added (avoiding an excess) and 4 grams meat or other fresh substance, or 2 grams dried food or feces were added to the peroxide solution. The crucibles were then allowed to stand on a steam-bath till the excess of water had evaporated, and then placed over the low flame of an alcohol lamp, heating the crucible very gradually, especial care being exercised when the substance became frothy not to have the flame hot enough to cause the frothy mass to ignite. This requires constant watching and for this reason the method is tedious in the extreme. When

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[1] This Journal, **24**, 142.

the frothy mass finally subsides, the flame is gradually raised till the mass in the crucible begins to show signs of fusion. The crucible is then removed from the flame, allowed to cool a bit, and more sodium peroxide added, and the crucible again heated. When, finally, an addition of a small amount of peroxide to the hot fusion produces no more action, the combustion is complete. The crucible is allowed to cool, hot water added, and the fused mass transferred to a beaker, the solution acidified with hydrochloric acid, and treated exactly as described in the preceding method.

The following are some of the comparative results, the figures representing the percentage of sulphur in the foods or feces.

Substance.	Berthelot.	Osborne.	Parr.
Cream of wheat	0.161	0.144	....
Beef steak	0.249	0.278	....
Pork chops	0.245	0.269	....
Veal cutlets	0.247	0.332	....
Roast beef	0.218	0.271	....
Ice cream	0.030	0.030	....
Oysters	0.164	0.193	....
Bread No. 1, dried	....	0.160	1.61
Bread No. 2, dried	....	0.152	0.162
Bread No. 3, dried	....	0.167	0.154
Bread No. 4, dried	0.142	0.178	....
Potato, dried	....	0.128	0.137
Chicken	0.247	0.329	....
Fish	0.139	0.185	....
Custard	....	0.279	0.262

Substance.	Laboratory No.	Berthelot.	Osborne.	Parr.
Dried feces	9102	....	0.582	0.593
" "	9103	....	0.611	0.583
" "	9104	....	0.591	0.600
" "	9105	0.548	0.588	0.578
" "	9106	0.585	0.682	0.679
" "	9107	0.511	0.600	0.579
" "	9109	0.480	0.573	0.562
" "	9055	0.469	0.511	....
" "	9060	0.527	0.540	....
" "	9078	0.558	0.603	....
" "	9089	....	0.496	0.487
" "	9195	....	0.529	0.524
" "	9060	....	0.540	0.515
" "	9088	....	0.562	0.594
" "	9051	0.573	....	0.627
" "	9052	0.513	....	0.620
" "	9057	0.431	....	0.527
" "	9058	0.616	....	0.661
" "	9059	0.483	....	0.627

From the above results it can readily be seen that something is at fault with the Berthelot calorimetric method as used by us. One cause of these low results by this method seems to be in the action of the nitric acid (formed in the combustion of foods and feces) on the lead gasket of the calorimeter, producing lead nitrate, which, in the presence of sulphuric acid in a dissociated state, forms lead sulphate. This is seen as a white layer or powder on the exposed surface of the gasket. No doubt, had it been possible and practical to remove, each time, this lead sulphate and to determine its amount, the results of this method would have been in closer agreement with those of the other methods. As this would have entailed fusion with sodium carbonate, and as the estimation of sulphur in the presence of lead is by no means an easy task, it was decided to use the Osborne method exclusively for our sulphur determinations.

While the results obtained by the Parr method are, for the mast part, in close agreement with those of the Osborne method yet, because of the small amount of substance which could be burned at one time, it was difficult to obtain good duplicates, and, therefore, it was decided to discontinue using this method, though we believe that with certain modifications (*e. g.*, making a cartridge large enough to burn 2 to 3 grams substance) it would prove itself as reliable as, and much more practical than, the Osborne method.

The sulphur in urine was determined throughout the experiment by the Osborne method. For this determination a smaller amount of water and a correspondingly smaller amount of sodium peroxide were placed in the nickel crucible, then 20 cc. of urine added by means of a burette. The solutions were allowed to stand on the water-bath till the excess of water was driven off, then burned over an alcohol flame, as previously described. This is a very rapid and satisfactory method for urine combustion, and if the precaution be taken to evaporate the excess of moisture, very little or no frothing is experienced.

Recently Barlow[1] has published a method for sulphur determinations, which, he claims, gives absolute results. Thus far we have been unable to give it a trial, and whether it will prove all that is claimed for it and, at the same time, be practical in making

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[1] This Journal, **26**, 341.

the large number of determinations, per day, necessary in some investigations, remains to be shown.

PHOSPHORIC ACID.

(*a*) Determination of phosphoric acid in foods and feces.

(*b*) Determination of phosphoric acid in urine.

(*a*) After combustion phosphoric acid was determined by the official volumetric method[1] with the following modification: Instead of heating to from 60° to 65°, precipitating and filtering after fifteen minutes, precipitation was made in the cold and the solutions allowed to stand at room temperature over night. In this way precipitation is complete and the close watchfulness necessary to prevent separation of molybdic acid by heating to too high a temperature is avoided. After filtering and washing about twelve times with cold water, the last 50 cc. being tested with a drop of standard alkali to ascertain the absence of acid, titration was conducted as usual.

Combustions were made by the three methods described above. A large number of comparative results were obtained, some of which are tabulated below. Generally speaking, one method seems to give as accurate approximations to the truth as the others. It proved more difficult, however, to obtain good duplicates when using the Atwater calorimeter, thereby necessitating the frequent repetition of analyses. The Parr calorimeter was more satisfactory in this respect, while Osborne's method, as used by us, was most pleasing of all, seldom requiring a triplicate combustion.

TABLE OF COMPARATIVE RESULTS OBTAINED WITH THE BERTHELOT, OSBORNE, AND PARR METHODS.

Dried feces. Number.	Berthelot. Per ct. P_2O_5.	Osborne. Per ct. P_2O_5.	Number.	Osborne. Per ct. P_2O_5.	Parr. Per ct. P_2O_5.	Number.	Berthelot. Per ct. P_2O_5.	Parr. Per ct. P_2O_5.
9062	7.41	7.34	9053	6.73	6.93	9059	8.26	8.33
9104	5.10	5.35	9054	4.70	4.60	9106	4.83	4.98
9100	5.95	6.03	9055	7.27	7.20	9107	7.43	7.43
9096	5.53	5.48	9060	5.56	5.55	9108	6.14	6.25

(*b*) Phosphoric acid in urine was determined by titrating with a standard solution of uranium acetate, using potassium ferrocyanide as indicator. The method is that given in Hammersten's Physiological Chemistry,[2] with the exception that 50 cc. of urine

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[1] Bull. 46, revised, Bureau of Chemistry, Dept. of Agr., p. 14.

[2] Hammersten's "Physiological Chemistry," pp. 514-515; Sutton's "Volumetric Analysis," 8th edition, pp. 315-316.

were diluted with 100 cc. of water before heating and titrating. A number of comparative titrations showed that the use of undiluted urine gives low results.

This modified method was compared with the official volumetric method for phosphoric acid and found to give concordant results. Combustions for the official method were made by the Osborne method.

Of the 46 samples of urine analyzed by the uranium acetate, and the official volumetric methods, the results obtained on 6 samples were identical; one sample showed a maximum difference of ± 0.015 gram P_2O_5, the mean of all the results being ± 0.004 gram P_2O_5 in 100 cc. urine.

[Contribution from the Biochemic Division, Bureau of Animal Industry, U. S. Department of Agriculture.]

ESTIMATION OF NICOTINE IN PRESENCE OF PYRIDINE.

By James A. Emery.

For several years past the writer has made examinations of tobacco powders and extracts submitted by manufacturers throughout the United States to the Bureau of Animal Industry for the endorsement of that Bureau, as to whether certain requirements, recommended by the United States government, were being complied with in regard to the percentage of nicotine present in their products. For the dipping of sheep for scab the dips now approved by the Department of Agriculture are "The Tobacco and Sulphur Dip," and "The Lime and Sulphur Dip."

The regulations, as established for the first named dip, require that it shall be "made with sufficient extract of tobacco or nicotine solution to be a mixture containing not less than five one-hundredths of one per cent of nicotine and 2 per cent. of sulphur."

For the convenience of stockmen, transportation companies, and others, who were compelled by the regulations as cited in the Bureau of Animal Industry, Order No. 108, to use a dip, certain manufacturers in the various States have prepared highly concentrated extracts of tobacco, containing varying percentages of nicotine to be diluted according to the directions accompanying them, so that the resultant liquid will conform with the requirements of the government demand.