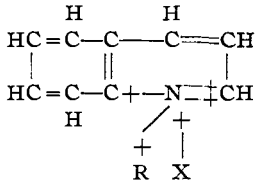
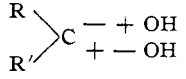
the nitrogen atom as fully reduced, *viz.*, $>C\overset{+}{=}\overset{-}{=}N-$ Therefore, in the cyclic compounds studied by H. O. Jones, we should have



In other words, the nitrogen atom is linked to only four different groups, since the linking $\equiv N\overset{-}{=}\overset{+}{=}C<$, is synonymous with Na_2bcd. It does not seem improbable, however, that some day conditions may be found to stabilize the system $\equiv N\overset{-}{\underset{+}{=}}C<$ in certain compounds, which may be expected to show optical activity. Furthermore, it may be predicted that compounds of the form



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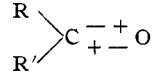

will be found to show activity if they are ever prepared. Many other possibilities of a similar character suggest themselves.

When the existence of isomers among organic compounds was first recognized, Faraday[1] said, "In reference to the existence of bodies composed of the same elements, and in the same proportion, but differing in their qualities, it may be observed, that, now we are taught to look for them, they will probably multiply on us." In the opinion of the author, we are in much the same position at the present time with respect to these new isomers which depend upon the distribution of electrons and their exchange; and it is not improbable that they will "multiply on us" in the future.

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FORMS OF SULFUR IN PLANT MATERIALS AND THEIR VARIATION WITH THE SOIL SUPPLY.[2]

BY W. H. PETERSON.

Received April 13, 1914.

Sulfur occurs in plant and animal tissues chiefly in the form of proteins, volatile compounds, as the mustard oils, and sulfates. In the proteins, it is known to exist as the amino acid, cystine, but in spite of much investigation, no other sulfur bearing compound has been isolated from the

[1] Faraday, *Phil. Trans.*, 440, (1825).

[2] This paper represents a thesis submitted to the Graduate Faculty of the University of Wisconsin in partial fulfillment for the degree of doctor of philosophy. Published with the permission of the Director of the Agricultural Experiment Station.

products of protein hydrolysis. Much evidence, however, has been gathered to indicate the existence of sulfur in other forms than cystine in the protein molecule. Johnson,[1] reviewing the literature on this subject, was led to believe that other sulfur linkages than that illustrated by cystine may occur in these substances. Arguing from a theoretical point of view, he pointed out the close analogy existing between oxygen and sulfur groupings, and the possibility of sulfur functioning in much the same way as oxygen in tying together the protein molecule. He has prepared many synthetic compounds showing this similarity, but, as yet, has not shown the existence of any new sulfur linkage in the protein itself.

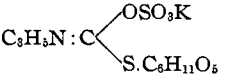
That sulfur does function in other protein linkages than the mercapto of cystine has been shown by Barger and Ewin,[2] who isolated a betaine closely related to histidine, in which the sulfur group (—CSNH) was found.

The recent work of Levene[3] in isolating a sulfatide from the brain, though not related to sulfur in the protein molecule, is closely associated with the larger subject of sulfur in plant and animal material.

Compounds Yielding Volatile Sulfur-Glucosides.—Besides sulfur in proteins, many plants contain volatile forms of sulfur, as is best illustrated by the mustard oil, allyl-*iso*-thiocyanate, the chief volatile compound existing in black mustard. Allyl sulfide, vinyl sulfide, mercaptan, and other volatile complex organic sulfides, have been obtained from onions and garlic. These volatile bodies are most abundant in the Cruciferae, but are contained also in many members of the Resedaceae, Capparidaceae, Tropaeolaceae, Liliaceae and other families.

The mustard oils do not occur as such in the plant, but as glucosides, the best known of which are sinigrin, found in black mustard, and sinalbin in white mustard.

That sinigrin contained sulfur was known to Fourcray and Tingry,[4] Henry and Garot,[5] Pelouze and Dumas,[6] Loewig[7] and others. Will and Körner[8] were the first to determine its composition and described a method for its preparation. Later, Gadamer[9] worked out its true composition and suggested as its formula



A sinigrin splitting enzyme, discovered by Ban Bussy,[10] which he named myrosin, decomposes the glucoside into allyl-*iso*-thiocyanate, glucose and acid potassium sulfate, according to the following equation:

$$C_{10}H_{16}NS_2KO_9 + H_2O = C_3H_5NCS + C_6H_{12}O_6 + KHSO_4$$

A very extensive study of myrosin was made by Grignard[11] with reference to its distribution, preparation, properties and activity. Sinalbin

and other sulfur-containing glucosides are much more complex, and less is known of them than of sinigrin. In many cases the quantity of glucoside present is small and its separation from other organic matter is very difficult.

The methods used for the determination of volatile sulfur all begin with grinding the material, whereby the enzyme, coming in contact with the glucoside, sets free the volatile mustard oil. This is then distilled out, with or without the aid of steam, and the sulfur in the distillate determined by various methods. The earliest method, that of Dietrich,[12] was to collect the distillate in a 10% ammonia solution, when the allyl-*iso*-thiocyanate combines with the ammonia to form allyl-thiourea, $C_3H_5NH.(NH_2)CS$. On adding silver nitrate to this solution, silver sulfide is precipitated; this is then filtered off and the sulfur therein calculated to the corresponding allyl-*iso*-thiocyanate.

Firbas[13] modified this method by using standard silver nitrate and, by titrating the excess of silver nitrate, obtained a check on his results. Vuillemin[14] obtained better results by adding alcohol to the ammonia, while Jorgensen[15] evaporated the solution of allyl-thiourea to dryness and then weighed and checked his results by determining the content of nitrogen in the dried residue.

An oxidation method was devised by Shiet,[16] who collected the distillate in alkaline permanganate, and, after removing the excess of permanganate with alcohol, precipitated the sulfates present with barium chloride.

Mann[17] extracted the mustard oil from the distillate by means of rhigolene, evaporated off the rhigolene in an ingenious apparatus and weighed the mustard oil directly.

The most widely used of these methods is that of Dietrich, with its several modifications. This is the method that was first employed in the experimental part of this paper; it was, however, abandoned, primarily because not all the volatile sulfur could be driven out by distillation. A blackening of the distillate appeared on the addition of silver nitrate long after a precipitate ceased to come down. The volume of the distillate made it exceedingly difficult to handle, so the oxidation in a combustion furnace, described later, was finally adopted.

Sulfates.—Arendt, Ulricht, E. Schulze, Berthelot and Andre, Fraps,[18] and recently Thompson,[19] have investigated the amount of sulfates in plants and found it to vary within wide limits, according to the amount in the soil, the parts of the plant, and the stage of growth.

Relation of Sulfur to Agriculture.—The determination of volatile sulfur has been limited almost entirely to mustard and rape seed. A pretty thorough review of the literature shows no data concerning the amount of volatile sulfur in the common farm crops such as rutabagas, turnips, sugar beets, cabbage, onions, etc. The question as to the amount of this

volatile sulfur, the amount of sulfur in the form of sulfates, and other possible forms of sulfur in the plant, has arisen as a result of the work done three years ago at this station on the amount of sulfur contained in farm crops and the supply of sulfur in the soil. In this paper, Hart and Peterson[20] showed that much larger amounts of sulfur were contained in plant material than had hitherto been suspected, and that the supply of this element in the soil was lower on the average than that of phosphorus, the necessity for conservation and maintenance of which in the soil has been generally recognized for many years.

Shedd,[21] in the analyses of forty Kentucky soils, has shown a loss of sulfur in practically every comparison of the cultivated soil with the virgin soil. This loss amounted to from 3–42% in the surface soils, and from 5–56% in the subsoils, and occurred in many instances even though the soils had been fertilized.

French investigators[22] have, for a long time, been experimenting with elemental sulfur and carbon disulfide in relation to increased crop production. The cause of increased yields has generally been attributed to an antiseptic action of the sulfur in the soil, whereby certain organisms unfavorable to the growth of the plant have been suppressed and the desirable ones permitted to develop. More recently the role of sulfur as a fertilizer has been considered by them.

The utilization, forms, and distribution of sulfur in plants, are questions which have arisen as a result of the increased importance of sulfur in relation to crops and soils, and the adjoined data have been gathered in an attempt to get more light on these questions.

Experimental Part.

It was planned to dissect the sulfur of the plant into four parts: volatile sulfur, sulfates, total soluble sulfur, and insoluble sulfur. The third of these includes the second, and by subtracting the sulfates from the total soluble sulfur, we have what is there probably in the form of soluble proteins. This, together with the insoluble sulfur, represents probably the total sulfur of the proteins.

Determination of Volatile Sulfur.—To get at the volatile sulfur in rutabagas, Dietrich's modified method was used at first, but because of the difficulties already mentioned, it was abandoned. Collecting the volatile sulfur in alkaline permanganate gave lower results than Dietrich's method, and the same objection, *i. e.*, a very large volume of distillate to deal with, operated here. For example, with 200 g. of green rutabagas, 2000 cc. of distillate did not seem to remove all the sulfur, since a blackening of the last 200 cc. ensued on the addition of silver nitrate. For this reason it was impossible to tell when to stop, and the discordant weights of silver sulfide obtained bore out the ocular evidence.

During these preliminary experiments, it was noticed that total sulfur,

determined on the green rutabagas and on the same sample after drying in the steam oven at 95°, gave uniformly lower results, indicating the loss of volatile sulfur on drying. This suggested the idea of drying the material in a closed receptacle and determining the sulfur in the evolved vapors. After numerous attempts, the following method was worked out and used subsequently in all analyses.

Two hundred grams of material were placed in a one-inch tube 25 inches long, having a right-angle bend at each end with 4 to 5 inches of upright—in other words, a long U tube. This was immersed in a trough containing a strong brine of calcium chloride, and the tube connected to the hard glass tube in a combustion furnace. This combustion tube was filled with copper oxide filings. To the combustion tube was connected a wash bottle, half filled with glass beads and strong potassium hydroxide solution, and immersed in a bath of cold water. The wash bottle was connected to the suction pump, so that air could be drawn through the whole system. To the other end of the drying tube was attached a wash bottle filled with strong sulfuric acid, and, preceding this, another wash bottle half filled with glass beads and potassium hydroxide. The air entering the apparatus then passed first through the potassium hydroxide, whereby any hydrogen sulfide and sulfur dioxide were removed. Then it was dried in the sulfuric acid bottle, passed through the drying tube, combustion tube, and absorption bottle successively. All stoppers used were of cork and, where rubber connections had to be employed, the ends of the glass tubes were placed in contact with one another so as to expose as little of the rubber tubing as possible to the gases. The rubber connection between the combustion tube and the absorption bottle was wrapped with a cloth and kept wet during the entire period. The whole system was tested for leaks before beginning the drying, by closing the entering tube with a pinchcock and then turning on full suction. A vacuum was thus created and any leaks disclosed. These were sealed with gelatine until no gas bubbles passed through the absorption bottle at the end of the apparatus. Heat was then applied to the calcium chloride bath, air admitted slowly to the apparatus, and the drying continued until the material in the drying tube appeared dry. This required from six to nine hours, and during this time a constant level of water was maintained in the bath and the temperature not allowed to rise above 95–100°.

After drying the material, the apparatus was cooled, disconnected, and the copper oxide filings removed from the combustion tube. Almost invariably several blue crystals of copper sulfate could be detected among the filings. These were boiled to dissolve out the copper sulfate, filtered and the sulfate precipitated with barium chloride.

The solution in the absorption bottle, now much increased by the con-

densed water from the green material, was washed out, combined with the washings from the copper oxide, concentrated on the water bath, filtered, cooled, and carefully neutralized in the cold with hydrochloric acid. This neutral solution was then poured slowly into bromine water, heated to oxidize any sulfites, and the resulting sulfates precipitated with barium chloride.

In the earlier determinations, the copper oxide washings and the absorption solution were combined and the sulfates determined in this solution, but in the later determinations the two were kept separate and the barium sulfate precipitated in each. It was found that most of the sulfur was fixed by the copper oxide and only where the total barium sulfate amounted to more than 50 mg. was there any sulfur in the absorption bottle.

Determination of Sulfates.—To determine the sulfates in the plant tissues, the dried material was removed from the drying tube and extracted with successive portions of water on a steam bath for 6–8 hours and the extract filtered into a volumetric flask. With 200 g. of green material, the extraction was continued until 2000 cc. had been obtained. An aliquot of this was then slightly acidified with hydrochloric acid and allowed to stand on the water bath for 24 hours to premit the precipitation of proteins and other organic materials. These were filtered off and a clear, brownish colored solution was obtained. This was heated to boiling, a slight excess of barium chloride added, and the beaker allowed to stand on the water bath over night. The precipitate thus obtained was generally somewhat discolored, but on ignition usually became white or grayish white. If it seemed to be contaminated, it was fused with sodium carbonate and reprecipitated.

Determination of Total Soluble Sulfur; Sulfates and Soluble Protein Sulfur.—Another aliquot, 250 or 500 cc., was placed in a volumetric flask, attached to a reflux condenser after adding bromine water and nitric acid, and kept warm for 10–12 hours. It was then boiled to remove the excess of bromine water and evaporated to dryness on a water bath. If very black, it was again taken up with water, more nitric acid added, and again evaporated. The residue, now slightly yellow to brown in color was dissolved in water, transferred to a nickel crucible, and evaporated; sodium peroxide was added, and the material fused over an alcohol burner. It was found quite impossible to get oxidation with nitric acid and bromine alone. Other wet oxidizing agents, such as strong hydrochloric acid and potassium chlorate or as alkaline permanganate, were tried without success. After three days' treatment with hydrochloric acid and small portions of potassium chlorate in a hot water bath, the solution still turned dark on standing, showing incomplete oxidation.

The Wolf-Osterberg[23] method for determining sulfur in organic material was tried many times and gave uniformly lower results than were obtained

with the peroxide treatment. Poor duplicates were also obtained by this method.

The determination of this soluble unoxidized sulfur gave a good deal of trouble, as here most of the sulfur was lost in the preliminary experiments. Only from 60–80% of the total sulfur could be accounted for, and it was only after the method described above was employed that the sum of the parts approached the total. While the procedure outlined is somewhat long and cumbersome, it is the only one that gave satisfactory results.

Insoluble Sulfur.—The extracted residue contained some insoluble organic sulfur, which was always determined by the sodium peroxide method. If to this is added the soluble unoxidized sulfur, *i. e.*, the total soluble sulfur minus the sulfate sulfur, we have what is probably the sulfur of the protein. In Table II, giving the distribution of sulfur, this value is entered in the last column.

The methods, as outlined above, were used in the analysis of different plant materials and the data thus obtained follow:

TABLE I.—VARIOUS FORMS OF SULFUR IN PLANT MATERIAL; CALCULATED AS PER CENT. SULFUR ON MOISTURE-FREE BASIS.

Material.	Moisture.	Soluble sulfates.	Volatile sulfur.	Soluble unoxidized sulfur.	Insoluble unoxidized sulfur.	Sum of various forms.	Total sulfur determined on green material.	Total sulfur determined on dried material.	Sulfur lost by drying.
Rutabagas, green 1	89.0	0.328	0.093	0.373	0.073	0.867	0.964	0.749	0.215
Rutabagas, green 2	88.9	0.248	0.098	0.293	0.103	0.644	0.717	0.590	0.127
Rutabagas, green 3	89.8	0.276	0.106	0.235	0.088	0.695	0.736	0.583	0.153
Rutabagas, green 4	90.1	0.327	0.087	0.256	0.098	0.768	0.784	0.657	0.127
Average	89.5	0.295	0.096	0.289	0.091	0.744	0.802	0.643	0.155
Cabbage, green	94.3	0.195	0.077	0.409	0.111	0.792	0.818	...	...
Sugar beets	74.3	0.018	..	0.015	0.020	0.053	0.053	0.047	0.006
Sugar beet tops, green	85.6	0.052	0.0031	0.183	0.165	0.403	0.433	...	...
Alfalfa hay	10.8	0.179	..	0.075	0.108	0.362	0.361	0.346	0.015
Alfalfa, green	77.5	0.003	0.0066	0.151	0.124	0.282	0.288	...	...
Dried rape (*a*)	..	0.051	..	0.193	0.075	0.319	0.422	...	...
Dried rape (*b*)	..	0.000	..	0.106	0.071	0.177	0.160	...	...
Dried rape (*c*)	..	0.600	..	0.200	0.076	0.876	0.940	...	...
Dried rape (*d*)	..	0.304	..	0.253	0.078	0.635	0.794	...	...
Radishes, green (*b*)	89.1	0.000	0.010	0.090	0.009	0.109	0.173	0.139	0.034
Radishes, green (*d*)	85.8	0.420	0.038	0.230	0.146	0.834	0.820	0.749	0.071
Clover, green (*b*)	65.6	0.000	0.004	0.024	0.092	0.120	0.112	0.120	0.008
Clover, green (*d*)	76.4	0.071	0.014	0.093	0.063	0.241	0.235	0.214	0.021
Clover, green (*e*)	80.0	...	0.0055	...	...	...	...	...	...
Clover, green (*e*)	79.5	...	0.0035	...	...	...	...	...	...
June grass (*e*)	72.2	...	0.0022	...	...	...	...	...	...
Milk	87.0	...	0.037	...	...	...	...	...	...
Oats (grain)	..	...	Trace	...	...	...	...	...	...
Wheat (grain)	..	...	Trace	...	...	...	...	...	...

(*a*) No fertilizer added to soil in greenhouse.

(*b*) Nitrogen, phosphorus and potassium added to soil in greenhouse.

(*c*) Nitrogen, phosphorus and potassium and sodium sulfate added to soil in greenhouse.

(*d*) Potassium, nitrogen, phosphorus and calcium sulfate added to soil in greenhouse.

(*e*) Sample taken from field.

TABLE II.—DISTRIBUTION OF THE SEVERAL FORMS OF SULFUR IN PERCENTAGES OF THE TOTAL SULFUR FOUND.

Material.	Soluble sulfate. A.	Volatile sulfur. B.	Total soluble sulfur. C.	Insoluble sulfur. D.	Sum of fractions. B, C & D.	Loss on drying.	Total unoxidized sulfur. (C−A)+D.
Rutabagas, average 4 samples	37	12	73	11	96	19	47
Cabbage	24	9	74	15	98	..	65
Sugar beets	34	..	62	38	100	11	66
Sugar beet tops, green	12	1	55	38	94	..	...
Alfalfa hay	50	..	70	30	100	4	50
Alfalfa, green	1	2	52	43	97	..	...
Rape, dried (*a*)	12	..	58	18	76	..	64
Rape, dried (*b*)	00	..	61	44	105	..	105
Rape, dried (*c*)	64	..	85	8	93	..	29
Rape, dried (*d*)	38	..	70	10	80	..	42
Radishes, green (*b*)	00	6	52	40	98	20	92
Radishes, green (*d*)	51	5	80	18	103	9	47
Clover, green (*b*)	00	4	21	82	107	..	103
Clover, green (*d*)	30	6	70	27	103	9	67
Wheat (grain)	Trace	..	..	..	...	..	...
Oat (grain)	Trace	..	..	..	...	..	...

(*a*) No fertilizer added to soil.

(*b*) Potassium, nitrogen and phosphorus added to soil.

(*c*) Potassium, nitrogen and phosphorus and sodium sulfate added to soil.

(*d*) Potassium, nitrogen, phosphorus and calcium sulfate added to soil.

In Table II we have the different amounts of sulfur found, calculated to show the distribution of this sulfur, *i. e.*, what part of the total sulfur exists in these various forms. A study of this table shows that in the case of rutabagas, about 11% of the sulfur exists in the form of volatile sulfur, 37% as sulfates, and about 48% as unoxidized sulfur. In the case of sugar beets no volatile sulfur was determined, as none was believed to exist there. The correctness of this assumption may be questioned, however, in view of what was found later in the case of clover, grass and sugar beet tops. Alfalfa hay contains one-half its sulfur in the form of sulfates, about one-fifth soluble unoxidized sulfur, and the remainder, three-tenths, as insoluble unoxidized sulfur.

The four samples of dried rape were grown in the greenhouse by other members of the department, and form part of a great many experiments to observe the influence of sulfur fertilization on various crops. Rape

sample (*a*) was the yield from a box of normal soil; sample (*b*) this same soil fertilized with potassium, nitrogen and phosphorus; samples (*c*) and (*d*) the complete fertilizer reinforced with sodium sulfate and calcium sulfate, respectively.

It is noticeable that where the rape, radishes and clover had only the residual sulfur in the soil to draw upon, but had an abundant supply of nitrogen, phosphorus and potassium and made vigorous growth, there were no sulfates in the tissues. Evidently the plants were utilizing all the available sulfur to form volatile sulfur compounds and build sulfur-containing tissue, and had none left as sulfates. Where sulfates were added to the soil, large quantities, 40–60%, were circulating in the plants as sulfates, and an abundant supply existed for building tissue and forming the volatile compounds of the plant.

It is important to notice (Table I) that where no sulfates had been added to the soil, the actual quantity of volatile sulfur found was only about one-fourth as much as where sulfates had been added. In the case of radishes (*b*) the volatile sulfur amounted to 0.010% and with sulfates added to the soil this rose to 0.038%. The figures for clover, under the same treatment, were 0.004% and 0.014%, respectively. The question may be properly asked as to whether this constituent of the plant is raised or lowered by a larger or smaller supply of sulfates in the soil. This increase of volatile sulfur on addition of sulfates does not appear in Table II, because here the very large amount of sulfates makes the percentage of volatile sulfur about the same in both cases. For a correct view of the matter, Table I must be examined.

An entirely unexpected discovery of volatile sulfur was made in the case of clover. So far as we are aware, no mention of a volatile sulfur compound in clover has been made in the literature. This seemed such a surprising result that to verify it determinations were made upon two samples of red clover from the field and upon grass (Kentucky blue grass). Several months later green alfalfa, sugar beet tops, cabbage and milk, were also examined for volatile sulfur. In every case volatile sulfur was found. Every precaution was taken to prevent the accidental entrance of any volatile sulfur into the drying apparatus. Potassium dichromate was added to the concentrated sulfuric acid to oxidize any sulfur compounds existing in the air that might pass through the first potassium hydroxide bottle. Another bottle of strong potassium hydroxide was placed between the sulfuric acid bottle and the drying tube to preclude the possibility of any volatile sulfur, given off by the acid, from passing into the drying tube. After all these precautions, volatile sulfur was found in these later determinations. As a further test for the correctness of these results, the apparatus with about 25 cc. of distilled water in the drying tube was run for eleven hours and no sign of sulfates was

found. Two sources of this volatile sulfur seem possible: the existence in the material of some compound similar to the mustard oils or the splitting off from the proteins of volatile sulfur, as hydrogen sulfide. It would seem rather improbable that by drying below 100° proteins would give off a volatile sulfide yet that seems to be the most probable explanation, in view of the results secured by Rettger[24] and other investigators, who showed that, when milk was heated to 85°, lead acetate paper was blackened when held in the vapors. From experiments made in this laboratory it was found that the amount of this volatile sulfur is considerable, 50 mg. barium sulfate being obtained on drying 300 cc. of fresh milk. Casein precipitated with acetic acid and washed free from acid, on drying in the tube gave about 20 mg. of barium sulfate from 250 g. of casein.

Summary.

1. A widespread interest in the question of sulfur from both a theoretical and agricultural point of view is evident. Numerous investigations show many new compounds, either synthetic or natural, that contain sulfur. The use of sulfur or its salts as a fertilizer, and the requirements of the plant and the soil supply of this element, are subjects of interest and investigation.

2. The determination of the volatile sulfur, sulfates, soluble unoxidized sulfur, and insoluble unoxidized sulfur in seventeen samples comprising seven kinds of plant material is given.

3. A new method for determining volatile sulfur in plants is described. The material is dried in a tube with sulfur-free air, and the gases passed over heated copper oxide and oxidized. With small quantities the sulfur is all fixed as copper sulfate.

4. The volatile sulfur in field samples of rutabagas and cabbage averages about 10% of the total sulfur. In radishes and clover grown in the greenhouse, the amount present is dependent on a liberal supply of sulfates, being four times as great with added sulfates as without them.

5. Volatile sulfur is lost on drying green material in the steam oven. In field samples of rutabagas this amounted to 20% of the total. On greenhouse samples this amounts to from 10–20%.

6. Volatile sulfur was obtained from red clover, alfalfa, June grass and sugar beet tops not known to contain any bodies yielding such compounds. The volatile sulfur in milk was also determined. The nature of this compound is not known. In rutabagas it is evidently of a sulfide form, as silver sulfide is obtained when silver nitrate is added to the distillate from rutabagas.

7. The sulfates in the field samples examined varied from 10 to 50%. Where large quantities of sulfates were added to the soil, there was a corresponding increase of sulfates in the plant tissue. Rape, radishes and clover grown in the greenhouse on soils low in sulfur, but supplied with

an abundance of nitrogen, phosphorus and potassium contained no sulfates.

8. Where no sulfates were added to the soil, 90% or more of the total sulfur was found in the form of unoxidized sulfur, probably in proteins. In the normally grown plants examined this was from 50–65% of the total. The plant was evidently very economical of its supply and made healthy, vigorous growth where no sulfates could be found in the tissue.

To Prof. E. B. Hart, at whose suggestion this work was undertaken, the author wishes to express thanks for his interest and encouragement.

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A METHOD FOR THE DETERMINATION OF FAT IN MILK (NEPHELOMETRIC METHOD).

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The methods for the determination of fat in milk, in common use at the present time, may be roughly classified as follows:

(1) Rapid methods, like the Babcock and Gerber methods, which de-