The determination of moisture, silica, iron, aluminum, calcium, and magnesiuim in the lungs of normal and lunger sheep
by Elwood P Wilson

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry
Montana State University

Abstract:
Both the experimental data for the testing of the methods of analysis and the actual results when these methods were applied have been included in this problem.

1. The "sliming", the nitric and perchloric acids, and the ashing methods of removing the organic matter have all been tried and the ashing method used because of its applicability to the problem. Occasionally the nitric and perchloric acids method was used to check the silica content as procured when the ash was dehydrated with HCl.

2. It was found that the phosphate had to be removed in order to make accurate analysis. Both the ammonium molybdate and the tin methods of removing the phosphate were tried, and the latter found the more applicable as well as giving the better results.

3. The tin method of removing phosphate has for the first time been shown satisfactory for quantitative work. Table VI shows its reliability in presence of relatively large amounts of phosphate and small amounts of Al, Ca, Mg and Fe. The molybdate method does not seem to be satisfactory (see Table IV) for the material in question where small amounts of Al, Ca, Mg and Fe must be determined accurately.

4. One of the most outstanding differences observed between normal and "lunger" lungs is that of the heavier weight of the latter's dried material. Although the percentage moisture is about the same in both cases, the "lunger" dry weight is 3.7 times heavier than that of the normal.

5. In the "lunger" lungs the calcium is about 2 times, magnesium about 1 1/2 times, and iron about 2 times as much as in normal lungs of the same size dry sample, while the silica and aluminum content seem to be about the same in both cases. When the weights of the mineral content of the entire dried lungs were calculated they were much greater in the diseased than in the normal lungs. The average of the mineral content of the total dried "lunger" lungs contains approximately the following over that in the normal lungs: silica 4 times, iron 5 times, aluminum 3 times, calcium 6 times, and magnesium 5 times.

6. Since blood contains considerable iron and calcium, the quantity of these two found in the analysis of the lungs would vary with the amount of blood present. The excess blood in the lungs was kept very low by observing precautions when removing the lungs from the sheep so as to keep its content as low as possible, 7. It is beyond the scope of the present problem to make any conclusions as to whether or not the increase in mineral content of the "lunger" over normal lungs could be the cause of the "lunger" disease of sheep, To determine whether the increased mineral content is due to inhaled dusts or pathological deposits is also excluded from the present investigation.

THE DETERMINATION OF MOISTURE, SILICA, IRON, ALUMINUM, CALCIUM, AND MAGNESIUM IN THE LUNGS OF NORMAL AND "LUNGER" SHEEP

by

Elwood P. Wilson, Jr.

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THE DETERMINATION OF MOISTURE, SILICA, IRON ALUMINUM, CALCIUM, AND MAGNESIUM IN THE LUNGS OF NORMAL AND "LUNGER" SHEEP

I. INTRODUCTION

"Lunger" disease of sheep, which has been described as chronic progressive pneumonia, is well known to sheep men of the Northwest, and to them the affected sheep are known as "lungers" or "heavey" sheep. As the name indicates, the disease is one affecting the lungs, producing a rapid, jerky, panting type of respiration which results in loss of condition, emaciation, exhaustion, and finally the death of the animal.

This is a disease of range sheep and so far as is known at present does not occur in sheep raised and maintained in small flocks on irrigated pastures. It is observed most commonly in ewes over four years of age, but it occurs in two, three, and four-year-old sheep, and in bucks and weathers as well as in ewes. Occasionally the symptoms are seen in yearlings.

Observations show that the disease occurs in Utah, Wyoming, Washington, Oregon, Idaho, and North Dakota, as well as in Montana. In Montana the loss from the disease in the average infected band runs about two per cent annually. Although there are some sheep outfits that are not known to have any "lungers" among their sheep, the disease is quite generally distributed and the total loss each year reaches a high figure.

Dr. Hadleigh Marsh and Dr. Howard Welch of the Montana Veterinary

Research Laboratory have carried out studies in connection with the "lunger" disease for fifteen years. They worked on the problem largely from the pathological and bacteriological standpoints and isolated from the lungs of "lunger" sheep three species of bacteria which were a Pasteurella, a diphtheroid bacillus, and Micrococcus catarrhalis (Pfeiffer). They succeeded in recovering in pure culture the diphtheroid bacillus and Micrococcus catarrhalis (Pfeiffer).

On inoculating normal sheep with the bacteria obtained in pure culture from the lungs of "lunger" sheep, the disease could not apparently be reproduced.

Dr. Marsh and Dr. Welch found from their observations that the normal sheep lung immediately after death is bright pink in color, tends to collapse in the chest cavity, is soft and elastic, and is easily compressed in the hand. The whole lung is even in texture and contains no lumps or variations in structure. The lung of a "lunger" sheep is gray in appearance, firm, heavy, and fills the chest cavity. It does not tend to collapse and is not elastic. In the smaller anterior lobes and along the lower margins of the main lobes, and occasionally in other parts of the lung, are solid darker areas. The gradual enlargement of these solid areas in the lungs and the gradual thickening of the walls of the air sacs throughout the lungs determine the course of the disease. As the consolidation progresses, the available air space decreases until finally there is not enough air-containing tissue to supply the necessary amount of oxygen to the blood and the sheep dies.

The whole pathological picture is that of a long continued irrita-

tion, either bacterial or mechanical. Microscopical examination of the lung tissue shows that there are present in the various parts of the diseased organ certain particles which give a greenish yellow refraction. It is thought that these particles may be foreign material, principally dusts of inorganic nature, and by either mechanical or chemical irritation of the lung tissue so react as to cause the "lunger" disease.

Because of the facts that the "lunger" disease of sheep could not be reproduced in normal animals by inoculating them with the bacterial species isolated from "lunger" sheep, and that histological observation showed the presence of particles of greenish yellow refraction in "lunger" lung tissue, the present study was undertaken with the object of determining the content of certain minerals in lungs of "lunger" sheep as compared with those in normal lungs.

II. REVIEW OF THE LITERATURE

The work of previous investigators which applies to the present problem may be divided into four groups: first, previous work done on "lunger" disease; second, foreign minerals that are likely to occur in the lungs of "lunger" sheep; third, the method of destroying organic matter present; and fourth, the method of analysis.

A. Previous Work Done on "Lunger" Disease

Previous work on the "Lunger" disease of sheep has been along the bacteriological and pathological lines. Welch and Marsh (42) have worked for a number of years on the bacterial infection in the lungs of diseased sheep and found no definite cause. Marsh (31 and 32) has described the disease as a progressive pneumonia of sheep. He isolated two species of bacteria which he believes to be factors in the etiology of the disease but could not reproduce the disease by inoculating normal sheep with the isolated organisms.

B. Foreign Minerals That Are Likely To Occur In Lungs of "Lunger" Sheep

Very little work has been done on the mineral analysis of sheep tissue, especially of the lungs. Laws and Gilbert (29) have made an inorganic chemical analysis of the entire body of thin, half-fat, fat and very fat sheep, and recorded them in percentage of live weight. For thin sheep they found 1.118 per cent H_3PO_4, 1.321 per cent CaO, 0.056 per cent MgO, 0.173 per cent K_2O, 0.120 per cent Na_2O, 0.037 per cent Fe_2O_3, and 0.021 per cent SiO_2; for fat sheep 1.040 per cent H_3PO_4, 1.184 per cent CaO, 0.048 per cent MgO, 0.148 per cent K_2O, 0.097 per cent Na_2O, 0.034 per

cent Fe_2O_3, and 0.026 per cent SiO_2. The results for half-fat and very fat sheep were very close to those already given above.

Weiske (43) has analyzed the blood of lambs in connection with a study of the effects of ingestion of acids. He found the following in per cent dry weight: 0.150 per cent P, 15.255 per cent N, 0.415 per cent K_2O, 2.220 per cent Na_2O, 0.066 per cent Ca, and 0.029 per cent MgO.

Any differences in the lungs of "lunger" and normal sheep other than pathological deposits might be the result of foreign material. With this fact in mind, as well as knowing something of the metabolism of the animal, the composition of the possible inorganic dust breathed by the sheep should be a determining factor in the cause of the "lunger" disease.

In the analyses of the soils of Montana, aluminum and magnesium silicates play an important part. Also volcanic ash is present in a considerable area in Montana soils. This ash according to Emerson (12) is largely composed of angular bits of glass and has abrasive properties which clays do not possess. Emerson (12) in an example of the composition of lava states that in solution it includes quartz, feldspar, mica, and hornblende. This would give lava soils the ingredients quartz, aluminum, potassium, sodium, and calcium silicates.

Hilgard (17) gives the following table for the oxides in the earth's crust:

SiO_2	57.78	MgO	3.81	P_2O_5	0.37
Al_2O_3	15.67	CaO	5.18	MnO	0.22
Fe_2O_3	3.31	Na_2O	3.88	FeS_2	1.03
				H_2O (acid)	0.36
FeO	3.84	K_2O	3.13	H_2O (base)	1.42

From this composition of the soil and from the metabolic ability of animals to throw off various mineral matter, the most likely foreign mineral elements that one would expect to find in larger quantities in the "lunger" lungs than present in normal lungs are silica, aluminum, iron, magnesium, and calcium.

This problem seems very closely related to certain mineral diseases in the lungs of human beings. Of these, silicosis is one of the most important. Fowweather (13) states that the silica content of normal human lungs varied from 0.12 per cent to 0.20 per cent (average 0.163 per cent) and in lungs of a railway man with marked anthrocosis there was 0.17 per cent silica. He said there is no relationship between the amount of silica present and the amount of pulmonary fibrosis and gives two possible answers to the question of silicosis: first, silicosis is determined not by the amount of silica present but by its nature and second, silicosis is determined not solely by the amount of silica but by certain conditions present in the body of the person.

Jones (22) in his analyses of silicotic lungs found that the silica was not there in a free state but there as potassium and aluminum silicates. His analyses of silicotic lungs are 9.59 per cent Al_2O_3, 48.02 per cent SiO_2, 8.43 per cent Fe_2O_3, and 2.16 per cent CaO, 1.64 per cent MgO, 6.42 per cent Na_2O, 16.47 per cent P_2O_5, and 6.07 per cent K_2O.

Stewart, Leeds, and Faulds (39) found in their analyses of Haematite Miners Lung that the average per cent of silica is 1.66 per cent and in normal lungs 0.16 per cent of the dry weight. They found quartz and sericite particles up to the size of ten microns and believe silica to

be the real cause of fibrosis. It is also stated in this article that haematite is a comparatively harmless dust which stimulates phagocytosis, and is rapidly removed from the lungs. This evidence should indicate that iron would probably not be an important factor in the cause of the "lunger" disease in sheep.

The difference in the silica content of the lungs of silicon workers and that in normal lungs has been shown also by Lieb and Schadendorff (30). They found the SiO_2 content of pathological lungs to be 4.6 per cent to 22.8 per cent of the ash tissue while the values for normal tissue were 0.08 per cent to 4.0 per cent.

McNally (34) in an article on the silicon dioxide content of lungs in health and in disease asserts that the silica content of lungs increases with age even in normal persons. He points out that 12.8 per cent of the earth's crust is free silica and in the combined form it is present to the extent of 42.5 per cent. He found the silica content of normal lungs to be 1.13 mg. per gram of dried lung and in lungs of eight persons working in dusty atmosphere the milligrams of silica per gram tissue ranged from 2.4 to 26.0 mg., depending upon the occupation.

Cummins and Sladden (5) in an article on coal miners lung found 2.36 per cent silica in lungs of coal miners who died of pulmonary disease after long disablement, and in coal miners not dying of pulmonary disease and not known to have any pulmonary disability--1.24 per cent. In non-coal miners they found 0.13 per cent silica. It is also stated in this article that an excess of iron is partially attributed to the excess of actual tissue material in the fibrotic lungs. They state that

iron in the lungs is due to three causes: first, "normal" tissue iron (including iron of the blood); second, iron in pigment of endogenous origin; and third, iron of external origin from inhaled dust. They found that the coaly material increased all out of proportion with the iron and therefore the latter has very little significant value in the cause of the disease.

Silicosis has been produced experimentally by Gardner (14) by exposing guinea pigs and rabbits to an atmosphere containing quartz dust eight hours a day for six days a week and over a period of one year or more. He produced lesions characteristic of silicosis in man by using a dust containing 90.75 per cent SiO_2 and whose particle size ran from 1 to 10 microns.

To quote from Gardner (14) in regard to inhalation of various dusts:

> "A comparison of the reaction to quartz dust with that of other types of dust, like carborundum, soft oval, asbestos, and granite, has indicated that there are definite differences in the response to different types of dust.
>
> In the case of quartz the activity of the phagocytes is responsible for the concentration of adequate quantities of an irritating chemical in direct contact with the connective tissues. Granite also contains free silica, but the elements in its composition appear to modify the effect of the silica upon the phagocytes and perhaps upon the connective tissue as well."

It is Gardner's opinion that the difference in the reaction to quartz and granite is not entirely due to the lower concentrations of silica in granite dust, but that the non-siliceous compnnents of this dust modify the behavior of the phagocytes so that they do not concentrate the irritating silica with the same rapidity that they do in the

case of quartz.

Iszard (21) in regard to the influence of an inspired dust on specific infection of the lungs found that calcium hydrate dusts are not harmful to lungs of humans. This article states that the silica content of an inorganic dust bears direct relationship to its harmfulness and that nonabsorbable dusts are the most injurious.

To quote from Collis and Greenwood (3) in regard to inhalation of inorganic particles:

> "Only particles which are insoluble in the fluids of the body when carried into the air passages remain as foreign bodies either to stimulate the ciliated epethelium to overaction for their expulsion, or, if they gain access to the lymph channels, it gives rise to a proliferation of connective tissues. Thus dusts of ivory, horn, bone, and other animal structures, and of calcium sulphate, of limestone, and of oxide of iron, are not associated with pneumonoconiosis in the way dusts of vegetable husks, emery, glass, sandstone, and flint are."

Landis (27) with reference to inorganic dusts states that inorganic dust from any source produces definite changes in the lungs. They differ only in the severity of the lesions which depend upon the hardness, sharpness, and chemical composition.

Iszard (21) found that calcium is partially removed by the blood stream and that the dissolved calcium is in the blood stream always as bicarbonate and phosphate, the excess, introduced by calcium dust, being deposited in those organs normally characterized by high calcium content such as bones, kidneys, and lungs.

Landis (27) states that organic dusts produce no lesions, but where lesions are encountered in the lungs of those who have been exposed to

organic dust, the lesions are to be ascribed to inorganic materials--in other words, a mixed dust. He states that the symptoms of inorganic dust are sneezing, coughing, tickling sensation in the throat, and vomiting.

In a paper in regard to relation of organic dusts to pneumonoconiosis, Landis (28) states that organic dusts do not have the power to establish the condition known as pneumonoconiosis. Quoting from Landis (28):

> "Disregarding the predisposing influence it may have on the development of tuberculosis, it should be clearly recognized that inorganic dust of itself can and does cause serious and disabling damage to the lungs."

C. Methods of Destroying Organic Matter

There has been a great deal of work done on the various ways of destroying organic matter in plant and animal matter. From the survey of the literature there seem to be six methods which are used to a considerable extent at the present. As Koch (24) points out, the method used depends upon the mineral matter that is to be determined. He suggests sulphuric acid plus a catalyst for nitrogen determinations; sulphuric acid and nitric acid for phosphorus, but not safe for nitrogen determinations; simple incineration of organic material in a muffle which is not safe for sulphur, phosphorus, chlorine, or nitrogen, but is useful for most metals; fusion with sodium carbonate and sodium hydroxide with or without oxidizing agents as potassium nitrate or sodium peroxide for sulphur determinations.

Nitric and perchloric acids were used by Gieseking, Snider and Getz (15) as well as Smith (37). They claim less loss of mineral matter by this method than by incineration. They also state that the silica is completely dehydrated without taking the solution to dryness and that the

perchlorate of the metals are much more soluble than the chlorides or oxides in ashed material. A "sliming" method which consisted of treating the wet or dry animal tissue with concentrated nitric acid for a period of seven to ten days was used by Jones (22). The insoluble material is filtered and the original cyrstal structure of the siliceous matter may be examined under a microscope since the nitric acid is believed to have no effect on the residue material.

King (23) and many other authors advise the use of platinum crucibles for the ashing process and usually used a sample of about 2 grams. A nickel crucible was used by Kroese and Nieuwenhuyzen (25) and they found that the nickel is not appreciably oxidized on heating and that it is only very slightly attacked by the charring of the organic matter. They put the dried ground organic material in a closed nickel crucible and incinerated in an electric oven at 300° to 350° C. while a stream of oxygen was passed into the nickel vessel. They obtained a chalk white ash in 3 to 4 hours.

D. Methods of Analysis

There is much literature on the methods of analysis for animal and plant materials as well as for certain inorganic elements. Many workers have given the method of analysis for certain of the elements sought in the present study, but few have given a method for the complete analysis.

Cummins and Sladden (5) in an article on Coal Miners Lung selected 300 to 400 grams of wet lung, cut the material into small pieces with scissors, and placed the tissue in a procelain dish. This was dried over a water bath until the weight was constant and then the dried pro-

duct was ground in a coffee mill. The analyst determined ash, silica, "coaly" material, and iron on the sample.

Kroese and Nieuwenhuyzen (25) in their estimation of small amounts of silica and calcium in the lungs of humans ashed the tissue in a nickel crucible. The ash was transferred to a platinum cricuble and fused with a NaOH-KOH mixture. This was then evaporated three times with concentrated HCl, dried at 110° and finally ignited. The residue was treated with dilute HCl, the silica filtered off, washed with hot water, and finally ignited in a platinum crucible to constant weight. The purity of SiO_2 was checked by evaporation with sulphuric and hydrofluoric acids and once more ignited to a constant weight. In the filtrate from the silica, the phosphorus was precipitated by addition of nitric acid, ammonium nitrate, and an excess of ammonium molybdate. The phospho-molybdate was removed by filtration and the excess molybdenum removed from the filtrate by saturation with H_2S, after first boiling off the nitric acid. The nickel was precipitated with ammonium sulphide after preliminary neutralization. The calcium was then precipitated as the oxalate and weighed as CaO.

Morgan and King (35) have given a method for micro determination of silica. They ashed the sample and used perchloric acid to dehydrate the silica in place of HCl They filtered off the silica, ignited in a platinum dish, and weighed.

Kuzirian (26) estimated the calcium in the ash of plant and animal carcasses. He removed the phosphorus with ammonium molybdate, precipitated the calcium as the oxalate, and weighed the latter as CaO. The calcium

oxide was then dissolved in dilute HCl, filtered, ammonium chloride and ammonium hydroxide added, boiled until odor of ammonia is faint, and iron and aluminum hydroxides removed. The calcium is then precipitated as the oxalate. He recommended not removing the excess molybdenum since it did not interfere with the precipitation of the calcium.

Stanfield (38) in his method for determining the molybdenum in plants and soils advises the precipitation of the molybdenum as the sulphide. This is accomplished by saturating a strong ammoniacal solution with hydrogen sulphide and pouring the solution into an excess of 6 N. sulphuric or hydrochloric acid, boiling to coagulate the precipitate, and filtering off the MoS_3.

McCrudden (33) has given experimental data for determination of calcium and magnesium in the presence of phosphorus and iron. His results with the method for calcium agree with those given by Official Methods of Agricultural Chemists (I, XII, 9)*, Halverson and Bergeim (16), Corley and Denis (4), Hillebrand and Lundell (19), and Breazeale (2). The calcium is precipitated as the oxalate in a slightly acid solution and then brought to slight alkalinity with sodium acetate. The magnesium is determined in the filtrate from calcium by evaporating to dryness, taking up with HCl and finally precipitating it as the ammonium phosphate. If iron is present 0.5 to 1.0 cc. of a 5 per cent sodium citrate solution is added before the magnesium is precipitated. The use of the citric acid to form complex ions with the iron and aluminum has also been verified by Curtman (7) and St. John (40) as well as Curtman and Dubin (8).

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* Denotes the division and determination number.

Dienes (9) has used the same essential methods for calcium and magnesium as did McCrudden (33), but the former's work was with microchemical analysis which involved some minor changes.

Elvehjem and Hart (11) confirmed the method for iron given in Official Methods of Agricultural Chemists (I, XII, 7) and Hillebrand and Lundell (18). Treadwell and Hall (41) give a method for the determination of iron and aluminum when they are procured as combined oxides. Winter and Patten (44) precipitated the iron and aluminum as phosphates and later determined the iron separately by adjusting the hydroxyl concentration by means of ammonium acetate. Myers, Mull, and Morrison (36) followed a similar scheme but used a colorimetric method for aluminum.

Edwards (10) found the amount of iron and aluminum in phosphates by removing the phosphate with citro magnesia and precipitating the iron and aluminum in a basic solution with hydrogen sulphide. The ferrous sulphide was washed, dissolved in dilute sulphuric acid, boiled, cooled, and titrated with standard permanganate. The aluminum is calculated by the difference.

Curtman (6) has given a method for removing the phosphate by use of tin in qualitative analysis and found it quite satisfactory. The method is simply adding tin to a concentrated nitric acid solution free from chlorides and removing the phosphate by filtering.

From the literature pertinent to the problem, it appears that considerable work has been done in analyzing the various inorganic materials present in human lungs and that the method used varies with the particular determinations sought. There is no literature on the determination

of the mineral content of lungs of "lunger" sheep.

III. CONDITIONS UNDER WHICH THE SHEEP WERE RAISED

All of the sheep whose lungs were analyzed in this experiment were raised under the supervision of the Montana Experiment Station. The sheep whose lungs were considered normal were raised either under flock conditions, on the range, or partly under both. The "lunger" lungs came from sheep who usually had been on the range for some period of time.

IV. PREPARATION OF SAMPLES

The procedure of sampling falls into three groups and will be considered in this manner.

A. Method of Removing Lungs

Dr. Marsh of the Veterinary Research Laboratory procured all of the lungs which were analyzed in this problem. In all but one case, in which the sheep died, they were killed by bleeding from the jugular vein and both lungs upon removal from the animal placed in a porcelain dish. The trachea as well as all other tissue other than that of lung was cut away.

B. Drying and Grinding

The drying was done in an electrically heated dryer provided with a fan for circulating the air. The temperature of the dryer ranged from 55° to 65° C.. After from five to seven days in this dryer, depending upon the time necessary for the material to come to constant weight, the sample was removed and ground in a Wiley mill.

C. Sampling and Preserving

The ground lung was then "tabled" by placing on a piece of paper about two feet square and pulling each corner of the paper in succession over the diagonal corner, thus causing the pieces to roll over one another. This was continued until each corner had been pulled across for ten times. The total dried material was then bottled up in an eight ounce bottle or quart canning jar depending upon the size of the lungs. Since the size of the lungs of some of the sheep was small it necessitated the keeping of the complete lungs. This practice was carried out with all the lungs.

V. METHODS OF ANALYSES

A. Qualitative Analysis

The general methods as given in Curtman's Qualitative Analysis were used to identify the various ions present.

B. Quantitative Analysis

The methods of analyses were modified to suit the determinations desired in a few cases, but in general the procedures of the Association of the Official Agricultural Chemists (1) were followed. This reference will be referred to as "Official Methods" in further citations. Usually a 12 gram sample of the dried lung material was taken and carried through the entire analysis, although in some cases other sizes were used. In all cases the determinations were run in duplicate. A large sample had to be ashed in order to receive sufficiently large amounts of inorganic materials in the ash.

Moisture in the Lungs

The lungs as received in a porcelain evaporating dish were weighed and the weight of the dish subtracted so as to obtain the weight of the wet lungs. After the lungs had been dried at a temperature of 55 to 65° C. to constant weight, the weight of the dish was again subtracted from the total weight in order to arrive at the dry weight of the lungs. The percentage of moisture was found on the wet basis.

Total Ash

Total ash for computations was carried out in four separate samples, two of which were normal and two of which were "lunger" lung samples. A 6 gram sample was weighed into a silica dish and then placed in an electric muffle. The temperature of the muffle was quite low at the start of the ashing process in order to prevent loss of material by flaming and also loss due to "puffing up" of charred material. The ashing was then continued at a temperature of 393° to 488° C. for 48 to 96 hours while a stream of air passed through the muffle. This temperature was the lowest at which ashing would take place completely. At a higher temperature the ash had a tendency to fuse.

Silica dishes were used in place of platinum dishes since a large sample had to be ashed in order to procure a sufficiently large ash and that such large samples when ashed may have destructive action on the platinum. Large platinum dishes were not available.

The ashing as carried out for the inorganic analysis was done in a nickel crucible as suggested by Kroese and Nieuwenhuyzen (25) by placing the vessel with the organic tissues in an electric muffle whose tempera-

ture was just high enough to char the organic matter and low enough to prevent the "puffing up" of the material as much as possible. After the material had stopped smoking the temperature of the muffle was raised to 393° to 488° C. for the remainder of the ashing process. Total ash could not be determined by this method since a certain amount of nickel oxide and salts were formed by the ignition of the ash in the nickel crucible. Silica dishes could not be used for ashing the sample for inorganic analysis because some silica may be removed from the dish and give false results in the silica determination.

Silica Determination

The siliceous matter was determined in the ashed material according to Hillebrand (20) and Official Methods (I, XII, 5), the ashed material being put into a beaker and evaporated three times with concentrated HCl and finally taking up the residue with dilute HCl. After filtering, the filtrate was evaporated to dryness but rarely ever gave any silica. The siliceous matter was filtered through a Whatman 42 filter paper and ignited in a platinum crucible to constant weight. The silica content was procured by evaporating the siliceous matter with sulphuric and hydrofluoric acids until constant weight was reached. The difference of weight of the siliceous matter before and after this treatment gave the weight of the SiO_2 present.

Iron Determination

The filtrate received after all the siliceous matter had been removed was made up to a volume of 100 cc.. A 90 cc. portion was placed in a beaker for the determination of aluminum, calcium, and magnesium.

The remaining 10 cc. were used for the iron determination according to the microcolorimetric procedure of the Official Methods (I, XII, 7) with the precaution taken of adding the same amount of nitric acid to both unknown and known solutions of iron.

Phosphate Removal

Since calcium as well as magnesium phosphate would precipitate when a solution containing the ingredients is made alkaline, the phosphate has to be removed. The method of removing the phosphate by ammonium molybdate according to Kroese and Nieuwenhuyzen (25) and Official Methods (I, II, 7) was tried, but difficulty was found in removing both the phosphate and excess molybdenum. According to Official Methods (I, II, 7) 70 cc. of molybdate solution has to be added for every decigram of P_2O_5 present in an HCl free solution. This would necessitate the adding of about 300 to 400 cc. of molybdate solution in order to precipitate all of the phosphate present. This dilution seemed to interfere with the precipitation of the phosphate. Also much difficulty was encountered on trying to rid the solution of the excess molybdenum by the method suggested by Stanfield (38).

In an effort to remove the phosphate the qualitative method of Curtman (6) was tried and found to give good results. This method consisted of evaporating the aliquot to dryness after the iron sample had been removed and taking it up with 10 cc. of concentrated nitric acid. This was taken to dryness and 10 cc. of nitric acid again added. After taking this to dryness the residue was taken up with concentrated nitric acid, heated until all material had gone into solution,

and about 2 grams of tin in the form of CP foil was added. This was boiled and evaporated to about 0.5 of a cc., 25 cc. of water were added, the solution stirred, allowed to stand, and finally filtered. The precipitate was washed from the filter paper into a beaker and 10 cc. of hot water added. This was again filtered, washed with water, added to the original filtrate, and the total volume evaporated to about 25 cc. Since some of the original precipitate tends to go through the paper, the solution is again filtered. The filtrate, although free from phosphate, usually contains small quantities of lead and copper originally present as impurities in the tin foil. To get rid of these and any tin that might be present, the solution is made alkaline with ammonium hydroxide, made slightly acid with HCl, 5 grams of ammonium chloride added, and finally saturated with hydrogen sulphide. The precipitate is filtered, washed with ammonium nitrate, and filtrate made strongly acid with HCl. This is boiled to get rid of excess hydrogen sulphide and the free sulfur which forms is filtered off and washed with hot water. The filtrate is evaporated to about 75 cc.

Determination of Aluminum

The concentrated filtrate obtained from above after the phosphate removal is treated for Fe_2O_3 and Al_2O_3 according to Official Methods (I, XXXVII, 56). The iron content in the combined oxides is found by using the procedure in Official Methods (I, XXXVII, 57). The amount of Fe_2O_3 is calculated from the amount of iron found. The quantity of Al_2O_3 is found by subtracting the amount of Fe_2O_3 from the weight of

the combined oxides. The amount of the aluminum is found by use of chemical factors.

Determination of Calcium

The ammoniacal filtrate from the R_2O_3 determination is saturated with hydrogen sulphide to remove the nickel. This is filtered off and washed with a 1 per cent ammonium nitrate solution. The filtrate is acidified with HCl and boiled. The free sulphur is filtered off, washed with hot water, and the resulting filtrate concentrated to about 200 cc. The calcium was determined according to Official Methods (I, XII, 9) except that the calcium oxalate was precipitated in a cold solution which was very slightly acid in reaction to methyl orange.

Determination of Magnesium

Magnesium was determined in a single precipitation according to Official Methods (I, XXI, 37).

VI. TABULATION AND DISCUSSION OF DATA

An important factor in getting good results in this problem is that of obtaining a method which will accurately and uniformly apply to all of the ashed samples of the lung material. With this fact in mind the various methods were tried in order to determine which would give the most accurate results.

A. Qualitative Analysis

A qualitative analysis was run on two normal and two "lunger" lung samples which were ashed in silica dishes. The methods given in Curtman's Qualitative Chemical Analysis were used and indicated the presence of SiO_2, aluminum, phosphate, iron, calcium, and magnesium in both normal and "lunger" lungs. No other qualitative tests could be gotten using these methods. Much time was spent trying to get the only available spectroscope in proper working condition in order to obtain the spectrum photograph of both the normal and "lunger" lung samples. It was finally decided that the instrument could not give fine enough lines for use in spectrum studies, and therefore could not be used in the present problem.

B. Quantitative Ash Determination

Table I shows the results obtained in ashing a 6 gram sample of two normal and two "lunger" ewes. The "lunger" average ashed weight was 0.1156 grams heavier or 41.8 per cent higher than the average weight of the normal lung ash. This determination shows that there is nearly 1½ times as much mineral matter in "lunger" as there is in normal lung samples.

Table I.

Weight of Total Ash in "Lunger" and in Normal Lungs

Sheep number	Size of samples	Weight of ash
	gms	gms
	Lunger Lungs	
617D	6	0.3731
557B	6	0.4124
Average	6	0.3928
	Normal Lungs	
430	6	0.2618
314	6	0.2926
Average	6	0.2772

C. Methods of Destroying Organic Matter

Three methods were tried for destroying the organic matter present and are namely the ash method according to Kroese and Nieuwenhuyzen (25); second, digestion of lung with nitric and perchloric acids according to Smith (37); and third, "sliming" method of digestion by means of nitric acid. Since the latter applied more to microscopical studies of the siliceous matter present than to chemical analysis, it was not used after preliminary experiment. The nitric and perchloric acids offered a quick and easy method of destroying organic matter as well as accurate results for silica since the perchloric acid dehydrates the silica without having to evaporate the solution to dryness. However, since the perchlorates formed by this treatment interfered with the tin method of removing the phosphate the method was used only to check the results on silica as obtained by the ash and HCl dehydration.

D. Methods of Removing Phosphate

In order to establish approximately the amounts of silica, aluminum, iron, calcium, and magnesium in sheep lungs the entire material of a normal ewe was ashed in a nickel crucible at 393° to 488° and the resulting ash analyzed. The SiO_2 was removed by dehydration with HCl and 5 equal aliquots taken from the filtrate for determinations on comparison of methods. Two of the aliquots were run on each method so as to act as checks on one another. In the first two the phosphate was left in solution and in the remaining two it was removed by use of ammonium molybdate solution. Table II tabulates the grams of each element found while Table III represents the percentages found. Table

Table II.

Weights of Aluminum, Iron, Calcium and Magnesium as Determined in a Normal Sheep's Lungs, With and Without Removal of Phosphate

Number of determinations*	Phosphate removed	Phosphate grams	Aluminum grams	Iron grams	Calcium grams	Magnesium grams
1	no	---	0.1089	0.012	0.0037	0.0008
2	no	---	0.0736	0.011	0.0034	0.0008
3	yes	0.0141	0.0507	0.010	0.0069	0.0008
4	yes	0.0134	0.0507	0.011	0.0064	0.0008

*Each sample was an aliquot containing 20.9 grams of material and represented one-fifth of the total dry weight of the lungs.

Table III.

Percentage of Aluminum, Iron, Calcium, and Magnesium as Determined in a Normal Sheep's Lungs, With and Without Removal of Phosphate

Number of determinations*	Phosphate removed	Aluminum per cent	Iron percent	Calcium per cent	Magnesium per cent
1	no	0.104	0.011	0.0035	0.0007
2	no	0.071	0.010	0.0032	0.0007
3	yes	0.048	0.009	0.0061	0.0007
4	yes	0.048	0.010	0.0061	0.0008

*Each sample was an aliquot containing 20.9 grams of material and represented one-fifth of the total dry weight of the lungs.

III is calculated from the data in Table II. It is obvious that in the cases where the phosphate was not removed the aluminum content is higher and the calcium content lower than in those where the phosphate was removed. These differences indicate that calcium phosphate must precipitate when the solution is made alkaline and therefore is weighed as Al_2O_3 thus increasing the amount of aluminum and decreasing the amount of calcium.

In order to test the accuracy of the various methods of analyses, a known solution was made up which contained the approximate quantities of the elements found in the normal sheep's lungs as tabulated in Table II. The stock solution of the known was made by dissolving ten times the amount represented below, making up to 100 cc. and taking 10 cc. aliquots.

Quantities Present in Known 10 cc. Portion

0.005 gm.	iron in form of C.P. wire
0.0500 gm.	aluminum or .3975 gm. $AlCl_3.6H_2O$
0.0100 gm.	calcium or .3065 gm. $CaCl_2.2N_2O$
0.0100 gm.	magnesium or .0846 gm. $MgCl_2.6H_2O$
0.3000 gm.	nickel or .6640 gm. $NiCl_2$
0.0199 gm.	phosphorus or .1324 gm. $NaNH_4HPO_4.4H_2O$

In the first determination of this known the phosphate was removed by ammonium molybdate solution and the excess of the latter as well as nickel not removed during the course of the entire analysis. The results are tabulated in Table IV under samples Nos. 1 and 2.

Another determination was carried out on the same known solution without removing the phosphate and the results obtained tabulated in

Table IV.

Contents of a Known Solution and Results Obtained by Various Methods

Sample number	Phosphate	Aluminum	Iron	Calcium	Magnesium
	grams	grams	grams	grams	grams
Known	0.0199	0.0500	0.0050	0.0100	0.0100
		Results Obtained			
1	0.0197	0.0511	0.0050	0.0107	0.0120
2	0.0208	0.0500	---	0.0109	0.0109
3	---	0.1318	---	0.0018	0.0051
4	0.0202	0.0514	---	0.0127	0.0089
5	0.0199	0.0445	---	0.0137	0.0114

Table IV under sample No. 3.

A method which consisted of removing the phosphate with ammonium molybdate and removing the excess of the latter as well as the nickel by precipitating with hydrogen sulphide in an ammoniacal solution brought back nearly to acidity with HCl was run on a known solution containing the same amount of all elements except no iron was present. The results obtained are tabulated in Table IV under samples Nos. 4 and 5. The best results seemed to be obtained when the phosphate was removed but the excess molybdate solution not taken out in the entire course of the analysis. This method was then used on the samples of the same lambs' lungs procured at a meat market. Various ways of destroying the organic matter were tried and the results of the analyses tabulated in Table V. Since the determinations were all run in duplicate and the weights of the various elements did not seem to check, other methods of removing the phosphate were sought.

Another known solution was made for testing the accuracy of the tin method of removing the phosphate. The known contained in each 10 cc. portion the following weights of each element weighed from the material as indicated.

Quantities Present in Known 10 cc. Portion

0.01	gm.	Iron from C.P. wire
0.05	gm.	Aluminum or .3975 gm. $AlCl_3.6H_2O$
0.01	gm.	Calcium or .0365 gm. $CaCl_2.2H_2O$
0.01	gm.	Magnesium or .0846 gm. $MgCl_2.6H_2O$
0.2169	gm.	Phosphorus or 1.440 gm. $NaNH_4HPO_4.4H_2O$

It was found that the iron was partially removed in some cases and

Table V.

Grams of Silica, Iron, Aluminum, Calcium, Magnesium and Phosphorus Pentoxide Found in Samples of Lamb* Lungs When Different Methods of Destroying Organic Matter Were Used

Sample number	Weight of sample	Method of destroying organic matter	SiO_2	Fe	Al	Ca	Mg	P_2O_5
	grams		grams	grams	grams	grams	grams	grams
1	10.00	ash	0.0020	0.0053	0.0030	0.0057	0.0063	0.2087
2	10.35	HNO_3 and $HClO_4$	0.0027	0.0040	0.0089	0.0100	0.0066	0.2411
3	10.35	sliming	0.0040	0.0036	0.0024	none	0.0100	0.2161
4	10.00	ash	0.0024	0.0072	0.0288	0.0108	0.0180	0.2175

*Three sets of lungs obtained from the meat market, history unknown.

entirely in others by the tin. Table VI shows the results when the known contained the quantity of ingredients as indicated above as well as when it contained twice as much aluminum with the other ingredients remaining the same. The values obtained seem to indicate the accuracy of this method.

Table VII shows the results when determinations were run on the same lungs using ammonium molybdate and tin methods of removing the phosphate. As can be seen much better results were obtained by use of the tin method. Great difficulty was encountered in the ammonium molybdate method in getting samples to check. The tin method is very easily and quickly applied as well as giving good results.

E. Results of Lung Analyses

Table VIII shows the percentage of each ingredient as obtained in analyses of normal and "lunger" lungs. The percentage is an average of two checks run on separate samples whose size is designated in the table. In most cases the analysis of each set of lungs was run three or more times and the figures reached most frequently used in Table VIII.

Tables IX and X tabulate the grams of each ingredient used to obtain the percentages in Table VIII. The size sample for iron and silica was the original weight of the sample since these determinations were run before any aliquots were taken. The size sample for aluminum, calcium, and magnesium was smaller because a 90 cc. aliquot of 100 cc. was used for their analysis.

Table XI represents the amounts of the various minerals in the

Table VI.

Determination of Known Solution When Phosphate is Removed by Tin*

Sample numbers	Aluminum grams	Calcium grams	Magnesium grams
1	0.0443	0.0100	0.0113
2	0.0494	0.0100	0.0115
3	0.0405	0.0100	0.0111
4	0.0440	0.0110	0.0113
5	0.0492	0.0107	0.0097
6	0.0490	0.0104	0.0098
7	0.0940	0.0100	0.0112
8	0.0988	0.0101	0.0126

*Analyses of known solution used on Samples 1 to 6, inclusive: Al 0.05 grams, Ca 0.01 grams, Mg 0.01 grams. For Samples 7 and 8: Al 0.10 grams and Ca and Mg same as above.

Table VII.

Comparison of Tin and Ammonium Molybdate Methods for Removal of Phosphate in the Same Lungs

Sheep number	Sample number	Method used	Aluminum per cent	Calcium per cent	Magnesium per cent
795N	1	Am. molybdate	0.010	0.482	0.073
	2	Am. molybdate	0.016	*	*
	3	tin	0.017	0.063	0.062
	4	tin	0.020	0.062	0.069
40F and 137F	1	Am. molybdate	0.020	0.560	0.070
	2	Am. molybdate	0.020	0.160	0.100
	3	tin	0.025	0.053	0.058
	4	tin	0.025	0.054	0.058

*Determination ruined because molybenum oxide formed when calcium precipitated.