

or 0.00361 gram to the creatinine value obtained. If it is impossible to keep the volume of the solution at 10 cc. it is advisable to use the correction factor.

4. The period of standing appeared to influence the creatinine values. The longer the solution stood the lower were the results. This point was worked out on commercial creatine and creatinine samples. It is advisable to allow the solution to stand five minutes after adding the reagents and read at once.

5. The presence of coagulable protein lowered the results in the case of solutions containing only creatine and creatinine, and on adding egg albumin to a beef extract solution the creatinine results were also lowered.

6. The presence of proteoses and peptones lowered the values for solutions of creatinine. This was also the case with commercial samples of creatine and on adding a proteose and peptone solution to a sample of meat extract the creatinine values were slightly lowered. The ordinary meat extract does not contain a sufficient quantity of protein bodies to seriously affect the creatinine results. In an exceptional case it is necessary to remove the protein bodies before applying this test.

7. The autoclave method of Benedict and Myers, modified so as to apply to meat products, gave the most satisfactory results in determining creatine.

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### CHEMISTRY OF ANIMAL FECES.

#### SECOND PAPER.<sup>1</sup>—THE DETERMINATION OF FATTY MATTER IN ANIMAL FECES BY ETHER AND CARBON TETRACHLORIDE.

BY A. D. EMMETT.

Received March 25, 1909.

In the first paper upon this subject, relating to the possibility of analyzing the fresh dung from cattle and swine without previously air-drying the samples, the determination of the fatty matter was reported as having been made with both anhydrous ether and carbon tetrachloride.

The use of the last-mentioned solvent was recommended by Bryant<sup>2</sup> as a substitute for ether in extracting fat from foods and feeding-stuffs, because of the great rapidity of the extraction, the decrease of danger from fire and the reduction of the expense. Lately, Herty, Stem, and Orr<sup>3</sup> have made use of carbon tetrachloride in determining the percentage of oil in cotton-seed products. They have found that this reagent, when compared with gasoline, removes quantitatively the same amount

<sup>1</sup> A. D. Emmett and H. S. Grindley, *THIS JOURNAL*, 31, 569 (1909).

<sup>2</sup> *THIS JOURNAL*, 26, 568 (1904).

<sup>3</sup> *J. Ind. Eng. Chem.*, 1, 76 (1909).

of fat from the various samples, and that, by estimating the specific gravity of the resulting tetrachloride solution, the results are as accurate as when the extract is evaporated and the residue weighed, and that, the method is very rapid.

In our work, in making a quantitative comparison of the solvent action of anhydrous ether and carbon tetrachloride with animal feces, it was found that the two methods gave quite different results. In the tests reported, the samples of excreta were obtained from two pigs which were being used in digestion trials at the experiment station. The feed, given the animals, was ground corn in the first experiment, and ground corn and middlings in the second one. The dried samples of feces were extracted with the two solvents in the regular Soxhlet apparatus. The length of the time of extraction was 12 hours for the carbon tetrachloride and 24 hours for the ether. Table I gives the data for each of the reagents.

TABLE I.—FATTY MATTER IN ANIMAL FECES (CALCULATED TO THE ORIGINAL FRESH SUBSTANCE).

Serial No.	Pig.	Experi- ment.	Fatty matter extracted by			Difference upon dry basis. Per cent.	Percentage difference due to	
			Carbon tetra- chloride. Per cent.	Ether. Per cent.	Differ- ence. Per cent.		Carbon tetra- chloride (gain)+. Per cent.	Ether (loss)-. Per cent.
2396	A	1 <sup>1</sup>	5.06	3.35	1.71	5.32	+33.79	-51.04
2400	A	2 <sup>2</sup>	3.68	2.95	0.73	2.52	+19.84	-24.74
2397	B	1	5.41	4.44	0.97	2.71	+17.93	-21.85
2401	B	2	4.50	3.09	1.41	4.27	+31.33	-45.63

From the table it will be seen that the ether removed considerably less of the fatty matter than did the carbon tetrachloride, the differences ranging from 0.73 per cent. for sample 2400 to 1.71 per cent. for sample 2396. Upon the dry basis, these differences show that they amounted to as much as 2.52 and 5.32 per cent., respectively, for the two samples of feces Nos. 2400 and 2396. If the variations between the carbon tetrachloride and the ether are calculated in per cent. of the respective total amounts extracted by each solvent, it is found that the carbon tetrachloride removed from 17.9 to 33.8 per cent. more of the fatty matter from the feces than did the ether, and further, that the ether extracted from 21.8 to 51.0 per cent. less of the fatty matter than did the carbon tetrachloride. In the final analysis then, it must be stated that carbon tetrachloride did not serve the purpose intended and that the results from its use did not compare favorably with those obtained by the official method, that is, using anhydrous ether.

Some tests were made to ascertain what might be the nature of the fatty matter extracted by the carbon tetrachloride on the one hand and

<sup>1</sup> Experiment 1, Ground corn. <sup>2</sup> Experiment 2, Ground corn and middlings.

the ether on the other, and among them the phosphorus content of the two extracts was determined. It was thought that perhaps the carbon tetrachloride might have removed some lecithin compounds such as Long<sup>1</sup> describes in his work upon human feces. Table II gives the data for the total phosphorus, as  $P_2O_5$ , in the fatty matter, calculated both in per cent. of the fresh substance and in per cent. of the fatty matter itself.

TABLE II—PHOSPHORUS CONTENT OF FATTY MATTER (CALCULATED AS  $P_2O_5$ ).

Serial No.	Fig.	Experi- ment.	In per cent. of fresh substance extracted by		In per cent. of total fatty matter extracted by	
			Carbon tetra- chloride Per cent.	Ether. Per cent.	Carbon tetra- chloride. Per cent.	Ether. Per cent.
2396	A	1 <sup>2</sup>	0.021	0.006	0.40	0.39
2400	A	2 <sup>3</sup>	0.032	0.020	0.81	0.74
2397	B	1	0.027	0.014	0.60	0.67
2401	B	2	0.031	0.022	0.72	0.71

From the above data, it is seen that the differences in the phosphorus content of the two extracts are too small to be of any special significance, either upon the fresh basis or that of the fatty matter itself, and, therefore, it is evident that the carbon tetrachloride did not remove any more phosphorus from the samples of dung than did the ether.

An approximate analysis of the material which was found to be insoluble in ether but soluble in carbon tetrachloride, showed that it contained 0.185 per cent. of nitrogen, 10.11 per cent. of mineral matter, and 9.12 per cent. of calcium, as the oxide. Other solubility tests were made upon this ether-insoluble substance and it was found to be insoluble in carbon disulphide, benzene, acetone, alcohol and a mixture of alcohol and acetone. Tests for proteins and bile salts were negative.

The nature of this difference in the action of the two solvents, carbon tetrachloride and ether, is being studied further.

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## THE PRESERVATION OF URINE BY THYMOL AND REFRIGERATION.

(Nutrition Investigations, Publication No. 25).

BY F. W. GILL AND H. S. GRINDLEY.

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In connection with a nutrition experiment of this laboratory, requiring the continuous daily collection of the urines from twenty-four sub-

<sup>1</sup> THIS JOURNAL, 28, 704 (1906). Long and Johnson, *Ibid.*, 28, 1499 (1906); *Ibid.*, 29, 1214 (1907).

<sup>2</sup> Experiment 1, Ground corn.      <sup>3</sup> Experiment 2, Ground corn and middlings.