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A QUANTITATIVE DETERMINATION
OF THE
AMOUNT OF EXTRACT IN THE HUMAN KIDNEY
AND AN
EXAMINATION OF ITS NATURE.

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A Thesis

submitted to the Faculty of the Graduate School

of the

University of Minnesota

by

Henry Joseph Hoffmann

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE.

May 1914.

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9H6759

R E P O R T

of

COMMITTEE ON THESIS

THE undersigned, acting as a committee of the Graduate School, have read the accompanying thesis submitted by Mr. Henry J. Hoffmann for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

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A Quantitative Determination of the Amount of Extract in the Human Kidney and an Examination of its Nature.

History and Criticism.

The fats in animal tissue and organs cannot be extracted quantitatively with the use of solvents only. Other substances than fat are always extracted, the fat itself more or less completely extracted and its nature more or less altered. The tissue must either be directly dried and powdered, when a partial oxidation of the more unstable unsaturated fats or other changes in the nature of some of the more complex fatty substances take place; or dried by means of alcohol, when the subsequent extraction always gives a high percent. of extract matter.

The determination of fat as such, therefore, is a very difficult task.

The most efficient solvents for animal fats, e.g., ether, chloroform, carbon tetrachloride and petroleum ether mix but slightly with water. It is therefore

necessary that in the direct extraction method the material be dried before extracting and the dried material powdered so as to give the solvent the best chance of reaching the soluble fats enclosed in the mass of insoluble substances composing the tissue. The energetic action of the solvents depends partly on whether they are miscible or non-miscible with water. In the first case the energy depends on the fineness and looseness of the material while in the latter, on the fineness and looseness of the material and on the amount of water. Complete drying of the substance is important, for in extracting the solvent takes up some water and this dissolves water soluble matters and increases the amount of extract.

The methods used in drying the materials are:

1. Drying in air or neutral gas either in vacuum or at atmospheric pressure.

2. Drying with alcohol.

In drying at a low temperature for a long time comparatively accurate results are obtained, while in

drying at a higher temperature for a shorter time less accurate results are obtained.

The readiness with which the unsaturated acids undergo oxidation when heated in the air makes it necessary to avoid that procedure which suggests itself most readily, i.e. heating the material in open vessels on water baths or in ovens. The unsaturated acids and combinations in which they are found in animal tissue and organs are partially converted when so treated into substances insoluble in petroleum ether, difficult, if at all, soluble in sulphuric ether and readily soluble only in alcohol. Where a small quantity of material only is used it may be advantageously dried under diminished pressure at a raised temperature.

In drying with alcohol the minced or pulped tissue is treated with an equal volume of alcohol and either evaporated directly to dryness on a water-bath or after some hours the alcoholic solution is poured off and the solution and residue dried separately. The treatment with alcohol may be repeated.

Mr. Tamura claims that by drying with alcohol the loss is less than by drying without alcohol. He found the loss on a 200 gram sample or less to be less than .5 per cent. and on a 300 gm. sample or over higher than .5 per cent. The variation was very small on individual tests. He is of the same opinion as Shimidzue that the extraction should be made on the wet sample.

Argutinsky recommends drying at ordinary temperature in vacuum over sulphuric acid and maintains that this method gives more exact results with muscle and other tissues than drying at a higher temperature.

Bogdanow dries the tissue according to one of the methods of Professors Zuntz and Hagemann for analyzing fat and excrement, that is, over sulphuric acid in a stream of illuminating gas, the gas passing first through sulphuric acid, then a solution of potash and then thro' a tower containing sticks of potash. He claims that on heating a fat it undergoes decomposition, increasing the volatile and free fatty acid contents.

Voit dries with alcohol. He claims that the alco-

hol introduces osmotic action by which the individual cells are more quickly and completely extracted. O. Frank also claims that the alcohol by diffusion empties the cells and facilitates extraction.

The three principal methods used in determining fat are:

I. Direct extraction methods,

II. Digestion methods,

III. Saponification methods.

I. Extraction Methods.

It was shown by Pflüger that extraction with ether, however prolonged, failed to give the full amount of fat present. Glikin, also Argustinsky, claims that all the fat can be extracted with ether.

Dormeyer claims that ether will not extract all the fat and says that the ether penetrates the substance too slowly, that the individual cells should be broken down. He is of the opinion that there are two extractable fats present; one easily extracted forming the reserve material in the interstices of the connective tissues, the other present in the muscular parenchyma tissue and is extracted only after this tissue is decomposed or broken down.

Bogdanow claims that the fat is held back mechanically. He showed that on heating a fat it underwent

a decomposition, increasing the volatile and free fatty acid contents. It is his opinion that there are two kinds of fat present.

Voit is of the opinion that it is not necessary to assume two kinds of fat.

Glikin extracts the powdered material with petroleum ether and purifies the extract with acetone, in which he claims that lecithine is insoluble or only slightly soluble.

Rosenfeld's method, which has in recent years been much used, consists in boiling the thimble containing the dry powder in a beaker covered with a watch glass containing alcohol, and heated on a water bath, then extracting the residue in a Soxhlet apparatus with chloroform for four to six hours, repeating the treatment with alcohol and the extraction with chloroform, evaporating the respective extracts to dryness, taking up the residue in petroleum ether, mixing, filtering, evaporating and drying.

J. B. Leathes says that the extract weighed as fat

by Rosenfeld's method is dark, and contains much that is probably not fat. The extract from the liver or heart contains more nitrogen on saponification and gives a lower percentage of fatty acids than if it were all lecithine.

Bogdanow removes the greater part of fat by covering the dried powder with ether and allowing it to stand three days. He then extracts with alcohol for three days and purifies the alcohol residue with ether.

Frank extracts the dried powder consecutively with alcohol and ether, and then repeats the extractions.

It is a well-known fact that the solvent which gives the largest yield of substances soluble in petroleum ether is alcohol, used at or near its boiling point. But the purified petroleum ether extract obtained in this manner is not as pure as that obtained by direct extraction of the dried powder with the petroleum ether.

II. Digestion Methods,

In the digestion methods part of the tissues are changed chemically.

The liberation of fat from the dried protein in

which it is entangled, or with which it has sometimes been, on insufficient evidence, supposed to be chemically combined, has been attempted by digestion with pepsin and hydrochloric acid after a preliminary extraction of the powder with ether by Dormeyer. The digested solution is then filtered, the filter and residue dried and extracted, and the filtrate shaken repeatedly with ether. The combined ethereal solutions yield ten per cent. more extract than can be obtained by simple continuous extraction with ether in a month, but, according to Rosenfeld, thirty per cent. less than can be obtained by his method.

Athanasiu shortened Dormeyer's method by digesting the wet tissue directly.

Nerking also shortened Dormeyer's method by using an automatic liquid extractor. He modified the method by boiling with two per cent. hydrochloric acid for three hours on a water bath and then extracting the solution with ether for forty-eight hours in his liquid extractor. The residue filtered off was either dried

and then extracted in a Soxhlet extractor or dissolved in a one per cent. soda solution added to the original solution and simultaneously extracted.

Schlesinger first digests, then filters the solution through a fat free filter, and extracts the dried residue and filter in a Soxhlet apparatus.

III. Saponification Methods.

Liebermann and Szekely saponified the dried powder directly with a strong solution of alcoholic potash. The fatty acids were then liberated with sulphuric acid and extracted with petroleum ether. An aliquot portion of the ether solution titrated with standard alkali,was evaporated to dryness, weighed, and the corresponding amount of triglyceriders calculated.

Kumagawa and Suto determine the higher fatty acids by saponifying the fresh tissue with alcoholic potash, liberating the fatty acids with sulphuric acid, extracting with ether, evaporating the ether extract to dryness, extracting the residue with petroleum ether and weighing the dried extract. They also compute the percentage of neutral fat by multiplying the weight of the petroleum ether extract by the factor 1.046.

Solvents and Reagents.

The petroleum ether was redistilled and the portion distilling between 55 and 65° C, used.

The sulphuric ether was first washed in a separatory funnel with two portions of distilled water, then placed in a large bottle, allowed to stand over fused calcium chloride for a month or more, then distilled and that portion distilling over at 34.6° C. used.

The chloroform was Merck's best grade redistilled, which boiled at 61.2° C.

The carbon tetrachloride was of a good grade, redistilled, and boiled at 77° C.

The 95 per cent. alcohol used was purified by allowing it to stand over sticks of potassium hyrdroxide for two or more weeks and then distilled from a flask containing a few crystals of silver nitrate. That which distilled over at about 78° C. was used.

The acetone used was of a good grade, redistilled,

and boiled at 56° C.

No solvent left a residue when a portion of it was evaporated.

Fat free cotton was prepared by boiling 20 grams of a good grade of absorbent cotton in a liter of two per cent. sodium hydroxide for a half hour, then pouring off the solution and retreating with a second liter of alkali of the same strength. The solution was poured off and the cotton washed free of alkali and then boiled for a short time with a .5 per cent. solution of hydrochloric acid. It was then washed free of hydrochloric acid with water, boiled with alcohol, the alcohol squeezed out, then dried and placed in a glass stoppered bottle.

The asbestos was prepared by first treating it with hot water, then warming on a water bath for a half hour with ten to fifteen times its weight of ten per cent. sodium hydroxide. It was then washed with water until neutral. The water was squeezed out and the fiber warmed for a half hour on a water bath with ten

to fifteen times its weight of aqua regia. It was then washed until neutral, boiled with alcohol, the alcohol squeezed out and then dried and placed in a glass-stoppered bottle.

Material.

The material used in the following experiments was the human kidney. All tests were made on the dry, finely pulverized substance. Its nature and source made it inconvenient and almost impossible to prepare one large stock sample from which portions could be taken for each test. For this reason all results cannot be compared quantitatively one with another, but comparative results are given on the individual kidneys.

Preparation and Drying of Sample.

The fresh kidney was first cut longitudinally into halves. The pelvis and hilum parts were removed from the cortical and medullary parts with the aid of a sharp dissecting knife, thus removing all the free fats and free fatty tissues from the cortical and medullary parts. These latter parts were weighed, then placed on a piece of glass and cut into small pieces not larger than one-eighth of an inch in diameter. This mass was then spread on the bottom of glass dishes and dried.

The drying was done in a vacuum oven at 60° C. in a current of carbon dioxide.

The cylindrical water-jacketed vacuum oven was provided with a glass opening at either end. The oven was kept at 60° C. and through it was passed a continuous current of carbon dioxide which was purified by first passing through distilled water, then concentrated sulphuric acid and finally through a ten inch tower containing fused calcium chloride. The carbon dioxide was introduced through a mercury seal at the front end of the oven by means of which its rate of flow could be nicely regulated. The outlet tube of the oven was in the farther end and connected with a water suction pump by means of a large-sized calcium chloride tube and suction flask. A manometer tube was attached to show the vacuum. The pressure in the oven was kept so low that the boiling point of water in the oven was always at 60° C. or lower. No trouble was experienced in keeping the oven at exactly 60° C.

When nearly dried the substance was removed from

the oven and pulverized very fine in an agate mortar. The powder was then put in wide-mouthed flasks provided with glass stoppers, and again heated in the oven to constant weight. In obtaining constant weight the last heating periods were of four hours duration. The powder was kept tightly stoppered in these flasks in a desiccator and portions were quickly weighed out for the various tests.

The following table gives the time required for drying the various samples to constant weight, and the percentages of moisture and solid matter found in each.

TABLE - NO I

Kidney	Weight of Fresh Kidney (gms)	Time of Drying Hrs	Weight of Dried Kidney (gms)	Percent of Solid Matter	Percent of Moisture
No. 1	63.3283	61	14.7138	23.250	76.750
No. 2	57.9581	$82\frac{1}{2}$	13.9866	24.100	75.900
No. 3a	96.0000	$152\frac{1}{2}$	21.4360	22.329	77.671
* No. 3b	-------	99	-------	-------	-------
No. 4	63.0154	75	16.0220	25.330	74.670
@ No. 1 &2A	138.9500	118	36.7060	26.410	73.590
# No. 3 &4A	144.5670	155	33.7961	23.239	76.761
			Average---	24.110	75.890

*- -

Note:
* No. 3b was chromated and was a mate to 3a.
@ Nos. 1 & 2A were mates.
# Nos. 3 & 4A were mates.

The greater part of this difference is due probably to the time lapsing before removing the kidney from the dead body, the amount of water and salts lost in preparing the sample and the loss in fine powder while pulverizing the partially dried material. Most of these conditions were not under the investigator's control.

I.

Determination of the Relative Amounts of Extract Obtained from the Same Kidney, respectively, with the Solvents, Petroleum Ether, Ether, Chloroform, Carbon Tetra-chloride and 95 per cent. Alcohol; and an Examination of its Nature.

A. Relative Amounts of Extracts.

From one and one-half to two grams of the dried powder were quickly weighed into a fat free extraction thimble of Schleicher and Shüll's make. The thimble was suspended from the cork by means of aluminium wire into a flask containing the solvent, and immersed to such a depth that the powder was completely covered by the solvent. To prevent the powder from floating on the solvent, a wad of fat free cotton was packed in the thimble over the material. The flask was then fitted up with a reflux condenser and heated on a water bath. After heating for a number of hours the extract was removed and the thimble placed in a Soxhlet apparatus and the extraction continued until complete. The extracts (those respectively from the hot and the cold extractions) were combined and the greater part of the

solvent removed by evaporation. The remaining portion was filtered through a fat free filter into a small tared flask and the filter paper washed with the solvent until free of fat. All but a few c.c. of the solvent was evaporated on a water bath and the flask placed in the vacuum drying oven above described and dried to constant weight at a temperature of 60° C. in a current of carbon-dioxide. Two hour periods were used in obtaining constant weight and from two to five periods were necessary.

The following table gives the weight and percentage of extract found in the dry powder and fresh kidney No. 3 a.

TABLE NO II.

Solvent	No. Det.	Weight of Sample (gms)	Time of Extraction Hot Hrs	Time of Extraction Cold Hrs	Weight of Extract (gm)	Percent Extract of Dry Powder	Percent Extract of Fresh Kidney
Pet. Ether	I	1.7960	52	300	.1786	9.940	2.220
	II	2.0425	96	322	.2038	9.978	2.228
	Av.					9.959	2.224
Ether	I	1.7322	53½	300	.2480	14.311	3.196
	II	1.5065	96	322	.2179	14.464	3.230
	Av.					14.388	3.213
Chloroform	I	1.6564	53½	300	.2790	16.844	3.761
	II	1.6848	96	340	.2845	16.886	3.770
	Av.					16.865	3.766
Carbon Tetra-Chloride	I	1.6088	53½	300	.2312	14.371	3.209
	II	1.5613	96	322	.2330	14.924	3.332
	Av.					14.648	3.271
Alcohol	I	1.5828	53½	420	.6376	40.283	8.995
	II	1.6725	96	480	.6767	40.389	9.018
	Av.					40.336	9.007

B. Saponification Number of the Extract.

The weighed extract was treated with 20 c.c. of a standard solution of alcoholic potash prepared by dissolving forty grams of potash in a liter of purified alcohol. This solution was standardized,every time it was used, against standard hydrochloric acid. The saponification was carried out by heating the mixture in a flask provided with a reflux condenser on a water bath for one hour. One-half c.c. of a one per-cent solution of pehnolphthalein was added to the soap solution and the excess of alkali titrated with a standard solution of hydrochloric acid. From the number of cubic centimeters of alcoholic potash used the saponification number of the extract was calculated.

Table No. 3 gives the saponification numbers of the extracts obtained,respectively,with the different solvents.

TABLE NO 3.

Solvent	No. Det.	Weight of Extract (gm.)	Weight of KOH (mgm)	Saponification No. of Extract	Av. Saponification No. of Extract
Pet. Ether	I	.1786	31.39	175.75	
	II	.2038	34.56	169.58	172.67
Ether	I	.2480	101.43	408.99	
	II	.2179	103.65	475.58	442.29
Chloroform	I	.2790	49.81	178.53	
	II	.2845	53.27	187.24	182.89
Carbon Tetra-Chloride	I	.2312	52.12	225.43	
	II	.2332	55.28	232.96	229.20
95% Alcohol	I	.6376	41.46	65.03	
	II	.6767	46.07	68.08	66.57

C. Unsaponifiable Matter in the Extract.

The neutral soap solution prepared in determining the saponification number was made slightly alkaline with a few drops of alcoholic potash and transferred to a separatory funnel. The flask was washed first with a small amount of 50 per cent. alcohol and then with 50 c.c. of petroleum ether, and the washings transferred to the separatory funnel, which was thoroughly shaken. When the petroleum ether layer separated, the underlying soap solution was drawn off with a beaker and the petroleum ether layer poured into a clean flask. The soap solution was extracted twice more in a like manner with fresh petroleum ether. The combined petroleum ether extracts were then returned to the separatory funnel and washed three successive times with 50 per cent.alcohol. The extract was poured into a flask and the greater part of the petroleum ether removed on the water bath. The remaining solution was then filtered into a small tared flask and, after removing all but

a few c.c. of the ether, placed in the vacuum oven and dried to constant weight at 60° C. in a current of carbon dioxide.

The following table gives the amount and percentage of unsaponifiable matter found in the extract, the dried powder and the fresh kidney.

TABLE NO 4.

Solvent	No. Det.	Weight of Sample (gms)	Weight of Extract (gm)	Weight of Unsap. Matter (gm)	Percent Unsaponifiable Matter in Extract	Percent Unsaponifiable Matter in Dry Powder	Percent Unsaponifiable Matter in Fresh Kidney
Pet. Ether	I	1.7960	.1786	.0528	29.563	2.944	.657
	II	2.0425	.2038	.0592	29.048	2.898	.647
	Av.				29.306	2.921	.652
Ether	I	1.7322	.2480	.0364	14.637	2.101	.469
	II	1.5065	.2179	.0309	14.181	2.051	.458
	Av.				14.409	2.071	.464
Chloro-form	I	1.6564	.2790	.0723	25.914	4.365	.974
	II	1.6848	.2845	.0667	23.444	3.959	.884
					24.679	4.162	.929
Carbon Tetra-Chloride	I	1.6088	.2312	.0690	29.844	4.289	.957
	II	1.5612	.2330	.0648	27.811	4.151	.927
	Av.				28.828	4.220	.942
95% Alcohol	I	1.5828	.6376	.1740	27.289	10.993	2.454
	II	1.6725	.6767	.1747	25.799	10.445	2.332
	Av.				26.544	10.719	2.393

D. Fatty Acids in the Extract.

The soap solution from the determination of the unsaponifiable matter in Section "C" was treated with 10 c.c. of approximately $\frac{N}{2}$ H_2SO_4 and warmed on a water bath for about ten minutes. The solution was then transferred to a separatory funnel and cooled, the beaker washed out with a little 50 per cent. alcohol and 50 c.c. of petroleum ether, and the washings were added to the funnel which was thoroughly shaken. When the ether layer separated, the underlying aqueous solution was drawn off into a beaker. The ether extract was poured into a clean flask. The aqueous solution was extracted twice more in like manner with fresh portions of petroleum ether. The combined petroleum ether extracts were returned to the separatory funnel and washed three times with 50 per cent. alcohol. The extract was then poured into a flask and the greater part of the petroleum ether removed on the water bath. Theremaining solution was then filtered into a small tared flask and after remov-

ing all but a few c.c. of the ether, put in the vacuum drying oven and dried to constant weight at 60° C. in a current of carbon dioxide.

Table No. 5 gives the weight and percentage of fatty acids in the extract, the dried powder and the fresh kidney.

TABLE NO 5.

Solvent	No.	Weight of			Percent	Fatty Acids	in
	Det.	Sample (gms)	Extract (gm)	Fatty Acids (gm)	Extract	Dry Powder	Fresh Kidney
Pet.	I	1.7960	.1786	.0898	50.280	5.000	1.116
Ether	II	2.0425	.2038	.1174	57.606	5.742	1.282
	Av.				53.943	5.371	1.199
Ether	I	1.7322	.2480	.0664	26.774	3.833	.856
	II	1.5065	.2179	.0720	33.093	4.779	1.067
	Av.				29.934	4.306	0.962
Chloro-	I	1.6564	.2790	.1214	43.513	7.329	1.634
form	II	1.6848	.2845	.1239	43.550	7.354	1.642
	Av.				43.532	7.342	1.638
Carbon	I	1.6088	.2312	.1064	46.021	6.614	1.476
Tetra-	II	1.5612	.2330	.0900	38.626	5.764	1.287
Chloride	Av.				42.324	6.189	1.382
95%	I	1.5828	.6376	.1296	20.326	8.188	1.828
Alcohol	II	1.6725	.6767	.1242	18.354	7.426	1.658
	Av.				19.340	7.807	1.743

II.

Comparison of the Amount of Extract in a Chromated and Unchromated Kidney.

Kidney No. 3b, mate of kidney 3a, was chromated and the results obtained compared with those of the unchromated.

The chromating process was carried out by treating thin sections of the kidney for twenty four hours in a ten per cent. solution of potassium bichromate, in a paraffin oven at about 57° C. (The Journal of Medical Research, Vol. XXIV, No. 3, pp. 539-546, June 1911.) After being washed over night in a stream of running water it was removed and cut into small pieces. The drying and pulverizing processes were those used in preparing the unchromated kidney, given on page 12. It was much easier to pulverize than the unchromated kidney.

The process of extraction was that used in extracting the unchromated kidney, page 17.

The results obtained are given in Tables Nos. VI, VII, VIII, and IX.

TABLE NO. VI.

Solvent	Weight of Sample (gms)	Time of Extraction Hot Hrs.	Time of Extraction Cold Hrs.	Weight of Extract (gm)	Percent Extract found in Chromated Kidney	Percent Extract found in Powder of Fresh Kidney*
Pet. Ether	1.4426	96	312	.0627	4.346	9.959
Chloro-form	1.7567	96	312	.1306	7.434	16.865
Carbon Tetra-Chloride	1.5232	96	312	.0978	6.421	14.648
95% Alcohol	1.5113	96	357	.2300	15.219	40.336

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* Note:
Results are average of two determinations.

TABLE NO. VII.

Solvent	Weight of Extract (gm.)	Weight of KOH (gms.)	Saponification No. of Extract.	Saponification No. Extract of powder of Fresh Kidney.+
Pet.Ether	.0627	16.124	257.16	172.67
Chloroform	.1306	25.914	198.42	182.89
Carbon Tetra-chloride	.0978	23.034	235.52	229.20
95 per cent. Alcohol	.2300	16.988	73.86	66.57

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\+ Note: Results are average of two determinations.

TABLE NO. VIII.

Solvent	Weight of Sample (gm)	Weight of Extract (gm)	Weight of Unsaponifiable Matter (gm)	Percent Unsaponifiable Matter in		
				Extract	Chromated Kidney	Powder of Fresh Kidney *
Pet. Ether	1.4426	.0627	.0232	37.001	1.608	2.921
Chloro-form	1.7567	.1306	.0423	32.389	2.408	4.162
Carbon Tetra-Chloride	1.5232	.0978	.0405	41.411	2.659	4.220
95% Alcohol	1.5113	.2300	.0452	19.652	2.991	10.719

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* Note: Results are average of two determinations.

TABLE - NO. IX.

Solvent	Weight of Sample (gms)	Weight of Extract (gm)	Weight of Fatty Acids (gm)	Percent Fatty Acids in Extract	Powder	Powder of Fresh Kidney *
Pet. Ether	1.4426	.0627	.0211	33.652	1.463	5.371
Chloro-form	1.7567	.1306	.0558	42.726	3.176	7.342
Carbon Tetra-Chloride	1.5232	.0978	.0394	40.286	2.586	6.189
95% Alcohol	1.5113	.2300	.0888	38,608	5.875	7.807

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* Note:
Results are average of two determinations.

III.

Determination of Relative Amounts of Extracts in Different Kidneys, Respectively with Petroleum Ether, Carbon Tetra-chloride and 95 percent. Alcohol.

The kidneys were prepared and dried as previously described on page 3.

The dried powder was weighed into fat free extraction thimbles of Schleicher and Schüll's make, covered and held in place in the thimble by wads of fat free cotton.

The extractions were made with both warm and cold solvents and the processes carried out in the usual manner except where the extractions were complete, when the determinations were carried out by the method described in detail in "I" page 17. To determine whether the extraction was complete, periods of from five to eight hours duration were used, and the extract obtained was weighed. The cold extractors were of the Soxhlet type, containing ground glass joints, and the hot extractors of the Wiley type, in which the substance to be extracted is suspended in the hot vapors of the solvent.

The extract was filtered by suction through a filter described by Kumagawa and Suto in the Biochemische Zeitschrift, 1908, Vol. 8, p. 212. The funnel was made by sealing a twelve inche piece of capillary tubing of 1.7 m.m. bore to an ordinary two-inch glass funnel, about two inches below the conical part. In this two inch space of the funnel was packed an inch column of prepared cotton which was covered with treated asbestos until the funnel was half full.

Most of the ether was removed from the filtrate by distillation and the residue washed into a small tared flask and heated to constant weight in the vacuum oven at 60° C. in a current of carbon dioxide.

The following tables give the relative amounts of extract in different kidneys,obtained respectively with petroleum ether, carbon tetra-chloride and 95 per cent. alcohol.

TABLE NO 10

Solvent - Petroleum Ether

No. of Kidney	Weight of Sample (gms)	Time of Extraction Hot Hrs	Cold Hrs	Test Portion Hrs	Weight of Test Portion (gm)	Total Extract (gm)	Percent in Powder	Extract In Fresh Kidney
3a	1.7960	52	300	5	.0000	.1786	9.940	2.220
3a	2.0425	96	322	5	.0000	.2038	9.978	2.228
4	3.4004	15½	145½	5	.0007	.3397	9.990	2.535
1 & 2A	3.2312	108	20	5	.0004	.2752	8.517	2.249
1&2A	2.3486	295	----	8	.0008	.1683	8.209	2.168
3&4A	3.1729	146	----	----	-----	.2829	8.916	2.072

TABLE NO. 11.

Solvent - Carbon Tetrachloride

No. of Kidney	Weight of Sample (gms)	Time of Extraction Hot Hrs.	Cold Hrs.	Test Portion Hrs.	Weight of Test Portion (gm)	Total Extract (gm)	Percent in Powder	Extract In Fresh Kidney
3a	1.6088	53-1/2	300	5	.0000	.2312	14.371	3.209
3a	1.5612	96	322	5	.0000	.2330	14.924	3.332
4	2.2079	28	----	---	-----	.2517	11.400	2.888
1&2A	3.1674	313	----	8	.0015	.4573	14.438	3.813
3&4A	3.0899	110½	----	---	-----	.3008	9.735	2.262

TABLE NO - 12

Solvent - 95 % Alcohol

No. of Kidney	Weight of Sample (gms)	Time of Extraction Hot Hrs	Cold Hrs	Test Portion Hrs	Weight of Test Portion (gm)	Total Extract (gm)	Percent in Powder	Extract in Fresh Kidney
3a	1.5828	53½	420	5	.0000	.6376	40.283	8.995
3a	1.6725	96	480	5	.0000	.6767	40.389	9.018
4 *	3.5382	107	16	5	.0008	1.3650	38.579	9.772
1&2A	3.1203	20	345	6	.0007	1.1580	37.112	9.801
1&2A	3.1086	298	---	8	.0008	1.1586	37.278	9.845
3&4A	3.2400	136	---	----	-----	1.1105	34.275	7.965

* Solvent was of equal parts of carbon tetrachloride and 95% alcohol.

IV.

Attempt to Separate the Neutral Fat from the Lipoids.

A. Carbon Tetra-chloride, 95 per cent. Alcohol, Water and Petroleum Ether Separation.

The weighed extract obtained with the different solvents in the previous extractions in Part"III" was transferred to a one hundred c.c. glass-stoppered extraction tube by means of 12-1/2 c.c. of carbon tetrachloride. The flask was rinsed out with 25 c.c. of 95 per cent. alcohol and the washings transferred to the tube. Twelve and one-half c.c. of water was added and the cylinder vigorously shaken; 35 c.c. of petroleum ehther was then added and the tube vigorously shaken for two minutes when it was allowed to stand until separation was complete.

A blow-off tube similar to the Werner-Schmidt blow-off tubes was inserted andthe ether layer cautious-

ly blown onto a filter and the filtrate collected in a clean flask. (In blowing off the ether layer care was taken that none of the carbon-tetrachloride layer was blown off, which may happen if a too close separation of the two layers is attempted. This was avoided by blowing slowly and observing if any bubbles rose in the tube. If bubbles were seen rising, the blowing was quickly discontinued and they were removed by gently sucking on the blow-off tube.) Five c.c. of petroleum ether was then placed in a small evaporating dish and gently drawn into the tube by sucking on the blow-off tube. After a few moments this ether, which had mixed with the thin ether layer in the tube, was blown off and filtered as before. Three c.c. of carbon tetra-chloride was then added to the contents of the tube which was thoroughly shaken, then 30 c.c. of petroleum ether was added and the tube again thoroughly shaken, then allowed to stand until separation was complete, and the ether layer separated and washed once as in the first blow-off. Another addition of 3. c.c. of carbon tetra-

chloride and 30 c.c of petroleum ether was made and the separation of the ether layer repeated as in the first blow-off. The filter paper was then well washed with small portions of petroleum ether, the flask connected with a condenser and most of the ether distilled off. The residue was washed into a small tared flask and heated to constant weight in the vacuum oven at 60° C. in a current of carbon dioxide.

A fourth blow-off was made in the same manner as the third. If a residue was found a fifth was made, and if necessary a sixth. In most cases but four were necessary.

Table No. 13 gives the weight and percentage of the extract dissolved in the petroleum ether in terms of the dry kidney and the total extract.

TABLE NO - 13.

No. of Kidney	Kind of Extract	Weight of Sample (gms)	Weight of Extract (gm)	Weight of Pet. Ether Extract (gm)	Percentage of Pet. Ether Extract	
					In Total Extract	In Dry Kidney
4	Pet. Ether	3.4004	0.3397	0.2675	78.746	7.867
1&2A	"	3.2312	0.2752	0.2600	94.840	8.046
1&2A	"	2.3486	0.1928	0.1683	87.295	7.161
4	Carbon Tetra-Chloride	2.2079	0.2517	0.2149	85.379	9.733
1&2A	"	3.1674	0.4573	0.3593	78.569	11.344
4	#	3.5382	1.3650	0.5239	38.381	14.807
1&aA	Alcohol	3.1203	1.1580	0.3710	32.038	11.889
1&2A	"	3.1086	1.1586	0.3215	27.749	10.342

Note: Equal parts carbon tetrachloride and alcohol.

B. Acetone Separation.

The petroleum ether extract obtained in the separation in "A" was treated with acetone. A precipitate was formed in every case. The precipitate was separated according to Glikin's method, Pflüger's Archiv. Vol.95, p. 107. The extract covered with acetone was allowed to stand twenty-four hours. It was then filtered through a small fat free filter paper, and the precipitate and filter thoroughly washed with acetone.

The filtrate was evaporated to complete dryness on a warm water bath, the residue treated with acetone and again allowed to stand for twenty-four hours when the precipitate was filtered off and washed, as previously stated. The filtrate was evaporated to complete dryness and the residue again treated with acetone, when no further precipitate formed.

The first acetone precipitate was dissolved on the filter paper with petroleum ether, evaporated to dryness on a warm water bath and again treated with acetone. The

precipitate formed was filtered, washed, dissolved in petroleum ether and the filtrate added to second acetone precipitate dissolved in petroleum ether. The acetone and petroleum ether solutions were transferred to platinum dishes and the phosphoruus determined in each.

The phosphorous was determined as $Mg_2 P_2 O_7$ and the process was carried out in the following manner:

Five c.c. of a solution of alcoholic potash, forty grams of potash per liter, was added to the solution in the platinum dish which was then evaporated to dryness on a water bath. The residue was charred by heating the dish very carefully over a thin asbestos board, and then moistened with dilute nitric acid. The residue was washed in a little hot water and decanted through a filter into a four hundred c.c. beaker. The filter was placed in the platinum dish and ignited until the residue was white, when it was treated with a little dilute nitric acid, filtered into the beaker, and the dish and filter washed. To the slightly acid solution (which was approximately 50 c.c.) 30 c.c. ammonium nitrate solution,

made by dissolving 340 grams of ammonium nitrate in a liter of water, and 15 c.c of dilute nitric acid were added, and the solution heated until bubbles began to rise. At the same time 120 c.c of three per cent. solution of ammonium molybdate was heated to boiling, when it was run in a thin stream into the middle of the phosphate solution which was stirred continuously. The yellow ammonium phosphomolybdate was at once precipitated. The contents of the beaker were kept in motion for about one minute more, then allowed to stand for a half hour or more when the clear liquid was poured through a filter, the precipitate washed once by decantation with 50 c.c. of the wash liquid, made by dissolving 50 grams of ammonium nitrate and 40 c.c. of nitric acid in a liter of water and then dissolved in 10 c.c. of eight per cent. ammonia.

To this solution was added 20 c.c. of the ammonium nitrate solution, 30 c.c. of water, and 1 c.c. of ammonium molybdate solution. It was heated, as before, until bubbles began to rise when the phosphoric acid was

reprecipitated by the addition of 20 c.c. of hot nitric acid, added drop by drop, the solution being stirred as it was before. The precipitate was immediately formed, and after standing a half hour or more, was filtered off and dissolved in warm 2-1/2 per cent. ammonia, after which nitric acid was added until the yellow precipitate formed dissolved only slowly on being mixed with the solution. Ten c.c. of"magnesia mixture" and one-third of the volume of concentrated ammonia were added. After standing four hours or more the supernatant liquid was poured off. The precipitate was washed four times by decantation with 50 c.c. of 2-1/2 per cent. ammonia, then dissolved in as little hydrochloric acid as possible, a few drops of "magnesia mixture" were added, the solution made alkaline with dilute ammonia and one-third of its volume of strong ammonia again added. After standing for another four hours the clear liquid was decanted through a filter, the precipitate washed three times by decantation with 2-1/2 per cent. ammonia, finally transferred to the

filter and thoroughly washed with dilute ammonia, dried at 100° C. and ignited to pyrophosphate in a platinum crucible.

The phosphorous was also determined in the carbon tetra-chloride, water and alcohol layer.

The following table gives the weight and percentage of phosphorous found in the carbon tetrachloride, water and alcohol layer, the acetone precipitate and acetone filtrate.

TABLE 14 - A.

No. of Kidney	Kind of Extract	Weight of Total Extract (gm)	Weight of Pet. Ether Extract (gm)	Weight of Phospherous in CCl_4, Water Alc. Mixt. (gm)	Weight of Phospherous in Acetone Pp't. (gm)	Weight of Phospherous in Acetone Filtrate (gm)	Total Weight P (gm)
4	Pet. Ether	0.3397	0.2675	0.00093	0.00137	0.00143	0.00373
1 & 2A	"	0.2752	0.2600	0.00132	0.00151	0.00109	0.00373
1 & 2A	"	0.1928	0.1683	0.00004	0.00173	0.00121	0.00298
4	Carbon Tetra Chloride	0.2517	0.2149	0.00168	0.00179	0.00126	0.00473
1 & 2A	"	0.4573	0.3593	0.00124	0.00098	0.00121	0.00343
4	#	1.3650	0.5239	-------	0.00160	0.00179	---------
1 & 2A	Alcohol	1.1580	0.3710	0.00384	0.00182	0.00271	0.00837
1 & 2A	"	1.1586	0.3215	0.00399	0.00187	0.00254	0.00840

Note: Equal parts carbon tetrachloride and alcohol.

TABLE 14-B.

No. of Kidney	Percent.P in Total Extract	Percent. P in Pet.Ether Extract	Percent. P in Acetone Precipitate	Filtrate
4	1.098	1.046	48.929	51.071
1-2A	1.424	1.000	58.077	41.923
1-2A	1.545	1.746	58.844	41.156
4	1.879	1.419	58.196	41.804
1-2A	.750	.609	44.749	55.251
4	------	.647	47.197	52.803
1-2A	.723	1.221	40.177	59.823
1-2A	.725	1.434	42.404	57.596

V.

Determination of the Higher Fatty Acids, Neutral Fat and Unsaponifiable Matter by Kumagawa and Suto's Method.

From two to five grams of the dried substance was treated in a small beaker with 25 c.c. of a 5 N sodium hydroxide solution. The mixture, covered with a watch glass, was heated in a water bath for two hours. The contents of the beaker were shaken during the heating by the boiling water. At the end of two hours all but a few flakes of the substance had dissolved and the hot solution was transferred to a 250 c.c. separatory bearing a glass stopper, and the beaker rinsed out with three successive 5 c.c. portions of water. The solutuion was then over-neutralized with hydrochloric acid of sp.gr. 1.1. In neutralizing the solution was cooled to about 40°-50° C., 20 c.c. of hydrochloric acid added and,after vigorous shaking, cooled under the water-tap, when 10 c.c. more of acid was added and the solution again vigorously shaken and cooled under the water tap. Seventy to

100 c.c. of ether was added, and the whole vigorously shaken. A separation of the ether and water layers took place immediately, between which collected a layer of precipitate. The water layer was run off into a beaker and the ether layer poured into a flask. The precipitate was treated with two successive portions of 5-10 c.c. of ether and the extracts were added to the first ether portion. The precipitate was then dissolved in 5 c.c. of a normal sodium hydroxide solution, 30-50 c.c. of ether added, the whole vigorously shaken and the acid water solution first run off, added. The mixture was then vigorously shaken, the ether layer separated and added to the other ether portion and evaporated to dryness. The residue was extracted with absolute ether, filtered through asbestos, the filtrate evaporated to thorough dryness at 50° C. and then extracted with petroleum ether. In extracting, 20-30 c.c. of ether was poured into the flask, agitated and allowed to stand from one-half to one hour. A cloudiness ap-

peared first, then a resinous like substance separated and settled to the bottom of the flask. The ether solution was filtered and dried to constant weight at 50° C.

A complete drying of the ether extract before extracting with petroleum ether is absolutely necessary in order to avoid dissolving anything but the acids.

Separation of the Unsaponifiable Matter from the Higher Fatty Acids and its Quantitative Determination.

The higher fatty acids and unsaponifiable matter obtained as above were dissolved in 50-70 c.c. of petroleum ether. From 30-40 times the volume of acids of $\frac{N}{5}$ absolute alcoholic potash was added and the whole vigorously shaken, when a complete solution of the fat took place. A volume of water equal to the volume of alcoholic potash was added and the mixture thoroughly shaken. There was an immediate separation of the solution into an ether and alcohol layer, the unsaponifiable

matter dissolving in the ether layer. The ether layer was separated and the alcoholic solution again shaken with 30-40 c.c. ether. The ether layer was separated, combined with the first.portion and evaporated to dryness. The residue was dissolved in a small amount of alcohol .5 to 1 c.c. of $\frac{N}{2}$ Sodium hydroxide solution added, the whole shaken and the alcohol evaporated on a water bath and the residue dried at 100° C. for fifteen to thirty minutes. The residue was then extracted with petroleum ether, filtered through asbestos, evaporated and dried at 100° C, to constant weight.

The neutral fat was calculated by multiplying the amount of higher fatty-acids by 1.046.

The following table shows the results obtained on different kidneys.

TABLE NO. 15.

No. of Kidney	No. Det.	Weight of Sample (gms.)	Weight of Acids (gm.)	Per cent. Acids	Weight of Fat (gm.)	Per cent. Fat	Weight of Unsap. Matter (gm.)	Percent. Unsap. Matter in Sample	Percent. Unsap. Matter in Extract
3a	I	2.0789	0.2310	11.11	0.2416	11.62	0.0242	1.16	10.476
	II	2.0248	0.2299	11.35	0.2404	11.87	0.0296	1.46	12.874
	Av.			11.23		11.75		1.31	11.675
4.	I	3.1196	0.3960	12.69	0.4142	13.27	0.0413	1.32	10.429
	II	2.8054	0.3553	12.66	0.3716	13.24	0.0410	1.47	11.229
	Av.			12.68		13.26		1.40	10.829
1-2A	I	3.0059	0.2839	9.44	0.2969	9.85	----	----	------
	II	3.0290	0.2859	9.44	0.2990	9.85	----	----	------
	III	3.0229	------	-----	-----	-----	0.0345	1.14	
	Av.			9.44		9.85		1.14	
3-4A	I	3.0638	0.2941	9.60	0.3176	10.04	0.0424	1.39	14.416
	II	2.9669	0.2855	9.62	0.2986	10.06	0.0410	1.39	14.325
	Av.			9.61		10.05		1.39	14.371

Conclusions:-

I.

Under certain conditions all neutral fats and fat-like substances can be extracted completely from animal tissue by direct extraction with the use of proper solvents.

II.

Acetone will not completely precipitate lecithine from neutral fat or other extract matter.

III.

The carbon tetra-chloride, alcohol and petroleum ether separation gave a fairly uniform separation of the phosphorous-bearing compounds, as is shown by the per cent. of phosphorous found in each portion.

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