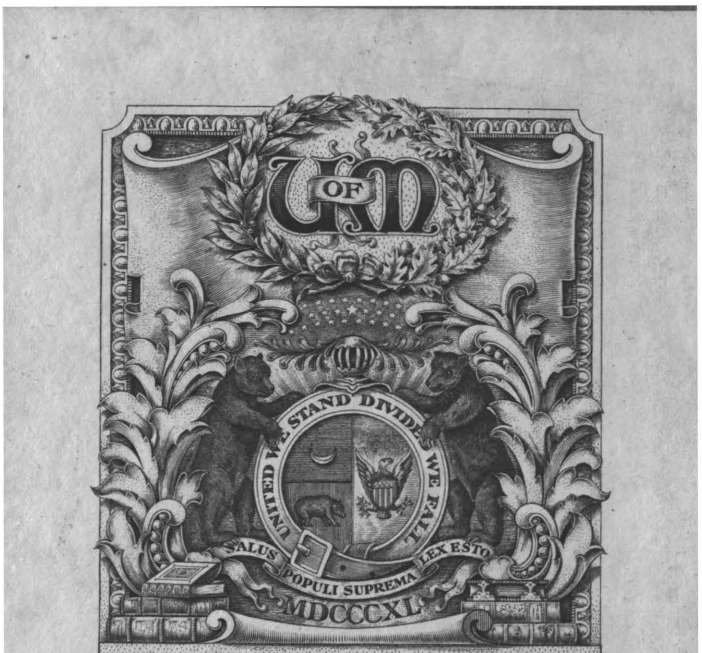

THE DETERMINATION OF THE LECITHANS IN THE TISSUES AND FLUIDS OF THE ANIMAL BODY.

by

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Review of Previous Methods.

The methods previously used in the determination of the lecithans are three, the first being that of Hoppe-Seyler(1), the second that of Thudichum(2) and the other, that of Koch(3).

Method of Hoppe-Seyler.

Extraction of Lecithans.-- In Hoppe-Seyler's method the finely divided tissue is extracted with alcohol and the alcoholic extract filtered off; in the case of liquids, alcohol is added and the solution filtered from the precipitate obtained. The material remaining on the filter in either case is extracted several times with alcohol at 50-60 degrees centigrade. The filtered extracts are combined with the first filtrate and the whole concentrated by careful evaporation; during this evaporation the solution is kept neutral(4) by the addition of acetic acid or of sodic

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1. Hoppe-Seyler: Handbuch der Chemischen Analyse, Seventh edition by Thierfelder, 1903, S. 159.
2. Thudichum, J. L. W.: Die Chemische Constitution des Gehirn des Menschen und der Thiere, 1901, F. Pietzcker, Tuebingen. S. 282.
3. Koch, W.: University of Chicago, Decennial Publications, 1902, X, p. 95; Ibid. American Journal of Physiology, 1904, XI, 3, p.320.
4. Alkalies hydrolyse lecithans rapidly even in the cold. Hoppe-Seyler: loc. cit. S. 159.

carbonate, as its reaction demands. The residue is extracted with a mixture of alcohol and ether, the filtrate freed from ether by distillation and the alcoholic solution further concentrated by evaporation. The syrupy residue is extracted several times with ether, the filtered extracts combined and the ether distilled off. The residue thus obtained contains besides lecithans, always cholesterin, usually fats, more seldom other substances, but is said to contain no inorganic phosphates.

The objections to this method of extraction are the possible incompleteness in the extraction of the kephalins by the alcohol(5), with which alone the original material is extracted and the possible presence of inorganic phosphates and organic phosphoric compounds other than lecithans, such, for example, as the sulphur compound(6), in the residue finally obtained.

Estimation of Lecithans.-- The estimation of the lecithans is made by oxidizing the organic residue, making a quantitative determination of the phosphorus in the inorganic phosphates thus obtained and calculating the amount of lecithans from the result. The determination of the phosphorus is made by either of two principal methods, the first being the gravimetric method of Hoppe-Seyler(7) and the other,

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5. Thudichum: Gehirn S. 133.
6. Koch, W.: Am. Jour. Physiol., 1904, XI, 3, p. 311.
7. Hoppe-Seyler: loc. cit. S. 405.

the alkalimetric one of A. Neumann(8).

Gravimetric Phosphorus Method of Hoppe-Seyler.

Oxidation of Material.-- In Hoppe-Seyler's method of determining phosphorus by weighing as magnesic pyrophosphate, the dried material is thoroughly mixed with ten to twenty times its weight of a mixture containing one part of sodic carbonate and two parts of potassic nitrate and carefully ignited in a platinum dish.

The objection to this method of oxidation is its slowness and the difficulty of determining when the destruction of the organic matter is complete.

Determination of Phosphorus.-- The mass remaining in the dish after ignition is dissolved in water, large excess of nitric acid added, the solution heated to drive off the nitrous fumes, somewhat concentrated and the chloridion present removed as silver chloride. After addition of ammonic nitrate to this solution, the molybdic solution(9) is added to precipitate the phosphorus as ammonic phosphomolybdate. For each decigram of phosphoric acid (P_2O_5) at least 80 c.c. of the molybdic solution is added and the amount of ammonic nitrate added is such that the solution from which the ammonic phosphomolybdate separates will contain 15%. The

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8. Neumann,A.: Archiv fur Anatomie und Physiologie. Physiologische Abtheilung, 1900, S. 159; Hoppe-Seyler: loc. cit. S. 406; Zeitschrift fur Physiologische Chemie, Bd. 37, S. 115, 1902-03; Ibid. Bd.43, S. 32, 1904.

9. This is the same as the nitric acid solution of ammonic

solution is well shaken, allowed to stand for twelve hours at a temperature of about 40 degrees centigrade and the supernatant liquid poured off through a small filter. The precipitate is washed repeatedly with a solution containing 15% of ammonic nitrate and 1% of nitric acid, decanting all the wash water through the original filter, until the filtrate remains perfectly clear on addition of ammonic hydroxide solution. The precipitate is dissolved in a solution containing 2% of citric acid and 2.5% of ammonia, the solution warmed somewhat and a slight excess of magnesia mixture(10) added to the rapidly whirling solution. After standing twelve hours this is filtered, the precipitate washed on the filter with a 2.5% solution of ammonia, dried, carefully ignited in a porcelain crucible, oxidizing completely with nitric acid, and weighed. From the weight of magnesic pyrophosphate obtained may be calculated the amount of lecithans in the material analyzed.

The only criticism of this method of determining phosphorus is in regard to the wash water used. As recently worked out by Dr. R. M. Bird of the Missouri Agricultural Experiment Station, the yellow precipitate of ammonic phosphomolybdate is insoluble in a wash water containing only

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molybdate of the Association of Official Agricultural Chemists, published in Bulletin No. 46 of the U. S. Dept. of Agriculture, the formula being 50 gram of molybdic acid, 200 grams of 10% ammonia, and 75 grams of nitric acid, specific gravity 1.20. Hoppe-Seyler:loc. cit. S. 587.

10. The formula for the solution used is 110 grams of

one gram of ammonic nitrate per liter. This latter is a most economical wash water as well as an efficient one, while that of Hoppe-Seyler is many times more expensive.

Alkalimetric Method of Determination of Phosphorus by A. Neumann.

Oxidation of Material.-- In Neumann's method of determining phosphorus, the organic material is destroyed by heating it in a one-half to three-fourths liter Jena flask with a mixture of equal volumes of concentrated sulphuric acid and concentrated nitric acid, specific gravity 1.40, adding 5-10 c.c. of the mixture at the beginning and subsequently small successive portions by drops from a dropping funnel, until oxidation is complete, carefully heating intermittently until the brown nitrous fumes are cleared away, but not until charring occurs. The oxidation is complete when the colorless or bright yellow solution does not darken from charring, on heating until the white fumes are evolved from the sulphuric acid. The dropping funnel(10a) advised to be used in this process is one of 100 c.c. capacity, its stem having been bent twice at an angle of about fifty degrees, once near the funnel and once near the point

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crystalline magnesic chloride, 140 grams of ammonic chloride in 1300 c.c. of water and 700 grams of 10% ammonia solution. Hoppe-Seyler: loc. cit. S. 587.

10a. Neumann, A.: Ztschr. f. Physiol. Chem. 43,32, 1904.

and the point drawn out to small caliber. With this the operator's hand is not exposed to the fumes from the oxidation flask and the acid may not be run into the flask too rapidly. The volume of the oxidation mixture must be known approximately and must not exceed 40 c.c.(11). The process is carried out under a good draft.

Determination of Phosphorus.-- In the determination of the phosphorus in the solution obtained, the solution is diluted to 150-160 c.c. (proportionately more, if more than 40 c.c. of the oxidation mixture has been used) and such a volume of a 50% solution of ammonic nitrate added that one-fifth of the amount added will remain in the solution as such(12). This solution is heated to 70-80 degrees centigrade, 40 c.c. of a 10% aqueous solution of ammonic molybdate added for every 60 milligrams of phosphoric acid (P_2O_5) present, the whole vigorously shaken for half a minute and set aside for the precipitate to settle. After fifteen minutes the supernatant liquid is carefully decanted off without interruption through an ashless filter of 5-6 cm. radius and the precipitate washed in the flask three to four times with 150 c.c. portions of cold water, decanting through the filter each time until the washwater

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11. This limit is undoubtedly too high, as will be explained later.

12. Neumann, A.: Ztschr. f. Physiol. Chem. 43, 32, 1904.

is no longer acid, as indicated by its reaction to litmus. The washing being complete, the filter and contents are placed in the flask with the bulk of the precipitate, about 150 c.c. of water added and the whole vigorously shaken in order to unfold the filter and to distribute the ammonic phosphomolybdate more equally. This being done, a half normal solution of sodic hydroxide is added from a burette until all the yellow precipitate dissolves on shaking. 5-6 c.c. in excess is added and the solution carefully heated and boiled over a free flame until all the ammonia is driven out, as indicated by the reaction of the vapors to litmus. This requires about fifteen minutes. The solution is cooled by allowing a stream of cold water to flow over the flask. When cool, 6-8 drops of a 1% alcoholic solution of phenol-phthalein are added and the excess of sodic hydroxide titrated with a half-normal solution of sulphuric acid. The end point is sharp. The number of cubic centimeters of half-normal solution of sodic hydroxide added minus the number of cubic centimeters of half-normal solution of sulphuric acid added gives the number of cubic centimeters of half-normal solution of sodic hydroxide used in dissolving the yellow precipitate. 1 c.c. of half-normal solution of sodic hydroxide is equivalent to 1.268 milligrams of P_2O_5. This last gives for the formula representing the composition of the yellow precipitate obtained by Neumann's method the

expression $(NH_4)_3PO_4.12MoO_3.2HNO_3$ (13), which is the same as that obtained by Hundeshagen(14) for the ammonic phosphomolybdate precipitated from nitric acid solution.

Neumann ascertained the number of milligrams of P_2O_5(15) to which 1 c.c. of half-normal sodic hydroxide solution is equivalent by adding to varying definite volumes of a known solution of di-sodic hydric phosphate 10 c.c. portions of the acid mixture and determining the phosphoric acid by the method described. His work is open to the criticism that 10 c.c. of the mixture is much less than is ordinarily used in carrying out an oxidation and, second, the solution obtained by thus oxidizing contains no nitric acid.

The primary shortcoming of the Hoppe-Seyler method or any method based on a determination of total phosphorus is that it does not distinguish between the two kinds of lecithans--lecithins and kephalins--merely determining the total lecithans in a given tissue or fluid.

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13. Neumann, A.: Ztschr. f. Physiol. Chem. Bd. 37, S.131.
14. Hundeshagen: Ztschr. f. Analyt. Chem. Bd. 28, S. 141, 1889.
15. Neumann, A.: loc. cit. S. 130.

Method of Thudicum.

Extraction.-- According to Thudichum's method of analysis of the brain, a weighed brain with coverings removed is finely divided, hardened with alcohol, crumbled and worked through a sieve. The finely crumbled material is extracted once with 85% alcohol and finally so washed that a liter of alcohol, heated with the entire mass, yields after filtration and distillation only an insignificant residue.

The first alcoholic extract is allowed to stand and cool. A mass of white material separates out.

Analysis of the White Material.-- The white material which separates out is extracted first with cold ether and then with hot ether. The extraction with hot ether is made in a special reflux extraction apparatus described by Thudichum(16). In this the ether vapors pass upward around a creased filter and the condensed hot ether falls back through the material on the filter, carrying the ether extract into the flask below.

Treatment of the Ether Solution. Separation of its Ingredients from one another.-- The ether extracts are combined and the ether distilled off entirely. The liquid residue is mixed with a solution of lead acetate in excess of alcohol with shaking and heated for sometime, using a reflux condenser. The mixture is allowed to cool and stand for a

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16. Thudichum: Gehirn, S. 75.

long time. The precipitate, consisting of the lead salts of kephalin and myelin, settles and is later covered by the cholesterin which crystallizes out. The mixture is moderately and slowly warmed in the water bath, in order to dissolve and pour off the cholesterin. The treatment with alcohol and warming is repeated in order to make the plumbic kephalin and the plumbic myelin as clumpy and coherent as possible, so that later, when all the warm alcoholic solutions are filtered together through a warmed filter, as little as possible of these compounds will get onto the filter. The residue of lead salts is then heated for a long time with alcohol, filtered and washed. The filtrates contain all the lecithin, paramyelin, cholesterin, amino-fats and cerebrin and a portion of the plumbic myelin from the white material. The insoluble residue consists of all the plumbic kephalin and most of the plumbic myelin.

Separation of Plumbic Kephalin and Plumbic Myelin.-- These are separated by absolute ether, in which plumbic kephalin is completely soluble, while the plumbic myelin remains behind as an insoluble white residue. The latter is washed with ether by decanting with a siphon and air pressure, finally placed on the filter, dried and weighed. This constitutes the first portion of the plumbic myelin. The solution of plumbic kephalin and all the ether used in washing the plumbic myelin is distilled from a tared flask

and the residue reckoned as plumbic kephalin. It contains all the kephalin which was present in the white material.

Lead, phosphorus or nitrogen may be determined both in plumbic kephalin and in plumbic myelin and the amount of pure kephalin or myelin calculated from the result.

Treatment of the Alcohol-soluble Portion.-- The combined and heated filtrates and the alcoholic washings from the lead precipitates are mixed with as much hot water as will be taken up without a precipitate forming and allowed to cool slowly. Cholesterin crystallizes almost completely. The portion of plumbic myelin in the solution also separates from it. Lecithin, paramyelin and some other materials remain in solution.

Separation of Cholesterin and Plumbic Myelin.-- The cholesterin is separated from the plumbic myelin by suspending the isolated, pressed, crystalline, dried material in ether and shaking well. The cholesterin dissolves, while the plumbic myelin and a small portion of cerebrosid remains undissolved as a white precipitate. The extraction of the cholesterin is continued by the repeated use of large volumes of ether until complete. The combined extracts are distilled to dryness; the residue is dissolved in hot, dilute alcohol and hot water is added to the solution until it becomes cloudy, in order to reduce the solubility of the cholesterin to a minimum, and the whole is set aside to crystallize. The cholesterin is filtered off, dried and

weighed. The insoluble white salt remaining after the ether extraction of cholesterin is the second portion of plumbic myelin. The cerebrosid sometimes mixed with it is extracted with hot alcohol.

The dilute alcoholic mother liquor of the cholesterin may still contain some cholesterin, but nothing else of significance save coloring matter. It may therefore be added to the main mother liquor from which the cholesterin and myelin were separated.

Alcoholic Solution of Lecithin, Paramyelin, Cerebrin, etc., and some Cholesterin(?).-- The lecithin remains in the dilute alcohol uncombined with lead. It may be separated from the trace of cholesterin in combination with a precipitant or hydrolysed and determined by means of its splitting products, namely, glycero-phosphoric acid, neurin, and oleic and margaric(?) acids. For precipitation an alcoholic solution of cadmic chloride is best. This precipitates both lecithin and paramyelin as chlorcadmic compounds, which are soluble in hot alcohol, insoluble in cold. The two chlorcadmic compounds are separated by benzol, the chlorcadmic lecithin being dissolved by cold benzol and the chlorcadmic paramyelin by hot benzol, the latter separating out in the cold.

Treatment of the Alcoholic Solution from which the White Material Separated.-- The solution and all the alcoholic extracts, obtained by repeated washing of the brain

material, are combined, distilled to moderate concentration and cooled. There separates out a buttery material which consists of lecithin, paramyelin, myelin, kephalin, cholesterin and traces of some other ingredients.

Treatment of the Concentrated Alcoholic Solution from which the Buttery Material has Separated.-- This solution is heated on the water bath until all the alcohol is driven out. On the resulting watery solution there floats a sort of oil, which either stiffens or remains oily on cooling, but is opaque and cohesive, absorbs water and distributes itself in flocks in the solution. This is mainly cerebrin with bregenin and amino-fats. Still mixed with these are small portions of lecithans and cholesterin. The whole product is called the final oily material. It is advised to free it from the aqueous solution mechanically, without using a filter, by collecting it on the walls of the vessel, pouring off the aqueous solution, washing it quickly with water. The buttery material is then added to it.

Analysis of the Combined Buttery and Final Oily Materials.-- The mass is dissolved in a slight excess of hot alcohol, precipitated with alcoholic lead acetate solution and the analysis completed in the manner above described for the ether extract from the white material. In addition to the constituents of the latter, the mixture of buttery and oily materials contains also amido-myelin.

Precipitation of Lecithans.-- From the alcoholic solution from which the lead salts of kephalin and myelin have been separated, all the lead is removed by heating with excess of ammonia. To the cold solution is then added an alcoholic solution of cadmic chloride, as long as a precipitate forms, and then one-half as much in excess. The precipitate is collected on a cloth and well pressed. It is then recrystallized from alcohol, washed with ether, and finally, the three lecithans separated by the above mentioned process with benzol. In this manner is obtained chlorcadmic lecithin, chlorcadmic paramyelin and di-chlorcadmic amidomyelin, all as crystalline, well defined compounds which are well dried and weighed.

One may also use hydrolysis in a quantitative analysis of particular constituents, even in a mixture; thus, for example, in a determination of lecithin. This lecithan is so far the only constituent of the brain that yields fatty acids on hydrolysis. A mixture may therefore be hydrolysed with baric hydroxide, the fatty acids set free, combined with lead and the lead salts extracted with ether or benzol. Thus the amount of lecithin present may be reckoned from the weight of the lead salts of the fatty acids obtained.

Chlorplatinic neurin may also be used as a means of approximate quantation of the lecithans, which contain the neurin radical; together with phosphorus it forms a good criterion.

Thus did Thudichum estimate the more abundant lecithans, cholesterin, cerebrin, etc., in the brain. His method of determining the other constituents of the brain does not concern us here.

The advantage of Thudichum's method is that by it a distinction is made between the lecithins and kephalins. The objections to it are the large amounts of alcohol and ether required in the various processes, its tediousness and the consequent extreme difficulty that would be encountered in mastering the technique to the extent necessary to obtain accurate results, especially when making analyses of comparatively small amounts of materials containing a small percentage of lecithans. Hence the method,as outlined by Thudichum,is not practically applicable to the determination of the lecithans in the various tissues and fluids of the body.

Method of Koch.

In determining the lecithans in the brain and spinal cord, Koch extracts alternately with hot alcohol and ether. The special feature of his method, however, is that he determines the lecithans in the residue from the alcohol-ether extract by virtue of methyl groups split off from nitrogen upon heating with hydriodic acid, according to the method of Herzig and Meyer(17). Briefly, in this method, the residue to be analyzed is heated with hydriodic acid and ammonic iodide in a double glass bulb placed in a sand bath. The reaction products are driven over by a current of carbon dioxide through a 100 c.c. flask, through a vertical condenser with its jacket kept filled with water at 40-50 degrees centigrade, and through a solution consisting of one part of potassic arsenite, one part of sodic carbonate and ten parts of water in a Liebig bulb surrounded by water kept at 40-50 degrees centigrade. This removes the hydriodic acid and iodine, the methyl iodide being carried on into an alcoholic solution of silver nitrate in a 100 c.c. flask, where it reacts with the silver nitrate, forming silver iodide, which precipitates out. The alcoholic solution and the precipitate of silver iodide are transferred to a beaker, the solution diluted with much water and heated

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17. Herzig und Meyer: Monatshefte fur Chemie, Bd. XV, S. 613.

on the water bath for several hours to remove the alcohol. Strong nitric acid is then added and the silver iodide filtered into a Gooch crucible, dried and weighed.

In this process methyl iodide is formed continuously from the temperature of 160 degrees centigrade to that of 240 degrees centigrade, as shown by the cloudiness of the silver nitrate solution. At 240 degrees centigrade the reaction ceases, as shown by the settling of the silver iodide precipitate, leaving the solution clear. The precipitation flask may now be replaced by another. On raising the temperature to 280 to 320 degrees centigrade a second reaction is brought about, more methyl iodide is formed and a final portion of silver iodide obtained. The first portion of silver iodide represents one methyl group from the kephalins present and one from the lecithins, the second portion representing the remaining two methyl groups from the lecithins and none from the kephalins. From these facts and the weights of silver iodide obtained may readily be calculated the amounts of lecithins and kephalins present in the residue analyzed, and hence the percentage in the sample taken.

Investigation of Methods.

The Hoppe-Seyler method not offering a means of separation of the lecithins from the kephalins it was not experimentally investigated. In attempting to apply the method of Koch to other tissues than the brain and spinal cord more silver iodide was obtained than could possibly be accounted for by lecithans. This being true, the source of error was investigated.

Estimation of Lecithans in Presence of Fats by Determination of Methyl Groups.

That the difficulty is due to the fat present in the other tissues I showed by the following experiment made with butter fat, which contains no lecithans.

Apparatus.-- A kipp carbon dioxide generator with two Wolff bottles, the first containing some water to wash the gas and the second, some concentrated sulphuric acid to dry it; next, a 100 c.c. reaction flask; after this, a 100 c.c. condensing flask in a beaker of water; following this, a set of Liebig bulbs containing the solution of one part of potassic arsenite, one part of sodic carbonate, and ten parts of water, the bulbs being suspended in a beaker; and finally, two 100 c.c. flasks, each containing 50 c.c. of the alcoholic solution of silver nitrate, made by dissolving two grams of silver nitrate in 5 c.c. of water and pouring this into 50 c.c. of alcohol.

Experiment.-- Placed 0.50 grams of butter and 5 c.c. of previously prepared crude hydriodic acid in the reaction flask. Connected up the apparatus and started a slow current of carbon dioxide through it. With a Bunsen flame heated the water in the beaker containing the condensing flask to 40-50 degrees centigrade and kept it at about that temperature. Poured water of the same temperature into the beaker surrounding the bulbs and kept it so continuously by further addition of hot water whenever necessary. With a Bunsen flame heated the contents of the reaction flask to boiling and kept it so until the reaction ceased. The silver nitrate solution in the first flask soon became cloudy, but after some time it cleared up again, owing to the cessation of the reaction and the subsequent settling of all the silver iodide formed in the flask mentioned. No precipitate formed in the silver nitrate solution in the second flask, this serving merely as a guard. The reaction having come to an end, removed the flames and cut off the current of carbon dioxide. Transferred the contents of the flask containing the precipitate to a beaker, added a relatively large quantity of water, evaporated off the alcohol on a water bath and added a little strong nitric acid. Filtered through a dried and tared Gooch crucible, washed the precipitate until the washings gave no test for silver nitrate, dried it in an air bath for half an hour at about 115 degrees centigrade

and weighed. The weight of silver iodide obtained was 46.6 milligrams. This corresponds to 2.97 milligrams of methyl, which is 0.59% of the weight of butter used and would account for 175 milligrams or 3.48% of kephalin.

The result of this experiment shows that, even at temperatures below that at which it reacts with kephalin, hydriodic acid reacts, perhaps with glycerin formed by the decomposition of the fats, yielding methyl iodide.

The error introduced into the kephalin determination by the presence of fat is so difficult of correction that the method is rendered at least impractical, if not inaccurate, save in the absence of fats. The presence of fat in most of the tissues and fluids of the animal body thus offering an unavoidable difficulty, the Koch method is not practically applicable to the determination of the lecithans in them.

Neither the method of Hoppe-Seyler nor of Koch for the estimation of the lecithans being applicable to the determination of the lecithins and kephalins in the various tissues and fluids of the body, that of Thudichum was investigated. Thudichum's method of separation of kephalins from lecithins being based on the fact that the kephalins react with lead acetate with the formation of insoluble lead salts(18) whereas the lecithins do not do this(19), the efficiency of this means of separation was tested experimentally.

18. Thudichum: Gehirn, S. 129, S. 283.
19. Thudichum: Gehirn, S. 121, S. 283.

The Separation of Lecithans by Lead Acetate.

The experiments made were the following:

First Experiment.-- Took 151 milligrams of lecithin prepared from a brain and analyzed it for phosphorus. Found 4.9 milligrams of phosphorus, which figure divided into 151 gives 30.8 as the phosphorus factor for brain lecithin. Analyzed 290 milligrams of kephalin prepared from a brain for phosphorus and found 9.86 milligrams of phosphorus, which figure divided into 290 gives 29.4 as the phosphorus factor for kephalin. Analyzed 299 milligrams of lecithin prepared from egg yolk and found 10.43 milligrams of phosphorus, which figure divided into 299 gives 28.7 as the phosphorus factor for egg lecithin.

Second Experiment.-- Weighed into one flask 100 milligrams of brain lecithin and 229 milligrams of brain kephalin; into a second flask, 110 milligrams of brain lecithin and 162 milligrams of brain kephalin; and into a third flask 303 milligrams of egg lecithin and 184 milligrams of brain kephalin, all from the preparations mentioned above. Added 100 c.c. of alcohol to each flask and heated to boiling. This dissolved all of the lecithin and part of the kephalin. To each I added 10 c.c. of a hot, saturated alcoholic solution of lead acetate and shook the flasks well. Flocculent precipitates were formed, which, on being allowed to stand, settled out in a few

minutes, leaving a tolerably clear solution. Very soon, however, the solution clouded up again, probably as a result of the cooling, causing the separation of the compound of lead acetate and alcohol which is probably analogous to the ordinary crystalline lead acetate(20); or it may have been due to the formation of some other lead compound(21), or to a further precipitation of the lead salt of kephalin(22). After twenty-four hours the clear supernatant liquids were decanted through small filters into flasks and the precipitates and filters each washed once with a few cubic centimeters of alcohol, this wash alcohol being combined with the filtrates. The filtrates were evaporated to dryness and both the residues and the precipitates analyzed for phosphorus. Number one gave 6.37 milligrams of phosphorus from the residue and 5.27 milligrams of phosphorus from the precipitate. The first, multiplied by the factor 30.8, accounts for 196 milligrams of lecithin and the second, multiplied by the factor 29.4, accounts for 155 milligrams of kephalin. Number two gave 5.1 milligrams of phosphorus

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20. On allowing a hot, saturated alcoholic solution of lead acetate to cool, the whole of it solidifies, forming a white crystalline mass. When again heated on the water bath this crystalline mass melts, giving the original colorless liquid.

21. 10 c.c. of a hot, saturated alcoholic solution of lead acetate added to 100 c.c. of hot alcohol resulted in the formation of a white precipitate on cooling. On filtering after twenty-four hours a clear filtrate was obtained, but this soon clouded up again and more of the precipitate settled out. These precipitates were not completely soluble on heating.

22. On filtering the supernatant liquid off from a keph-

from the residue and 3.4 milligrams of phosphorus from the precipitate. This accounts for 167 milligrams of lecithin and 100 milligrams of kephalin. Number three gave 10.2 milligrams of phosphorus from the residue and 4.82 milligrams of phosphorus from the precipitate. 10.2 multiplied by the factor 28.7 accounts for 292 milligrams of lecithin; and 4.82 multiplied by the factor 29.4 accounts for 141 milligrams of kephalin. The result may be noted more readily from the following table:

	Lecithin taken	Lecithin found	Kephalin taken	Kephalin found
No.1	100 mgms	196 mgms	229 mgms	155 mgms
No.2	110 "	167 "	162 "	100 "
No.3	303 "	292 "	184 "	141 "

There are two possible explanations for the fact that all the kephalin was not accounted for and that the lecithin was more than accounted for. One is that the kephalin is not all precipitated by lead acetate in twenty-four hours, under the conditions used, as I have shown (Note 22, p. 22); and the other is that the substance called kephalin contains a phosphoric compound not precipitated by lead acetate.

Though the precipitation with lead acetate does not

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alin precipitate after twenty-four hours and allowing the filtrate to stand another day, further precipitation resulted. This precipitate was not soluble on heating and furthermore it yielded an appreciable amount of phosphorus on analysis.

give a quantitative separation of the kephalins from the lecithins, it was adopted as a means by which approximate and comparable results may be obtained, more especially since, according to Thudichum, the addition of ammonic hydroxide makes the precipitation more complete(23).

With this method, however, the presence of the sulphur compound, mentioned as forming a part of the alcohol-ether extract(p. 2) would introduce an error into the determination of the lecithans, as it does in the case of the spinal cord, since it contains about 2% of phosphorus as well as the 4% of sulphur(24).

Correction for Error due to Sulphur Compound.

To ascertain the value of this correction in the case of the different tissues determinations of the organic sulphur were made in the lipoid precipitate(25)from the alcohol ether-extract from a sample of each of various tissues. Since the sulphur compound contains about half as much phosphorus as the lecithans, these containing about 4%(26), the percentage of sulphur compound is about twice the correction necessary to be made in a lecithan determination made by virtue of the phosphorus content of the

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23. Thudichum: Gehirn, S. 129, S. 135.
24. Koch, W.: Am. Jour. Physiol. XI, 3, 1904, p. 311,p.325.
25. This is the precipitate containing lecithans, fats, sulphur compound, etc., obtained by adding acid and chloroform to the water emulsion of the residue from an alcohol-ether extract.
26. Thudichum: Gehirn, S. 116, S. 132;
Hoppe-Seyler: loc. cit. 160;
Koch, W.: Univ. of Chicago, Decen. Pubs.X,1902,p.100.

lipoid precipitate.

As may be noted from the following table, this correction is found to be very slight, save in the case of the spinal cord. The percentages of sulphur compound in the tissues recorded in the table were calculated on the basis of all the organic sulphur present being in the form of sulphur compound. The first column of figures represents the number of parts per million of sulphur found in the lipoid precipitate, the second gives the calculated amount of sulphur compound in per cent of total substance and the third, the average percentage of total lecithans found in the tissues in later work.

Tissue.	Parts per million of sulphur	Sulphur compound. In %.	Total Lecithans. In %.
Submaxillary gland	48.	0.12	1.8
Testicle	59.	0.15	2.2
Kidney	85.6	0.21	2.7
Lung	84.4	0.21	2.2
Heart muscle	96.	0.24	1.7
Voluntary muescle	21.	0.05	1.3
Liver	96.	0.24	2.6
Spinal cord	668.	1.67	

The lead acetate method giving an approximate separation of the lecithans and the sulphur compound offering no hindrance to its use, the problem is reduced to the estimation of the phosphorus in the lecithin residue and the kephalin precipitate obtained. For this purpose the method of Hoppe-

Seyler and that of A. Neumann were considered.

No attempt was made to use the very excellent method of Hoppe-Seyler, because of the objection mentioned in regard to the oxidation process and primarily, because a volumetric method was preferred to a gravimetric one. The method of A. Neumann offering an easy, rapid means of absolutely complete destruction of organic matter and promising an accurate and economical volumetric means of determination of the phosphorus in the solution obtained, a faithful attempt was made to use it.

Difficulties of Neumann Method.

The difficulties encountered in the application of the Neumann method of determining phosphorus are those briefly mentioned by its author(27). If more than 15-16 c.c. of sulphuric acid are present in the solution,precipitation becomes slower and more or less incomplete and, if more than 20 c.c. are present, complete precipitation is prevented, the solution remaining yellow. Reasonable dilution and proportionate increase in the amount of ammonic nitrate added do not, contrary to Neumann's assertion, satisfactorily counteract the unfortunate effect of excess of sulphuric acid, but I find that the careful addition of a few cubic centimeters of ammonic hydroxide solution,1 c.c. at a time, with subsequent shaking and settling

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27. Neumann, A.: loc. cit. S. 129.

is effective(28). On the contrary, however, if too little acid be present, as is sometimes the case, when little has been required to complete the destruction of the organic matter, the separation of the yellow precipitate is accompanied, or at least soon followed by the separation of a more or less heavy white precipitate of molybdic acid.

As a result, if the solution be too acid, filtration after fifteen minutes, or perhaps several times that period, is followed by a further separation of the yellow precipitate in the filtrate, necessitating refiltration and destroying confidence in the determination, especially when, as is often the case, this yellow precipitate in the filtrate is accompanied by the white precipitate. In this last case,the phosphorus in the precipitate must be determined as magnesic pyrophosphate in the usual manner. And, if the solution be not acid enough, the precipitate may, after fifteen minutes, contain a little easily noticeable molybdic acid, or as often happens, a more or less heavy white precipitate may form even immediately in the filtrate, destroying confidence in the determination or necessitating determination of the phosphorus as magnesic pyrophosphate.

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28. This may possibly be due partly to the reduction of the acidity, but it is due mainly to the reduction of the interfering action of the sulphuric acid.

Also, as mentioned by Neumann(29), ammonic phosphomolybdate is slightly coluble in cold water and this solution reacts acid to litmus. This causes possible loss of precipitate and consequently necessitates a tedious and difficult determination of just when the precipitate has been washed sufficiently, in order to prevent appreciable loss.

This last difficulty is readily avoided by using the wash water containing 0.1% of ammonic nitrate (Bird, p. 4), a few drops of this not affecting phenol-phthalein in dilute solution, a slightly alkaline solution of which may accordingly be used in testing the wash water to ascertain when the precipitate and filter have been sufficiently washed. The difficulties of precipitation are not so easily overcome, however. These might be disposed of by evaporating off the sulphuric acid after oxidation, as suggested by Neumann(30) for some other determinations, but the difficulty of the evaporation renders this means unsatisfactory. By using the means suggested by Neumann(31) for certain other determinations, the difficulties might be many times decreased by approximate neutralization of the sulphuric acid with ammonic hydroxide. The phosphoric acid in the solution could then be precipitated by acidify-

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29. Neumann, A.: loc. cit. S. 132.
30. Neumann, A.: loc. cit. S. 138.
31. Neumann, A.: loc. cit. S. 120.

ing with nitric acid and adding a nitric acid solution of ammonic molybdate, containing a little ammonic nitrate, were it not for the contrary action of the excessive amount of ammonic sulphate which would often be present. One might counteract this effect by dilution and addition of a larger percentage of ammonic nitrate. This has been done by B. L. Hartwell, A. W. Bosworth, and J. W. Kellogg, of the Rhode Island Experiment Station(32), who, having found Neumann's method to be inaccurate, usually giving them low results, succeeded in developing a method, based on the above principles, which gave them accurate results. Their method is as follows:

Dilute the sulphuric acid solution with water, neutralize with ammonic hydroxide, acidify with nitric acid, warm to 70-75 degreés centigrade, add excess of the standard nitric acid solution of ammonic molybdate of the Association of Official Agricultural Chemists, to which has been added such an amount of ammonic nitrate solution (or solid) that the large volume of liquid from which the yellow precipitate is to separate out, will contain 15 grams of ammonic nitrate per 50 c.c. of solution. The resulting solution is kept at 60-65 degrees centigrade for several hours in order to secure complete precipitation of the phosphorus as ammonic phosphomolybdate. This means of

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32. Jour. Am. Chem. Soc., XXVII, 3, 1905.

getting the phosphorus precipitated quantitatively being effective as asserted by its authors and, as indicated by my experience, this principle of separation was adopted.

I am strongly inclined to think, however, that a saving modification of the Neumann method would be to add 10-20 c.c. of the mixture of concentrated sulphuric acid and nitric acid, specific gravity 1.40, to the mixture to be oxidized, heat as directed and complete the oxidation by careful subsequent successive additions of about 1c.c. portions of nitric acid, specific gravity 1.48, by drops from the dropping funnel. In this way the amount of sulphuric acid in the solution obtained may be far more easily controlled. To the solution obtained should be added from 2 to 6 c.c. of nitric acid, specific gravity 1.40, the volume varying with the volume of sulphuric acid used. This would give rather favorable conditions for the precipitation of ammonic phosphomolybdate, according to Neumann's directions, and moreover, would give very similar conditions to those under which he determined the equivalent of 1 c.c. of half-normal sodic hydroxide solution in terms of P_2O_5.

I did not adopt the use of the aqueous solution of ammonic molybdate of Neumann, because, as is well known, the use of a nitric acid solution precludes the probability of the separation of molybdic acid, while the former does not.

Foundations of Method Devised.

Considering now the method of determination of the lecithans in its entirety, the following are the principles upon which the work here presented is based.

Principles.-- Proteids are insoluble in alcohol, being coagulated by it; water and lecithins(33) are extremely soluble in warm alcohol, kephalins(34) also to a certain extent; both lecithins(35) and kephalins(36) are extremely soluble in ether; the lecithans readily form an emulsion with water(37); the addition of a little acid and chloroform to the emulsion causes complete precipitation of the lecithans(38) and other colloids, but not of the inorganic salts and water extractives; the lecithans are hydrolysed only with extreme slowness by dilute acids, even by dilute sulphuric acid(39); the kephalins are precipitated from alcoholic solution by lead acetate and ammonia(40), whereas the lecithins are not(41); organic matter is rapidly and completely destroyed by heating it with a mixture of concentrated sulphuric acid and strong nitric acid; lead sulphate is formed when other lead salts react with sulphuric acid and this lead

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33. Thudichum:Gehirn,S.121. 34. Thudichum: Gehirn, S.133.
35. Thudichum:Gehirn,S.121. 36. Thudichum; Gehirn, S.133.
37. Thudichum:Gehirn,S.126, S. 132; Koch W.: Univ. of Chicago, Decen. Pub. 1902, X, p. 94.
38. Thudichum:Gehirn,S.134; Koch W. Am. Jour. Physiol. XI, 3, p. 316, 1904.
39. Even 10% sulphuric acid hydrolyses lecithans only gradually. Hoppe-Seyler, loc. cit. S. 159.
40. Thudichum: Gehirn, S.129. 41. Thudichum:Gehirn, S.129.

sulphate is least soluble in dilute sulphuric acid containing one part of the concentrated acid to twenty parts of water(42); phosphorus is precipitated quantitatively as ammonic phosphomolybdate from a large volume of solution containing a large percentage of ammonic nitrate, even in the presence of ammonic sulphate, by a nitric acid solution of ammonic molybdate, when kept warm for several hours(43); ammonic phosphomolybdate is insoluble in a 0.1% solution of ammonic nitrate, a few drops of the latter also not affecting phenol-phthalein in slightly alkaline dilute solution(Bird, p. 4); ammonic phosphomolybdate being dissolved in half-normal sodic hydroxide solution, 1 c.c. of the half-normal solution is equivalent to 1.268 milligrams of P_2O_5 (0.553 milligrams of phosphorus) (44).

The following is the method developed, based on the above principles.

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42. Talbot: Quantitative Chemical Analysis, p. 51.
43. Hartwell, B. L., Bosworth, A. W., Kellogg, J. W.: Journal American Chemical Society, XXVII, 3, 1905.
44. Neumann, A.: loc. cit. S. 132.

The Method of Determining Lecithans Used in this Research.

Extraction.

Apparatus.-- For making an extraction with ether three Hopkins condensers are connected compactly in series, the connections being made with short pieces of heavy rubber tubing. Each of the two outer condensers is supported in a vertical position by a clamp fastened to a ring-stand, the middle one being supported by the rubber connections. Near the lower end of each condenser tube have been bored(45) three small holes equally distributed around the tube. Each condenser has been carefully fitted with a good cork of the proper size. From each condenser a ring made of copper wire is suspended by fine copper wires passing through the small holes in the tube. In this ring there is to hang a Gooch crucible and over the crucible and the cork adapted to the condenser tube is to fit snugly a carbon dioxide flask. Three flasks thus attached to the condensers hang immersed to the neck in a galvanized iron water bath made of the proper dimensions. This bath has a cover (to conserve heat as well as to prevent escape of water vapor)

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45. This is easily done by the use of a broken end of a file moistened with a solution of camphor in turpentine.

in which at the proper places are three holes slightly larger than the flasks. The bath is supported on two rings, one being attached to each ring-stand. A low form Bunsen burner is conveniently used under the bath.

Process.-- (a) Solids.-- A 10-15 gram sample having been taken from, for example, the liver of a dog, freshly killed by anaesthetizing and thoroughly transfusing with Ringer's solution(46), the sample is cut into small pieces, using scissors for this purpose, placed in a tared 200 c.c. flask, weighed, covered with 100 c.c. of 95% alcohol and heated to 50-60 degrees centigrade on the water bath for an hour. The sample may now be set aside indefinitely, the flask being corked. When ready to continue the extraction, the contents of the flask are again heated to 60 degrees centigrade on the water bath and filtered into a glass dish through a Gooch crucible having its bottom completely covered with a piece of filter paper. The undissolved portion of the tissue is cut up, using a pair of small scissors, into very small pieces, all placed in the crucible and a piece of perforated porcelain laid on top to distribute the ether in the subsequent extraction. The crucible is now placed in the wire ring suspended from one of the condensers and the carbon

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46. The solution used contained 0.7% sodic chloride, 0.03% potassic nitrate and 0.026% calcic chloride.

dioxide flask containing 25 c.c. of ether slipped over it and pressed tightly over the cork. There being three such samples in the apparatus, the water is heated to the temperature necessary to keep the ether evaporating at the desired rate, the heating flame regulated and the extraction allowed to continue for ten hours. A stream of cold water is, of course, run through the condensers during the extraction. The contents of the glass dish having been carefully evaporated to small volume on the water bath, the ether extract is carefully and completely transferred to the glass dish by the use of ether and alcohol and a flattened, sealed glass tube with a small piece of rubber tubing on the flattened end to assist in cleaning the flask. The undissolved residue in the crucible is replaced in the original extraction flask, covered with 50 c.c. of alcohol and the contents of the flask heated almost to boiling on the water bath for about an hour. After this the flask and contents may be set aside for a time. The contents of the glass dish having evaporated somewhat, the contents of the flask are heated almost to boiling on the water bath for about an hour and filtered into the glass dish, thoroughly washing the flask and residue with hot alcohol and a little ether.

The first and last extractions may be hastened by heating the samples in the alcohol for about four hours and three hours recpectively and filtering at once. These extracts may advantageously be filtered into the carbon dioxide flask used in the ether extraction and the alcohol and the ether evaporated from that as from the glass dish. Also, the final extraction with alcohol may be made in the same manner as the ether extraction, merely evaporating off the ether from the carbon dioxide flask, adding 25 c.c. of alcohol and keeping the bath at the proper temperature for four hours or longer.

In the case of milk, however, for example, the above method of extraction is necessarily modified in certain details, partly on account of the presence of phosphoric compounds, other than lecithans, soluble in dilute alcohol. Accordingly the following method of extraction is used:

(b) Liquids.-- 100 c.c. of milk being placed in a tared 350 c.c. flask and weighed, 200 c.c. of 95% alcohol is added and the flask placed on the water bath, contents heated to 50-60 degrees centigrade for at least an hour and filtered into a glass dish. The precipitate is transferred to the filter and the extract allowed to drain into the glass dish. When no more drains off, the bulky precipitate is replaced in the flask, 50 c.c. of ether

added and the flask stoppered, shaken and set aside for two days. The bulk of the contents having been carefully evaporated from the glass dish, the ether extract is decanted through a filter into the glass dish, the filter being carefully washed with ether. 50 c.c. of alcohol is now added to the flask, the flask placed on the water bath, and the contents heated almost to boiling for two hours. The ether having evaporated from the glass dish, the final extract is filtered into it, and the flask, insoluble residue and filter carefully washed with hot alcohol and with a little ether. The combined extracts are now evaporated very near to dryness on the water bath. The residue is thoroughly extracted with 50-60 c.c. of ether in five or more successive portions, the residue being carefully worked up with a small spatula, and the extracts filtered into a 100 c.c. glass stoppered flask.

The combined extracts obtained may advantageously be evaporated to dryness, the residue finely divided and extracted with ether in the extraction apparatus and the extract in the carbon dioxide flask carefully and completely transferred to a 100 c.c. glass stoppered flask.

The combined extracts thus obtained contain all the lecithans, some water extractives, some inorganic salts, usually fats. and sometimes the sulphur compound.

Emulsification and Precipitation.

The combined extracts in the glass dish or in the glass stoppered flask having been carefully evaporated very nearly to dryness on the water bath, distilled water is added. After several hours, not exceeding twenty-four, because of danger of bacterial decomposition, the contents of the dish are completely transferred to a 100 c.c. glass stoppered flask with the aid of distilled water and a sealed, flattened glass tube with a small piece of rubber tubing on the flattened end(47). The contents of the flasks are made up to about 90 c.c. with water and the organic material emulsified by shaking well. After emulsification 1-2 c.c. of concentrated hydrochloric acid and 2-4 c.c. of chloroform are added(48), the volume made up to the mark with water and the flasks shaken vigorously for a minute or two to hasten the forma-

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47. In case much fat is present it is very difficult to remove it mechanically from the sides of the dish, but it is readily removed with the aid of 2-3 c.c. of chloroform, which is afterwards transferred to the flask to aid in the precipitation.

48. The amount of chloroform necessary to produce complete precipitation increases with the amount of fat present. If too much chloroform be added, however, it makes subsequent decantation of the supernatant liquid very difficult, consequently excess is to be avoided by adding too little rather than too much at first and adding more later if necessary. Also, the rate of precipitation and settling increases with the amount of hydrochloric acid added. Too much must not be added, however, because of the possibility of a slight amount of hydrolysis resulting. I add one c.c. at first and one c.c. more later, if appearances so indicate.

tion and clumping of the precipitate. The flasks are set aside for the precipitates to settle. This settling requires a varying number of days (one or more), the time depending upon the amount of fat present(49). The precipitate finally settles completely, however, leaving a clear supernatant liquid. This precipitate has been designated as the lipoid precipitate (p. 24), since it consists almost wholly of lecithans and fat, containing also cholesterin and the sulphur compound, when the latter is present in the tissue extracted. The slight error sometimes introduced by the sulphur compound may be corrected for in the manner hereinbefore indicated (p. 24). The supernatant liquid contains the extractives and inorganic salts. Thus the lecithans are obtained practically free from all other phosphoric substances save the sulphur compound, when that is present in any appreciable amount. The practically negligible portion of inorganic phosphates still contaminating the precipitate may also be estimated, however, and corrected for by making a determination of the amount in the decanted supernatant liquid.

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49. If little or no fat is present, this precipitate is rather firm and somewhat adherent to the sides of the flask, allowing of easy decantation. If much fat is present, however, the precipitate is oily, rendering decantation more or less difficult.

Separation.

The lipoid precipitate having settled completely, the clear supernatant liquid is carefully decanted off through a 6-8 cm. ashless filter(50) and the precipitate washed by vigorously shaking in the flask with one 10 c.c. portion of water containing 1% by volume of strong hydrochloric acid. The precipitate settles again in a few minutes and the wash water is also decanted through the filter. The precipitate in the flask is dissolved in hot alcohol, the solution poured through the filter, through which the filtrate and wash water have just been decanted, into a 300-500 c.c. long necked Jena flask, the glass stoppered flask and the filter thoroughly washed with successive portions of hot alcohol, and finally rinsed with a small portion of ether to dissolve the last trace of the kephalins. The volume of the solution is made up to 100 c.c. with alcohol, the solution heated on the water bath(51) and 5 c.c. of a hot, saturated alcoholic solution of lead acetate added to the rapidly whirling

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50. The filtrate should be tested for further precipitation by addition of 1 c.c. of hydrochloric acid and 1 c.c. of chloroform and allowing to stand.

51. This removes most of the small portion of ether present, which would interfere with the complete precipitation of the kephalins, the lead salts of these being slightly soluble in ether.

solution(52). It is replaced on the water bath for about ten minutes, 1 c.c. of strong ammonic hydroxide solution added and the whole vigorously shaken. After remaining on the water bath about five minutes longer, it is set aside to cool. After a definite time, when cooled(53), the clear supernatant liquid is recanted through as small an ashless filter as is practical for the case in hand (usually 6-8 cm.) into a 300-500 c.c. long necked Jena flask and the precipitate washed with a few cubic centimeters of alcohol, the wash alcohol being combined with the filtrate. The filter with the portion of the precipitate on it is placed in the flask with the major portion of the precipitate. Or the flask containing the major portion of the precipitate is placed under the funnel, a hole punched in the bottom of the filter and the portion of the precipitate on it completely washed into the flask, using as little hot water as possible. This water is carefully evaporated off over a

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52. The alcohol solution of the lipoid precipitate from the emulsion of the alcohol-ether extract of egg yolk gives a precipitate when treated in this manner. Since a qualitative preparation of kephalins cannot be made from egg yolk, the precipitation in this case is probably due, not to the presence of kephalins, but to some hydroxyl modification of lecithin, the pure lecithin itself not forming a precipitate under these conditions. An alcoholic solution of commercial lecithin made by the Actien Gesellschaft fur Analin also gives a very slight precipitate on addition of lead acetate and ammonic hydroxide. This too may be due to the presence in slight amount of some hydroxyl modification of lecithin and not to kephalin.

53. Twenty-four hours is the time I allow, but I think

free flame without charring the organic matter in the flask. In the latter case the necessity of burning the filter paper is eliminated. The solution of lecithins in the flask is evaporated to dryness in the water bath, the flask being turned on its side as much as possible in order to allow the vapors to flow out through the neck readily.

Oxidation.

Apparatus.-- For destroying the organic matter in the residues the following described piece of apparatus was set up. The stem of a 250 c.c. dropping funnel was bent at a right angle about an inch below the stop-cock. The point of the stem was drawn out to a small caliber and, the funnel being in the upright position, the stem was bent again downwards at a right angle nearly two inches from the point. This dropping funnel was placed in a ring clamped to a ring-stand and held firmly in the vertical position by a burette clamp fastened to the ring-stand and clamped around the stem of the funnel between the bend and the stop-cock. A ring of suitable size with a copper gauze on which to place a flask was adjusted to the ring-stand so that the tip of the funnel stem would be barely within the mouth of a flask placed upon it. A joint of stove-pipe, with elbow, being fitted in

there is little advantage in waiting this long, especially if only comparable results are sought.

the usual manner to one of the flues of the hood, a narrow vertical slit about two inches long was cut in the base of the pipe. The horizontal stem of the funnel was passed through the slit, the whole being so arranged that the vertical tip of the stem in the vertical axis of the stove-pipe extended just below its base. With such an apparatus the operator's hand is not exposed to the fumes from the oxidation flask, when turning the stop-cock, no difficulty is experienced from material falling into the flask from the elbow, when care is taken, and the draft is excellent, the fumes being carried away completely. The use of any sort of stove-pipe is not recommended, however, when it can be avoided, because of the possibility of material falling into the flask from the elbow.

Process.-- To the flask containing the lecithin or the kephalin residue 10-15 c.c. of a mixture of sulphuric acid, specific gravity 1.84, with an equal volume of nitric acid, specific gravity 1.42, is added. The flask being placed on the gauze with the funnel stem extending barely into its mouth, it is very carefully and slowly warmed, using a Bunsen flame. If sufficient care be not taken at first, the reaction will become too violent, and too, the flask may crack, because of the too rapid heating. When the brown fumes have cleared

away and the liquid has become only slightly colored from charring, the flame is removed, fuming nitric acid very carefully running by drops and the process continued until the organic matter is completely destroyed, as indicated by the failure of the clear, colorless or bright yellow solution to become dark as the result of the charring of the organic matter, when strongly heated, with the evolution of white fumes(54). About twenty minutes is usually required to carry out an oxidation, the time varying with the amount of organic matter to be destroyed. The solution obtained is set aside to cool in a draft that will prevent the fumes from diffusing into the laboratory.

Estimation of Phosphorus.

Reagents used.

1. Dilute sulphuric acid, made by adding 100 c.c. of sulphuric acid, specific gravity 1.84, to two liters of water.

2. Ammonic hydroxide solution, specific gravity 0.90,

3. Nitric acid, specific gravity 1.42.

4. Crystalline ammonic nitrate or a 60% solution, made by dissolving 600 grams of the solid in 400 c.c. of water and filtering.

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54. The small volume of nitrosyl-sulphuric acid remaining may well be partially evaporated off, if desired.

5. Molybdic solution, made, according to the formula of Olsen, by dissolving 75 grams of crystalline ammonic molybdate in 500 c.c. of water and pouring this solution into rapidly whirling dilute nitric acid (250 c.c. nitric acid, specific gravity 1.42 plus 250 c.c. water) in a bottle or beaker and the mixture vigorously shaken or stirred. This is kept in a warm place for several days, until a portion heated to 40 degrees centigrade gives no precipitate. The solution obtained is filtered or decanted into a glass stoppered bottle.

6. Ammonic nitrate solution, made by dissolving one gram of the solid in one liter of water.

7. Phenol-phthalein solution, made by dissolving one gram of the solid in 100 c.c. of alcohol.

8. Half-normal solution of sodic hydroxide, standardized by titrating against half-normal oxalic acid solution containing 31.51 grams of Kahlbaum's special oxalic acid per liter and against the half-normal solution of sulphuric acid prepared.

9. Half-normal solution of sulphuric acid standardized by precipitating as baric sulphate and weighing the ignited precipitate obtained.

Filtering Apparatus.-- For making subsequent filtrations six 350 c. c. filter flasks, fitted with rubber stoppers and small funnels, are connected abreast by

T-tubes and short pieces of rubber tubing. The set is connected by a glass tube and rubber connections to a filter pump. The number of flasks in the set is readily changed by varying the number of T-tubes.

Procedure.-- When cool, the solution obtained by oxidation of a lecithin residue or a kephalin precipitate in the manner described is diluted by the careful addition of 50 c.c. of water, filtered under pressure to remove the lead sulphate, formed by the reaction of the lead salt in the residues with sulphuric acid, and the flask, lead sulphate and filter carefully washed free from phosphoric acid, using as little of the dilute sulphuric acid (one volume of acid to twenty volumes of water) as possible for washing, the wash water being combined with the main filtrate. The filtrate is carefully transferred from the filter flask to a 300-500 c.c. flask, rinsing the filter flask several times with a little distilled water.

The filtered solution in the flask is neutralized with ammonia water, specific gravity 0.90, and acidified with strong nitric acid, adding about one c.c. in excess. To this is added about 50 grams of dry ammonic nitrate, or a volume of the 60% solution containing that amount (65 c.c.), and the volume made up to 200-225 c.c. After heating to 70-75 degrees centigrade on the water bath,

25 c.c. of freshly filtered molybdic solution is added, the flask well shaken, replaced on the water bath and kept at 60-65 degrees centigrade for six hours. Removing the flask from the water bath, the contents are quickly decanted into a filter flask through an ashless filter paper supported by a cone of hardened filter paper or of cheese cloth, using pressure. The precipitate, flask and filter are now washed with successive 10-20 c.c. portions of the 0.1% ammonic nitrate solution until freed from acid, as indicated by the reaction towards phenol-phthalein of the last few drops from the funnel. The washing being complete, the filter with the portion of the precipitate on it is placed in the flask containing the other portion of the precipitate, about 150 c.c. of water added and the flask shaken to unfold the filter paper and distribute the precipitate more loosely. Half-normal sodic hydroxide solution is now added from a burette until the precipitate all dissolves on shaking. 4-5 c.c. in excess is added and the solution carefully heated over a free flame, protected by a gauze, and boiled until all the ammonia is driven off, as indicated by the reaction of the vapors to litmus. This requires about fifteen minutes. It is next let stand until cool or cooled under a stream of cold water, 6-8 drops of the alcoholic solution of phenol-phthalein added and the excess of sodic hydroxide titrated with half-normal sul-

phuric acid. The total number of cubic centimeters of the sodic hydroxide solution added minus the number of cubic centimeters of sulphuric acid added gives the number of cubic centimeters of half-normal sodic hydroxide solution required to dissolve the precipitate. This, multiplied by the equivalent of of 1 c.c. of half-normal sodic hydroxide solution in terms of phosphorus, gives the amount of phosphorus found. This, multiplied by the factor for lecithin or kephalin, gives the amount of lecithin or kephalin found. And this, divided by the weight of the sample taken, gives the percentage of lecithin or of kephalin in the tissue analyzed.

Results Obtained.

In the course of the work some comparable results were obtained which I will record as being suggestive. Not yet having experimentally determined the exact equivalent of 1 c.c. of half-normal sodic hydroxide solution in terms of phosphorus, when it is precipitated as ammonic phosphomolybdate under the above conditions, Neumann's factor was used in calculating the results recorded in the appended table. According to this, 1 c.c. of half-normal sodic hydroxide solution is equivalent to 0.553 milligrams of phosphorus (Log. of factor equals -1.743). The factor by which the amount of phosphorus found was multiplied to obtain the amount of lecithin or kephalin was 25.75 (Log. equals 1.411).

From the table it is noticeable that heart muscle contains a greater percentage of lecithans than does voluntary muscle; that submaxillary gland contains more than heart muscle; that lung tissue contains more than submaxillary gland tissue; that the percentage in the testicle varies somewhat inversely with the age of the dog from which the testicle is taken, the average being about the same as that of the lung; that the kidney contains probably more than the average testicle; that the liver contains about the same as the kidney; and finally, that very little is found in cow's milk. It may also be observed that a greater percentage of lecithins than of kephalins were found in the voluntary muscle while the reverse was true in the case of the heart muscle; and that the lung and submaxillary gland more nearly resemble the voluntary mucle in this respect, while the testicle and kidney resemble the heart muscle.

Analyses of the lecithans in the pancreas, quadratus lumborum, egg yolk, egg white, and in a sample of human milk are not yet completed. These analyses will [illegible] be finished and more interesting and concordant results are expected, now that the technique of the method is more thoroughly worked out than when the analyses recorded were made.

RECORD OF

Tissue	Weight in grams	c.c. $\frac{N}{2}$ NaOH neutralized by lecithin phosphorus	c.c. $\frac{N}{2}$ NaOH neutralized by kephalin phosphorus
Masseter muscle	8.91	4.7	3.45
" "	7.65	3.75	3.1
Heart muscle	18.30	7.85	12.95
" "	16.97	7.1	13.3
Submaxillary gland	12.34	8.4	7.5
" "	11.74	8.5	5.85
Lung	10.35	8.9	7.7
"	13.55	9.8	9.7
2 testicles	2.95	2.8	2.95
1 testicle	7.98	5.65	6.55
1 "	9.14	6.15	5.7
1 "	12.05	5.7	7.6
Kidney cortex	12.14	10.35	11.6
" "	13.98	12.75	13.9
Liver	12.70	7.2	13.65
"	12.11	12.5	11.15
Cow's milk	102.96	3.55	2.7
" "	103.16	2.6	3.3

A N A L Y S E S.

Lecithin found. In %.	Kephalin found. In %.	Total Lecithans found. In %.	Correction for Sulphur Compound. In %.
0.75	0.55	1.30	0.025
0.70	0.58	1.28	0.025
0.61	1.01	1.62	0.12
0.59	1.12	1.71	0.12
0.97	0.86	1.83	0.06
1.03	0.71	1.74	0.06
1.22	1.06	2.28	0.105
1.03	1.02	2.05	0.105
1.35	1.43	2.78	0.075
1.01	1.17	2.18	0.075
0.96	0.89	1.85	0.075
0.67	0.90	1.57	0.075
1.22	1.36	2.58	0.105
1.30	1.42	2.72	0.105
0.81	1.53	2.34	0.12
1.47	1.31	2.78	0.12
0.049	0.037	0.086	
0.036	0.045	0.081	

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