A THESIS

THE INFLUENCE OF FEEDING FOODS RICH IN LECITHIN AND PHOSPHOPUS CONTENT UPON THE BRAINS OF DOGS, CATS, AND GUINEA-PIGS.

BY

CHARLES S MOORE.

1921

TABLE OF CONTENTS

—DEDICATION—

To Dr.Hilton Ira Jones,whose hearty co—operation and untiring interest in the supervision of this re—search,has made it altogether possible,the following pages are respectfully dedicated.

INTRODUCTION

There has been a deep-seated superstition for a long period of years, and which is nearly universal today, to the effect that certain substances are brain foods. Fish are generally believed to belong to this class, not withstanding the fact that it is not observable, nor has it ever been, that fish eaters are men of marked superiority of intellect. However, in America this phosphorus-delusion has twined itself around a saying quoted (rightly or wrongly) from Professor L. Agassiz, to the effect that fishermen are really more intelligent than farmers, because they eat so much fish, which contains so much phosphorus. William James, Professor of Psychology at Harvard University, doubts the above statement, however, and seems to hold to the view that the intelligence of the being is not necessarily increased by eating food rich in phosphorus content. There is, moreover, in the language constant reference to brainy people as those who, as Elbert Hubbard says, have phosphorus plus, as though the possession of the phosphorus in some way or other measured or indicated the possession of superior mentality. A very old German addage is that ''Ohne Phosphor, kein Gedanke;' which literally means that, ''Without phosphorus, no thoughts!' This was a noted war-cry of the 'materialists' during the excitement on that subject wich filled Germany in the '60s. The brain, like every other organ of the body, contains phosphorus, and a score of other chemicals besides. Just why the phosphorus should be picked out as its essence, no one knows. It would be equally true to say, 'Ohne Wasser kein Gedanke' meaning 'without water no thoughts!' or even 'Ohne Kochsalz kein Gedanke' meaning 'without sodium chloride no thoughts'; for the thought would stop as quickly if the brain should dry up or lose its sodium chloride as if it lost its phosphorus.

The work of this research was undertaken primarily to answer two very important and fundamental questions-first, to confirm the results already worked out answering the question as to whether a definite relationship exists between possession of superior intelligence and a high phpsphorus content in the brain; and second, is it possible by feeding to influence the phosphorus comtent of the brain. The main part of the research, however, will be towards the answering of the second question, as the first has practically been answered by the work of Dr. Koch, Dr. Hilton Ira Jones, and others at the University of Chicago. These men had the opportunity of the examination of brains of some twelve hundred individuals for the determination of lecithin and phosphorus, running all the way from guinea pigs brains to the brain of Marshall Field, one of Chicago's greatest business men. Their results show it to be an apparent fact that the intelligence of the subject varies in proportion to the lecithin content of the brain., and they also found that the lecithin extract contained all the phosphorus in the brain, inother words, brain phosphorus is all ecithin phosphorus. So the question regarding the superstition that superior intelligence is accompanied by high phosphorus content is based on fact, and some experiments in this research will confirm the above results.

In the face of these determinations, it would seem therefore, that it might be possible by feeding those foods which are high in lecithin, that is brain phosporus, to increase the phosphorus content of the brain and therefore the intelligence of the individual. Without doubt the question as to whether it is true that there is a relationship

between possession of superior intelligence and a high phosphorus content in the brain is already settled, namely that such a relationship does exist. The principal problem, therefore, in this research is to prove or disprove the fact that phosphorus content in the brains of dogs, cats, and guinea-pigs may be increased by feeding them foods rich in phosphorus and lecithin.

HISTORICAL.

Numerous chemical researches have been made on brains, not only human brains, but also of lower animals, such as the dog, cat, guinea pig, and rabbit especially. The main purpose of such work was to ascertain if possible exactly what constitutes the brain, and to make a comparative analysis of the human brain with that of certain of the lower animals.

Much of the work done thus far has been performed in foreign countries, Germany in particular. However, America has done her share in this great research, especially in the past decade.

(1) Waldemar Koch, while analyzing sheep' brain precipitated out a substance known as kephalin from an etherea extract of the brain. The formula for it is $C_{42}H_{82}O_{15}NP$ and it is probably dioxyes-stearyl monomethyl lecuthin. It swells and forms an emulsion with water like lecithin. The lecithin separated out yielded choline and fatty acids in such a proportion that probably there was a mixture of three possible lecithins. Cerebrin was obtained in a crystalline condition, and analytical figures agreed very well with those obtained by Thierfelder.

Waldemar Koch and his sister Miss Mathilde Koch have probably done as much work in the line of chamical research on the brain as any of the recent Scientists. Another investigation carried on by the Kochs (2) was on the chemical differentiation of the brain of the albino rat during its growth.

(1) Lecithin Kephalin and Cerebrin from Brains--W. Koch.
(Test, Physical Chemistry 1902,36, 134-40)

(2) Chemical Differentiation of the Central Nervous System,3.
" " " " Brain of Albino Rat During Growth. --
W. Kiock and M. L. Koch (J. Biol. Chem., 1913.15, 423-8)

he found the princi le chemical changes which occur in the rat's brain during growth are: a decrease in the water which begins before medullation sets in; a relative fall in protein due to appearance of lipoids. The lipoids which appear with medullation are cerebrosides and sulphatides. The phosphatides increase before medullation, and occur both in cells and sheaths. The increase of colloidal matter, which is relatively in active supporting matter, is one factor in the showing of Metabolism which characterizes senesence.

Some very excellent comparative examinations of the chemistry of the brain of different animals were conducted by Fraeukel and Lenvert[3] in which the authors endeavored to establish the normal relations of the lipoid constituents of the brain. The dried petroleum ether , $C_6 H_6$, abs.alc. and 80% Alc. The brains of the rabbit, cat, dog, pig, ox, horse, monkey and man gave results without significant differences in the total lipod content or the amounts of the different fractions. During growth the total lipiod content increases in both man and ox. The increase is much greater in man however, and marked differences in theman and the ox in the effect of maturity on the individual fractions occur. Different portions of the human brain differ decidedly. The acetone extract of the fray matter contained very little cholesterol, but chiefly an unsaturated phosphatide, while the acetone extract of the white matter was chiefly cholesterol.

M. Gobley[4] in his researches on the human brain made a complete chemical analysis of the brain. In his analysis he found that the brain contains two albuminoid matters.

(3) Lipoids. 11. Comparative Investigations on the Chemistry of the Brain. Sigmund Fraenkel and Kurt Lennert. Bioc Chemistry Z., 26,44-52

(4) Chemical Researches on the Brain. M. Gobley (J. Pharm. Chemistry. (4) 20,161-6)

3

One of which is soluble in water and does not differ from albumin, for the other, the name cephalin has been proposed. The fatty matter of the brain is formed principally of cholesterin, lecithin and cerebrin besides which there are traces of olein and margarine. The brain contains the ordinary salts of the human system, together with extractive matters, of which some are soluble in water and alcohol, others soluble in water and insoluble in alcohol. During putrefaction the cerebral pulp furnishes acid products among which are oleric, margerine, phosphoglyceric, and phosphoric acids. The following may be taken as the main % composition of the brains:

Water.	.80.
Albumin.	1.
Cephalin.	7.
Cholestern.	1.
Cerbrin.	3.
Lecithin	5.5
Olein and Magerine.	-
Inosite, Creatine.	-
Xanthine, etc.	-
Extracts Matters(aq. andalc.	1.50
Cl of K and Na. :	1
Po4 of K, Ca, Mg	
	100.00

Diacanon, Hoppe, Segler, and Hurdiclum have thrown doubt upon the existence of protayon, which Liebuich(Armalen, 134,24-44) discovered in the brain substance, and to which the formula C_{116} H_4 O_{22} P was attributed. In consequence of this protayon has since been regained or a mixture of lecithin and cerebrin, a portion which the physical properties of these bodies make,untenable, futher, the authors have prepared large quantities of protayon form brains of horses and dogs, by a modified form of Liebuich's method: the analyses lead to the adoption of C_{160} H_{308} N_5 PO_{35} for this body. Its physical properties are those attributed to it by Liebush. By long continued boiling with ether it is decomposed.

The components of these extracts and residues are as follows:

(1) Cold alcohol extract contains:

Substance insoluble in anhydrous ether
Substance soluble in " "

(2) Ether extract together with a (sic) contains:

Lecithin
Cholestern and fats.

(3) Warm alcohol extract contains:

Lecithin
Cerebrin

(4) Residue Contains:

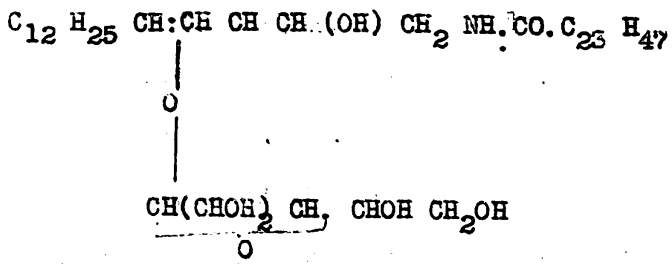
Albuminous substances
Salts

The method employed in estimating the lecithin from the magnasium phosphate was that recommended by Hoppe-Seyles.

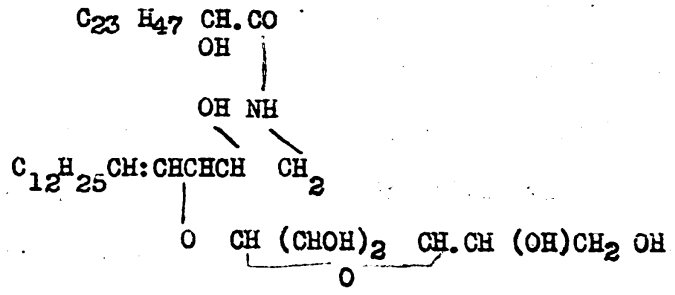
The dry grey substance appeared to consist of about one half albumin, one fourth cholestern and fats, very little cerebrin, its principal components being albumin and water. In the white matter, cholestern and fats much more than half the dried mass, albumin a quarter and cerebrin present in considerable quantities.

The galactosides of the human brain were studied extensively by Rosenherin, in which he studied the properties of phrenosin and kerasin. The result of his work showed that; phrenosin in dextro-radatory and on hydrolysis yields phrenosinic acid ($C_{25}H_{50}O_3$) aphingosine and galactose. Kerasin is levaratatory and on hydrolysis yields lignoceric acid($C_{24}H_{48}O_2$) splingosine and galactose.

The following constitutional formula is suggested for kerasin $C_{47}H_{91}O_8N$



The constitution of Phrenosin is thus given:



Investigation upon the chemical constituents of the brain were made by Barbieri(7) and Rielander. Barbieri discovered that, when brain matter was kept at 45° for 12-18 hours, carbon dioxide came off to the amount of about 1 c.c for each gram of brain taken. The other substances separated are loosely described as (1) the hydrochloride of a ptomaine, (2) a substance of phenolic nature, (3) a crystalline material intermediate between leucine and butalanine, (4) cholesterol, margarine, stearin, olein, (5) a substance with a fishy odor and (6) a residue which probably consists largely of keratin. Rielander(8) discovered that basic constituants precipitable by phosphotungstic acid were investigated after hydrolysis by HcL, Hestidine, arginine, lysine and sholine were obtained: also bases with heavier molecules than choline.

(6) Galactosides of Brain 4. Cons. of Phrinosin and Kerasin . Otho Rosenberin. Physiol. Laboratory. King's call, London. Bevchern J.10,142-159 1916.

(7) I The Brain. By N. Alberto Barbieri (compt.rend.,1900)

(8) Chemistry of the Brain. A. Rielander (Chemistry Zents 1908.137: from Zends Physiology 1908,22,377-380)

6

The nitrogenous constituents of the brain lecithin, form a very important part of the tissues, and there were carefully studied by Darrah and Mac Arthur(9)by using for experiment sheep and beef brains. Ground sheep and beef brains were allowed to stand two days in Me_2 Co pressed out, the Me_2 Co treatment repeated at least twice more, and dehydrated material dried in a current of air, shaken twice 2-4 hours with two volumes C_6 H_6(some extraction made at 65° c) the C_6 H_6 evaporated almost to dryness under reduced pressure in Co_2 and the residue dissolved in smallest possible amount of Et_2 O; in addition 2.5 volumes EtOH (ethel ether cephalin; the filtrate was evaporated almost to dryness under reduced pressure in CO_2 the residue dissolved in a little Et_2O and treated with two volumes Me_2 Co which precipitated lecithin and some "white material". After drying in a darkened vacum dessicator, the precipitate was allowed to stand over night in Et_2 O when the "white material " separated out.

The Et OH and Et_2O treatments had generally to be repeated to remove cephalin and "white material". The lecithems so albumied were hydrolyzed by boiling 15-20 hours in Co_2 with 3 per cent HcL or 1.6 per cent KOH. The presence of $NH_2CH_2Ch_2OH$ was demonstrated by means of the chloraurate and picrolonate which were found identical with the corresponding compound prepared from the synthelic alcohol cholin is isolated as the chloroplatinale.

In both sheep and beef brains the choline was about equal to the amount in Nh_2 CH_2 CH_2OH. The two comprising 85% of the total N in the lecithin; the other 15% is in the form of umhydralyzable residue.

(9) Nitrogenous Constituent of Brain Lecithin. J. E. Darrah and C. J. Mac. Arthur J. Am. Chemistry Soc.,38,922-30(1916)

If lecithin is a single substance(and it probably is not or it is much mor more complex than usually believed) it probably contains one molecule each of the above bases; This associated lecithin molecule is rather firmly combined with a salt nitrogluous compound which by law impossible to remove.

In his studies of the combinations of the phosphoric acid in the mervous system Jolly (10) found that phosphoric acid occurs in the nervous substance as glycero or oleophosphoric acid, and on ignition of the brain substance, a residue consists of phosphoric acid and alkaline phosphates and carbonates. The residue by ignition of 100 grams of the brain substance of Ox and Calf and spinal marrow of ox are as follows.

	BRAIN OF CALF	OX	SPINAL MARROW
Free Phosphoric Acid	------	0.095	.874
K Phosphate "	4.774	1.851	2.316
Na " "	0.104	0.206	0.105
Mg " "	0.054	0.178	0.076
Fe " "	0.088	0.309	0.154
TOTALS	5.020	2.639	3.519

These results show that in the young animal the brain is very rich in phosphates, while in the full grown animal the special cord contains more phosphoric acid, and then after the alkaline phosphates. Phosphate o Iron is most abundant.

Rosinheim and Tebb, in their researches on the brain lipoids, isolated the sphingomyelin which they studied.

(10) Combination of H PO in nervous substances. L. Jolly (Compt. Zend. 89,756-758.0)

Sphingonchin is the phosphorized constituent of so called protegon and may be separated from non-phosphorized constituent of this mixture by combining fractional precipitation by means of acetone and alcohol, cholroform solution with re-crystalligation from pyridine. The term galactoside is adapted for the non-phoslorized substanced just referred to.

Sphingonchin is a white, crystalline, non-phosphorized substance which exhibits the phenomenal previously described. It contains 4 per cent phosphorous and the P:N ratio is 1:2 ; it is therefore a diamino-phosphide. On hydrolysis it yields choline and forty acids, but not glycerol. On partial hydrolysis it furnishes a substance which has some resemblance to the simplest mucleic acids, but this on complete hydrolysis yields $H_3 PO_4$, a base, and a arystalline alcohol instead of a carbohydrate.

There are many different conditions and aspects in which the brain might be studied, and two of these, namely; the composition at different a ges, and also the difference in composition of the brain in normal and starving animals. The first condition was studied by Waldemar Koch and Sidney A. Mann (12) in which, three brains were examined, one at the age of nineteen years, one at six weeks, one at two years. With the growth of the brain a decrease of moisture, protein, extractives, and ash occurs, while there is an increase in cerebrins, lipoid sulphur, and cholesterol, that is the substances which predominate in the white matter.

(11) Lipoids of the Brainl M. Christine Webb. (Proc.physicology Soc.1909 J. Physical 38)

The composition of the brain under normal and starving conditions was investigated by Paladino(13).

In experiments carried out on dogs it was found that the water content was higher than that of normal animals. Parallel with the increase of water there is a dimunation of ether soluble substances. No difference could be detected in the quantities of other brain constituents (cholesteral, lethins, prolein) . Another similar examination under abnormal condition was made by Messing (14) on the mineral constituents of the normal and pathological brain. The water content shows only slight variation(from 77-78 12 per cent). The $P_2 O_5$ content increase with age and weight of brain, provided arteriosclerosis is not present, in which case it decreases.

The $P_2 O_5$ content decreases from the 60th year onward, this and the above behavior being also observed in pathological brains. The same relative values are found during infance and in adults. The $P_2 O_5$ content is inversely proportional to the CaO content while the content of Cl is directly proportional to the former.

(12) The differences in the composition of the Brain Substances in Normal and Starving Animals. Reffage Paladino(Biochern Tutsch 1912,38, 443-7)

(13) The Composition of Human Brain at Different Ages. Waldemar Koch and Sidney A. Mann. (Proc.physiol. Soc. 1907 36-38 J. Physiol.36)

(14) Some mineral constituents of the normal pathology of the brain. Basia Messing. Warswa Biological Chemistry. 14 Inaug. Diss. Zarich 1912 Zents.Biochen biological Chemistry 14.133

The lecithin content of the brain, human, was estimated by Cruckshank (14) as well as by Burrows, (15) the latter also investigating the amount in both brains and milk extracted by means of the ether-alcohol mixture and estimated from the amount of phosphorous in the extract. In different animals it was found that the amount of lecithin varies, its proportion becoming greater as the relative brain weight increases.

The following table gives results:

	CALF	DOG	MAN
Relative brain weight	1.37	1.30	1.7
Lecithin of milk in % of proteid.	1.40	2.11	5.05

In 100 grams of moist tissue human brain yielded 0.6, and other tissues varying amounts from 0.14 to 0.48 grams.

The cerebrosides of brain tissue were studied by Levene (16) which rapidly dessicated brain tissue was extracted with hot 95% Et OH. The deposit obtained on cooling was separated into a number of fractions, differing in their solubilities and optical activity. Both optically active and inactive cerebronic acid were obtained in hydrolysis. The more soluble fractions yielded an acid $C_{24} H_{48} O_2$. This is believed to be lignocenic acid, because the melting point of the free acids the PB salts and Et esters agreed closely.

(14) The Lecithin Content of Different Tissues. (P. A. Levend. Rock. Inst. Of J. Biol. Chemistry 15,359-64) John Cruchshank. Path. Bact. 1913.13

(15) Lecithin in Brain and Milk. Robert Burroro. Zent Physi ology Chem. 1900,30 .495-507

(16) The Cerebrosides of Brain Tissue. P. A. Levend. Rock. Inst. J. Biol. Chemistry 18,359-64.

Simonds (17) found that the protein of calf brain subjected to autolysis undergoes a change due to free existing enzymes. About 14 per cent of the organic phosphorous is converted to the inorganic soluble form of enzymes. The organic portion of the brain both soluble or insoluble in alcohol ether undergoes phosphorous cleavage change when subjected to auto-digestion. The lecithin and phosphatide content of the brain as well as of other organs is greatly affected by the administration of alcohol as Sieher (18) proved in his experiments upon dogs.

Dogs were fed alcohol daily for several months at the rate of 0.9-25 grams per kilo of body weight. Occasional periods of a few days rest were allowed. Thealcohol solutions were evaporated, the residue taken up with ether, and the phosphatides precipitated from ethereal soluble with acetone. No alcohol animals compared with normal ones showed in every organ except the kidney a phosphatide deficit. The first figure in each pair in the following list gives the average per cent of phosphatide present in the dry organs of three alcoholic dogs. The last figure represents the average of the three normal animals.

Brain	16.34-27.76	Heart	4.65-7.16	Lungs	5.81-7.15
Spleen	3.72-6.09	Kidneys	7.21-6.37	Blood	.061-085
Stomach Membrane	4.21-8.36	Liver	4.58-7.33		
Intestional "	4.08-7.3				
" Wall	2.66-3.76				
Stomach "	1.86-2.43				

(17) Autolysis of the Brain-Friedrick Simon. Chen. Abt. Pathology Just. Berlin. Zents Physiology Chemistry 72, 463-83.

(18) The Effect of Alcohol Upon the Phosphatide Content of Animal Organs. N. Sieher. St. Petersburg. Bio. Chemistry Zents 23,304-23.

However, similar experiments on dogs using morphin gave results somewhat different according to Biherfield (19). The estimation of certain lipoid constituents in the brain of dogs which had acquired a tolerance for morphine revealed no marked deviationfrom normal ones.

The cleavage of the glucosides by the brain was studied by Hess (20) in which he found that the brains of rabbits, guinea pigs, and man contained a substance which splits Beta glucosides, for example arbutein but not salicin nor Alpha methol Delta glucoside. The brain on being heated lost this cleavage power, indicating the enzymine nature of the reaction. The reaction was favored by a weakly acid reaction, but checked or completely stopped by an alkaline reaction. The cerebral fluid also splits Beta gucoside hence the enz yene was soluble in water,glycerol extracts were not acting.

The lipoids of the ancient Egyptian (21) brains showed that sholesteral of the Captic brain had undergone almost complete exterification, and in the oldest (600B.C.) 95% had disappeared. The per cent ofphosphorous ranged from 55-70 per cent dur probably to methods of preservation.

In a critical estimate of the work already accomplished Fraenkel (22) points out that cholerterol (possibly cerebron) is the only substance as yet isolated in chemical purity from brain substance, and Kauffman (23) also states that ox brains contain no suchcompound as free choline.

(19) The Relative Amounts of the Different Lipoids of the Brain in Dogs which have acquired a tolerance to Morphine. Johannes. Biherfeld(Biodiern. Zeits Chemistry 1915 70,158-63)

(20) The Cleavage of Glucosides by the Brain. L. Hess Lab. allgerm. Polykiln. Vienna Wienklin. Wochskr. 24,1009-11)

(22) Brain Chemistry. Sigmund Fraenkel Vienna(Ergeb. Physiol. 8,212-53)

(23)The Descovery of Choline in Ox Brains. Max Kauffman Haite Z. Chem. 74,175-8)

13

Investigations on lecithin and "myelin" substances in the brain w were done by Zuelzer. The method for the separation of myelin substances in the brain was as follows: The brain is at first extracted with ether. On the removal of this and addition of acetone, a voluminous precipitate is obtained; this is free from cholesterol, which remains in solution. The precipitate consists of several substances containing phosphorus; one of these, protagon, is insoluble in ether free from cholesterol; the part which is soluble in ether is divisible by addition of excess of alcohol into two parts; the one which remains in solution is lecithin, the other, which is precipitated, consists of two new myelin substances which have still to be fully identified. Egg-yolk gives some what similar results.

A. Buglia and D. Maestrinr (b) investigated the phosphorous in the ventral and dorsal medullary fibers of the ox. The phosphorous present in the dorsal and ventral medullary fibers is mainly organic phosphorous and chiefly phosphatide phosphorous. The total phosphores of the dorsal fibers is uniformly greater than that of the ventral. The difference persists in the dry material and cannot be attributed therefore, to a difference in the water of inorganic content.

Phosphatide is approximately the same for each variety, but phosphorous in other types of compound is present in greater quantities in the dorsal fibers. While in the neutral fibers the difference in lipoid and organic phosphorous is scarcely noticeable. In the dorsal fibers it amounts to 3.5 per cent of the total phosphatide.

This points to the possibility that the dorsal fibers contain a small quantity of phosphatide in a special type of organic compound, in addition to the phosphatide.

The property which lecithin possesses of being so easily extracted and dissolved by alcohol and ether is made use of to a great extent in the preparation and extraction of lecithin and lecithin derivatives.

Lecithin emulsions were prepared by (24) Schiffers by dissolving a weighed quantity of lecithin in least amount of toluene, then mixing solution with sufficient (0.9) per cent Nacl solution to give required concentration.

After the mixture has been shaken thoroughly, a current H_2 passed through it until all the toluene has been removed, the emulsion then being submitted to centrifugal action, and filtered through cotton or wool. The following method may be employed for checking the strength of the emulsion; the method is based onthe amount of oxygen required to exidize the lecithin, 10cc. of the emulsion are heated to 90°C. for six hours in stoppered flask with 10 cc. of solution containing $K_2 Cr_2 O_7$ five grams, 38 % Kcl, 300cc. and water 700cc. After cooling 10cc of a 5% solution of KI are added, the mixture is allowed to stand for at least two hours, 30cc. water added and solution titrated with N/25 $Na_2 S_2 O_3$ solution. A similar titration is made by using 10cc. of the Nacl employed in making the emulsion, and the difference is the amount of throsirffate used in the titration if measure of lecithin present. Experiment shows that 1cc. of N/25 throsirffate equals 1.12 grams lecithin.

According to R. Cohn (25) the estimation of lecithin may be divided into three parts. Namely, the extraction of lecithin, purification, and estimation of phosphorous.

15.

For extraction, from 1-2 grams of commercial preparation of lecithin, of 5-10 grams of food containing lecithin are extracted for several hours with two successive quantities of 100 cc. of 96per cent alcohol, the first extraction being carried out at ordinary temperature and second at boiling temperature of alcohol, using the reflux condensation.

Lecithin was prepared by Mac Lean (26) in which he took crude lecithin obtained by extraction with alcohol and purified it. This was carried out by emulsification with water then treatment with acetone. Lecithin, after purification is easily oxidized, and many of the new lecithin like substances are merely oxidation products or consist of lecithin plus nitrogen based on solubility alone; the degree of oxidation and pre-sence of impurities influence solubility greatly.

The observation of the work of Guin and Kaele (27) show that in order to effect the synthesis of lecithin it is proposed to allow the components of choline to act in turn on di-glycerine-phosphoric acid. Ethylene glycol and phosphoric oxide acting on di-stearin produce almost quantitatively di-stearin ethylene-glycol orthophosphate.

(24) Method of Preparing Emulsion and the Subsequent Estimation of the strength of it. J. C. Schiffers Broihern Z. Chemistry 1912,40,189-192.

(25) Lecithin Properties and Estimation .R. Cohn A. Zeits Chemistry. Offento Chemistry. 1913, 19 54-62 Through Chemistry Zentralbl 1913 1. 1129-1130.

(9b) Chemistry of Nerve 2. Estimation of phosphorous in the ventral and dorsal medullary fibers of ox. G.Buglia and D. Maestrive Chemistry Z. 1915 336 from Andi. Farm specum. 1914,18,221-224.

(26). A Simple Method for Preparing Lecithin. Hugh Mac Lean (J. Path. Bact. 1914, 18,490-4.

(27) Albegd Synthesis of Lecithins-Adolph Guin and Fritz Kaele. Ber. 1912 45,3367-3376.

$C_3 H_5 (O.CO.C_{17} H_{35})_2 O.PO(OH).O.C_2 H_4 OH$

When ethylene chlorolydine is used, the reaction takes place in two directions, the glycol ester as well as the B-chlorolthyl enter.

$C_3 H_5 (O.CO.C_{17} H_{35})_2 O.PO(OH)O.C_2 H_4 Cl$ being formed. This compound reacts with trimethyl amine, forming the trimethyl ammonium salt.

$C_3 H_5 (O.CO.C_{17} H_{35})_2 O.PO.(O.C_2 H_4 cl) O NH Me_3$ and no more energetic action of excess of trimethylanine this undergoes rearrangement to lecithin hydrochloridd, $C_3 H_5(O.CO.C_{17}H_{35})_2 O PO(O.C_2 H_4 N Me_3 cl)OH$ the final product obtained was a mixture of both compounds together with an intermediate form.

The synthetic lecithin hydrochloride (Destrarin choline phosphoric acid ester) product is a soft waxy compound which changes at 69° to a clear, viscid liquid or oil which becomes mobile at 64-65° and opaque at 74.°

Plants, vegetables and seeds ahve been used as a source of obtaining Lecithin as well as egg yolks, milk, brains and other substances. Schulze and Franksfurt (28) found that in order to obtain lecithin from seeds, they should be finly ground, treated with ether, water should now be added to saturation and the emulsion which is thus formed must be cleared by the addition of salt; after separation the ether is evaporated and the lecithin which is left is purifiedl,by re-dissolving it in alcohol. To work quantitatively the $H_3 PO_4$ must be determined in the alcohol extract and the lecithin calculated therefrom (factor:magnesium pyrophesphate x 7.2703)

(28) Lecithin in Vegetable Substances E. Schulze and E. Frankfurt.(Lauden Versuls Sbat. 1893 307-378)

17

Schulze[29] working by himself, prepared lecithin and other phosphatides from plant seeds. By this method of ether extraction (crude fat) the lecithin is separated by treatment with acetone which dissolves the fat. The crude lecithin remaining is dissolved in ether and precipitated with acetone. Employing this method lecithin was prepared from the seeds of Saga bispids and Lupimus lutens. The former contained 3.04 per cent lecithin and the later 3.09 per cent . The low phosphorous content is due to the presence of carbohydrates in the material. From phosealus multifolons a lecithin was isolated with 3.84% phosphorous and this product upon decomposition gave the same products as usual.

The phosphatides isolated from Castanea vesca and Aesculus hippocastamun possess respectfully 2.63 per cent and 2.46 per cent phosphorous.

Further extracts (30) are recorded to show that hens feed on a diet free from lipoids, produce eggs which contain lecithin or lecithins. These differ in the nature of their fatty acid radicles, and variation may be produced by the nature of the lipoids of the diet.

Many different methods of preparation are now patented, Germany, France and U. S. all having patents covering such processes. The preparation of lecithin by H. Buer (31) from the seeds of fruit and vegetable material containing lecithin by extracting the initial material with 96% alcohol in amount, about fifty times the ratio of the extracted substance contained in the crude material, at a pressure of .5 to 1 or with the employment of a reflux condenser to stand for some time at the ordinary temperature.

(29) Concerning Methods Employed in Preparation of Lecithin and Other Phosphatides from Plants Seeds. E Schulze. Agriculture Chemistry Poly teck. Zuna & Physiological Chemistry 55,338-51

18.

When the complete separation of fats, cholesterol and coloring matter takes plaee, then exaporate the alcohol solution which contains the bitter principles and the solution to stand for a long time at the ordinary temperature until the separation of the lecithin.

Another process of German origin by C;A. Fisher (32) for the manufacture of lecithin-rich preparations or of lecithin fatty-oil and cholesterol by treating animal or vegetable lecithin containing crude material of mixtures of these substances with an ester of the fatty acids such as Et O Ac., Me OAc. Me. Butyrate, or mixtures of these esters with heat. The cholesterol separated from the mixture of fatty oil and cholestrol by standing at the ordinary temperature is removed in the known names by centrifugation of by pressing out from the fatty oil, and crystallised from alcohol or benzene.

In the making of lecithin salts by Bergell(33) 1 molecule citric acid is mixed with a solution of 1,2 or3 molecules of lecithin, or 1 molecule of glycerol phosphoric acid is mixed with a solution of 1-2 molecules of lecithin and the resulting lecithin salts are separated by freez ing out, evaporating or precipitating from the solution.

Ziegler (34) for extracting a product containing alecithin and serving for the preparation of free lecithin consisting in defining the wheat germ of its moisture by dessication at 70°, removing its oil by solution.

(31) Germany 236,605 September 17,1910 K.Buer Koln. Preparation of Lecithin;

(32) Germany223 , 593 May 29,1907,C. A. Fischer. Berlin F. Haherman Brunn and Sˇephannine Ehrenfeld Wein.

(33) Germany 268,103 November 2,1912,P. Bergell.

(34) " Patent 364,896 April 4,1906.Ziegler.

It is then treated with about 40 pints of methyl or ethyl alcohol of 90-95 % and distilling offin vacums. The residue which contains sugar and lecithin is treated with $C_2 H_5 OH$ of 60-80 per cent and lecithin precipitated by $BaCl_2$ in hot aqueous solution of 10 per cent.

The method used by Bergell (35) for the preparation of lecithin solution consists of dissolving freshly lecithin in glycerol. The resulting solution can be very readily emulsified with aqueous or alcohol liquids, and may be used for subcutaneous or direct injection, and for other medicinal purposes. Glycerol serves as a preservative for lecithin.

According to Feudler (36) the purification of the lecithin preparation is obtained by treating yolk of egg with acetone . A very disagreeable odor caused by the solvent, clings very tenariously to the lecithin albumin and has a marked effect upon its applicability. The purification is effected by moistening with water or an aqueous liquid and drying.

(35) Patent Lecithin Solution. Process for Preparing B. Bergell German Patent. 231,233 June 10-1910.

(36) German Patent 272, 257 Feb. 15,1913 G. Fendler.

The German Patent No. 241 (37)-564 states salts of lecithin are obtained by means of dichlorohydrin or trichlorohydrin, which dissolves 50 per cent in the cold or monochlorohydrin which dissolves. Cold solutions are obtained in the best pure lecithin and mixtures of lecithin and albumins containing variable proportions of pure lecithin from dessicated yolk of egg is based upon the relatively great solution of lecithin in hot acetone in the presence of fatty matter contained marmaly in the egg. For example dessicated egg yolk 100 grams is extracted in a Sothlet appliance by acetone 40.5 kgs. (des sicated egg of commerce containing 15-18 % of lecithin) until the mixture has the required composition. When the acetone solution is allowed to stand at 5-6°, the greater part of the lecithin mixed with the fatty matter and cholesterol, separated out. The product is washed with cold acetone to remove the fatty acid and cholesterol, the residue is almost pure lecithin.

Lecithin is present in bone-marrow and the method employed by (40) Otoslki is a very good one for its extraction. The preparation of lecithin from bone-marrow by means of the method of Bergell was as follows: Extraction of the marrow with warm 96% alcohol, treatment of the alcohol extract with ether and separation of the insoluble substances by decantation, evaporation of the a lcohol, ether extract to dryness, solution of the later in ether from which the lecithin is precipitated with acetone.

(37) German 241,564,Nov.5,1910.Werke Vikstoria G.M. B. H
(38) " 237,029 Feb.21,1911 Chemistry.Fabrisk Gealeon Richter.
(39) Lecithin H. Martin Hall 2,583. September.5,1918.
(40) The " Content of Bone Marrow S. W. Otoskke. Chemistry . RaisedJ. medical Expert. M. St. Petersburg. Bio. Chemistry Z 4. 124-53.

Levene and West found that pure lecithin may be prepared by means of the Cd Cl2 , but it is necessary first to remove almost all the cephalin by EtOH precipitation. The Cd Cl_2 compound is recrystallined from EtOCc until free from Cn_2-N and then decomposed with $(NH_4)_2CO_3$.

The fact that egg yolks contain lecithin furnishes one of the best possible sources for the obtaining and subsequent purification of lecithin.

Mac Lean (42) prepared a sample of lecithin from egg yold which contained all its nitrogen as choline. Dried egg yolk was powdered and extracted with alcohol, to the alcoholic extract was added excess of alcohol solution of Cd Cl_2. The resulting precipitate was washed with alcohol and then rubbed up with about 15 times its volume of ether containing a trace of alcohol. A dense opalescent mixture was obtained which on centrifuging separated into a brownish deposit and clear supernatant liquid. The deposit was thoroughly washed with ether, dried and decomposed by boiling in alcohol with $(NH_4)_2Co_3$ according to the method recommended by Bergell 1902.

The alcohol was concentrated, the residue taken up with ether containing an excess of acetone. The precipitate obtained was emulsified with water and treated by the usual process for the purification of lecithin (Mac Lean 1912). The lecithin was dissolved in alcohol and again precipitated by Cd Cl_2 . The double compound obtained was recrystallized from the ethyl acetate mixture already described. Beautiful white feathery crystals of pure lecithin cadmin chloride were thus obtained.

(41) Lecithin 2 Preparation of Pure Lecithin. Composition and Solubility of lecithin Cadmiun Chloride. P. A. Levene and C. J. West Rock. Inst J. Biology Chemistry 3,4,175-86 (1918).

(42) Preparation of a sample of lecithin containing all its nitrogen a s choline (Mac Lean 1915, Volume 9, Page 374. Biochen Journal)

22

The analysisi of pure lecithin Cd Cl_2 gave:

Nitrogen(Kjeldahl) 0.5604 grams required 5.85cc N/10 H_2SO_4 =146 % N.

Phosphorous (Newnam) 0.2600grans required 15.8cc N/2 H_2SO_4 = 3.37%P.

N:P = 1:1.04

Bergell (43) in his preparation of lecithin believed that lecithin was best prepared by extracting egg yolk with 96% alcohol and preciptiating with Ca Cl_2 at 0°; the precipitate is then extracted with ether, and alcohol composed by boiling with alcohol and $(NH_4)_2Co_3$. The lecithin separates from the alcohol solution at 100° and may be purified by being dissolved in chloroform or precipitated with acetone, mother liquor. The yield is about 4 per cent of the yolk.

Lecithin contary to the statement of Dracancf can be powdered and preserved in air exhausted vessel. (Arnalen 1979,148,71)

When the egg yolks (44) are extracted with ether fully one half of the phosphorous is removed. The per cent of phosphorous calculated as P_2O_5 is as follows. Egg 0.26

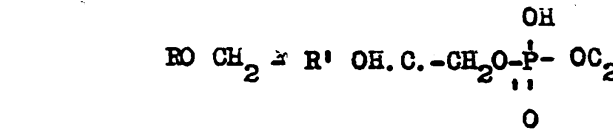
Pure lecithin free from cholesteral was obtained (45) by Riedel bu extracting egg yolk with cold Me OH. The latter substance which possesses the power of splitting lecithin albumin into its components after a short action. May also be used in the analysis of egg yolk.

(43) Preperation of Lecithin Peter Bergell Berlin, 1900, 33,2586-86

(44) Occurrence of Phosphorous in foods A. Balland. Compt. Rend 143.169.

(45) Lecithin of egg yolk. A. G. J. D. Riedel Bie. 1912.24-33 Zends Bio. Chemistry Bibly.13, 45

From the behavior of lecithin toward saponifying agents the form of the activity of glycerophosphoric acids obtained from lecithin R.iedel derives the following formula in which R and R' represent fatty acid radicate(paluntic, stearic, oleic, and lunoleic acids)



Lecithin[46] from egg-yolk was prepared and analyzed: hydrolyzed with $Ba(OH)_2$ or HCl. It gave 66 % of its N as choline. Lecithin from the heart muscle then similarly analyzed gave 42% of its N as choline, when precipitated as th Cd Cl_2 double salt, gave 75% of its N as choline and a fitrate containing N in non-choline form- showing that N is split off in forming the Cd Cl_2 salt. The difficulty was not the isolation of the choline, since when choline was mixed with glycerol, glycerophosphoric acid, P_2O_5, stearic and oleic acids, 94.5% of the choline was recovered as the chloroplatinate.

Amino ethyl alcohol was obtained by Ga rier (47) by hydrolysis of egg white. Lecithin with dilute H_2SO_4 .

Gobley (48) found both Lecithin and cerebrin a long time ago-in egg yolk and in brain substance, and lecithin afterward in human venous blood, bile, eggs, milk of carp, etc. Bouchardt also found it in milk.

(46)
Egg Yolk Lecithin H. Nac Lean Chemistry Abt. Physiol. Inst. Univ.Berlin. Z. Physiol. Chem ., 59,223-9

(47)
Extraction of Amino C2 H_3 -OH from Egg Lecithin George Garier Zurick Z. Physiology Chemistry 76,496-8.

(48)
Lecithin and Cerebrin. M Gobley. J. Chemistry 4. 19 .346-354.

24

In 1886 Liebuch of Berlin announced his discovery in brain matter of a substance containing Phosphorous and Nitrogen and to which he gave the name of protagon. When boiled twenty-four hours with $Ba(OH)_2$ it was said to yield glycerothophanic acid, fatty acids and a particular organism called neurnie. The author states that protogon is formed of the two distinct bodies, lecithin and cerebrin, and that neurine is a product of the decomposition of lecithin.

Work done by Mac Lean (49) on this phosphatide (lecithin) shows that some of the neurine is present in form of choline and part in unknown form. Control experiments show that loss of choline duting analytical methods used will not account for residual N (50). He has also shown that the extract of egg yolk contains ordinary lecithin and also a mono-amino diphosphate which is what different from amorin of heart muscle. The difference probably depends on the presence of different fatty acid radicles. The extract also contains pure tripalmitin.

Eppler (51) has shown that the products of hydrolysis of that portion of the phosphatides of egg lecithin which is not precipitated by $Cd\ Cl_2$ consists of amino-ethyl alcohol in addition to choline extraction. The phosphatide solution in alcohol after complete extraction of egg-yolk with ether is a monaminophosphatide.

(49)Lecithin of Egg Yolk Hugh Mac Lean. A. Zeits Chemistry 1909.59,223-229.

(50)A Mono- aminodosphosphatide in Egg-yolk. Hugh Mac Lean (Bio. Chemistry J. 1909,4, 168-174.

(51) Phosphatides, Particularly Those in Egg-yolk. Julius Eppler. Zeits Chemistry. 1913,87, 233-254.

Zorman states (53) that lecithin is not constant, but rather the proportion of lecithin occurring in egg yolks varies considerably. The yolk of eggs which have apparently kept well, exhibit, after sometime, substantial alteration in their composition especially in respect to the quantities of lecithin and cholesterol they contain. In this regard, too, marked differences are found between fertilized and unfertilized eggs.

Roaf (54) and Edie prepared lecithin and estimated the phosphorous. The egg yolks were repeatedly extracted with alcohol, the alcohol from the united extracts was distilled off under reduced pressure. To the syrupy mass so obtained, a little ether was added and the solution precipitated with acetone. The precipitate was reheated on a steam bath to remove ether and acetone and weighed, and the lecithin in it detected by phosphorous determination.

Haberman and Ehrefeld (55) patented a process for obtaining lecithin from egg yolk by separating from the lecithin and lecithin albumen and fat, cholestral coloring matter associated therewith and aromatic substances.

(53) Lecithin and Other Compounds of Egg Yolk E. Zorman. Biol. Chemistry Farms. 1909 48, 520-21.

(54) Preparation and Estimation of Lecithin. Herbert E. Roaf. F. E. S. Edie(Thompson, Yates, and Johnson, Lab. Rep. Liverpool 1905,6, 201-203)

(55) N. S. 987. 133. March 21 Joseph Haberman and Richard Ehrenfeld, Brum, Austria Hungary.

26.

This is done by extracting with cold EtO Ac. collecting the residue of mixed lecithinand lecithin albumin, extracting lecithin there-from in hot EtOCc and separating the pure lecithin by cooling the solution. 100 grams of yolk yield about 9-10 kilo-grams of lecithin.

B Barbiere[56] gives an account of his work in which he found that the yolk of egg, glandular fat or lipoids soluble in alcohol consisting of tri-palmitin, oleopalmitin, and coochromin; also nitrogenous and phosphorous substances. These can easily be separated without breaking up the lipoids. Egg yolk also contain non-glamular or somatic fats, which consist of tri-stearin and olen-stearin. These are mixed in alcohol. He[57] also separated the lecithin fractions of the yolks of 3000 eggs by dialysis and cooling to 0^{o} each separate fraction analyzed. It was found that soma bases not containing choline were present and were held in solution by the fats. Phosphorous is not united to glycerol, being di-anlyzable and either wholly or in part in the form of soluble phosphates.

Among the methods so far desined for the determination of lecithin and related compounds, the one suggested b · Hoppe-Seyler, depending on the determination of the ether-soluble phosphorus has been most commonly used. Like most of the other methods it does not however, distinguish between the two main groups of lecithins, the lecithins andkephalins.

(56) Non-existence of Lecithin in Egg Yolk and in Biological Chemistry structures. N. A. Barbiere Compt. Rend 155,312,14.

(57) Non-existence of Lecithin in Egg Yolks, Free or Combined. N. A. Barbiere. Compt. Rend. 151,405-7.

Thudichum[2] has suggested a method depending on the differences in solubility of the lead salts of the various lecithins in alcohol and ether. On account of the complicated manipulations and the amount of material required, it has never come into use. The method of Koch (3) which depends of the determination of the methyl group, split off above and below 240 degrees C, with hydriodic acid, is also rather complicated, but does not require much material.

As very little is known at present of the relative amounts of lecithins and kephalins in the various animals and vegetable substances, an attempt was made to extend the method of Koch to ether tissues besides the nervous system. The very first trials with milk (which does not contain much lecithin) gave, however, such unexpectedly high results that we were led to seriously doubt the accuracy of the method. A trial of the reaction with pure butter-fat revealed the fact that fat alone will split off with hydriodic acid either methyl iodide, ethye iodide, or some other iodide which interferes with the determination. This method was therefore abandoned and an attempt made to simplify the method of Therdichum.

The precipitate formed in an alcoholic solution of crude brain lecithin with alcoholic lead acetate has been previously shown by Koch to consist almost wholly of kephalin, as it gives off above that temperature. A crude preparation of egg lecithin, from which no kephalin can be separated by precipitation with alcohol also gives a precipitate with lead acetate. As there is a possibility that the precipitate in this case may be a modification of lecithin, it was tested for methyl groups.

The result was the same as with the substance from nerve tissues, only very little methyl iodide could be split off above 240°c. with hydriodic acid. We may assume ,therefore, that the lead precipitate always consists of a kephalin. As the formation of the precipitate takes place, however, somewhat slowly, it must be hastened by boiling and by the addition of a little ammonia, as described in detail later. The filtrate on standing will still continue to deposit small amounts of precipitate, but the reaction is practically complete and can be used for comparative investigations. The compounds found in the filtrate invariably contain the methyl groups in the proper proportion as required for lecithins.

The lecithins , therefore are calculated from the amount of phosphorus in the filtrate and the kephalins from the phosphorus content of the precipitate. The lecithins and kephalins before the precipitation with lead acetate are separated from inorganic and extractive phosphates by precipitation with chloroform in acid solution. A careful examination of this precipitate is called the lipoid precipitate, as it contains all the fat-like constituents in the case of all tissues investigated.

S. W. Johnson and E. W. Jenkins have devised a method for estimating phosphoric acid which is said to require less than half the time and labor necessary for the molybdic acid method, to be scarcely less accurate and generally appliable. Stolba has shown that the pure ammonio-magnesium phosphate can be determined by tiration as well as by weighing, one molecule of pure salt requiring two molecules of hydrochloric acid to destroy its alkaline reaction.

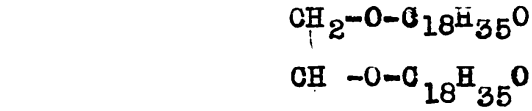
(Determination of Phosphoric Acid-S. W. Johnson and E. W. Jekins (Chem. News, 40,39-40)

The authors have taken advantage of this corcumstance. The standard acid used in other volumetric work answers perfectly for this work. A strong nearly saturated solution of ammomium tartrate free from carbonic acid and a solution of magnesium salt are also necessary. The latter is prepared by dissolving 10 grams of $MgSO_4$ and 195 Grams NH_4Cl in 1 leter water. 10 cc. of this solution contains twice the amount of magnesium necessary to precipitate 0.1 gram H_3PO_4. A suitable amount of phosphate is dissolved in HCl, the solution nearly neutralized with ammonianand ammonium tartrate solution is added, 10cc. at a time until the solution remains perfectly clear when alkaline. A suitable quantity of magnesium mixture is then added and the liquid either vigorously stirred with a rod, or if precipitation is made in an assay flask, shaken occasionally. When the precipitition is nearly complete enough ammonia must be added to make the liquid strongly alkaline, and let rest six to twelve hours, then filterthe precipitate and wash with equal parts of strong alcohol (85-90) and water. When the dish and precipitate are washed until the washings are no longer alkaline, the filter and precipitate are returned to the beaker or flask, a little water and few drops of cochineal tincture added, and whole is titrated. This is done by adding excess of standard acid at once, stirring to wet all the precipitate with it, and after standing a few minutes, titrating back with standard alkali.

Ammonim citrate may be substituted by ammonim tartrate for bringing the precipitate or reverted phosphates into solution. Since ammonium magnesium phosphate if largely soluble in ferric and aluminic solutions, containing insufficient ammonium tartrate, it is necessary, in presence of iron to add ammonium tartrate, more then enough to produce a reddish-yellow solution, enough, in fact, to produce a greenish-yellow solution.

CHEMISTRY OF AND GENERAL PROPERTIES OF LECITHIN.

Lecithins are ester compounds of glycerophosphoric acid substituted by two fatty-acid radicals with a base called choline. According to the kind of fatty acid contained in the lecithin molecule it is possible to have various lecithins, such as stearyl-, palmityl-, and oleyl-lecithins. According to THUDICHUM, two different fatty acids may exist simultaneously in one licithin, and according to him every true lecithin always contains at least one oleic-acid radical. All lecithins are mononitrogenous monophosphatides, which contain one atom of nitrogen for every atom of phosphorus. As an example of a lecithin we give the one closely stidied by Hoppe-Seyler and Diaconow, called disteryl-lecithin,



According to Henriques and Hansen the iodine equivalent of the fluid fatty acids obtained from egg as well as brain lecithin is higher than that of oleic acid,hence it follows that the lecithins contain other fatty acids besides stearic, palmitic, and oleic acids

Erlandsen in a specially thorough and careful investigation has studied the phosphatides of the ox heart and ox muscles. The lecithin had the same composition as that from the egg-yolk. The iodine equivalent as well as the analysis show that the fatty acids occuring in

the lecithin molecule are very poor in hydrogen and belong in part to the linolic or linolenic acid series. Diaminomonophosphatides, i.e., compounds in which the relationship N:P is not, as in lecithin 1:1, but 2:1, occur in the muscles but chiefly in the heart muscle. These phosphatides are isolated as metallic salts, and the cadmium compound of the diaminomophosphatide obtained from the heart had the composition $C_{40}H_{75}N_2PO_{12}.2CdCl_2$. Erlandsen has isolated a new phosphatide from the heart, which he calls "cuorin" and which belongs to the group of monaminodiphosphatides in which the relation of N:P is 1:2 This cuorin, which occurs only in traces in other muscles, contains two phosphoric-acid radicals which in part are united with glyceryl. Besides these it contains two residues of strongly unsaturated fatty acids and a basic radical, which is not identical with choline. The empirical formula is $C_{71}H_{125}NP_2O_{21}$. Cuorin is soluble in ether but insoluble in alchohol, and is characterized by a very great autooxidizability. It is obtained in the amorphous state. The monaminophosphatides (lecithin and cuorin) can be directly extracted from the air-dried and finely divided organs, and to all appearances occur in the free state. The diaminophosphatides are also soluble in ether, but cannot be directly extracted by either, but only after a previous treatment with alchohol, and therefore probably exist in combination with proteins.

Winterstein and Hiestand, and presious to them Schulze and Winterstein, have isolated from different parts of plants, lecithin preparations with are poorer in phosphorus than the ordinary lecithin, containing as a maximum 2.74 per cent phosphorus, and which on clevage with dilute iineral acids yielded, besides fatty acids, clycerophosphoric

acid, and choline, also considerably quantities of hexoses, indeed 16 per cent. The hexoses are d-glucose and d-galactose, and besides these small quantities of pentoses were found. These phosphatides seem to be widely distributed in the plant kingdom.

On saponification with alkalies or baryto-water, lecitin yields fatty acids, glycerophosphoric acid, and choline. It is only slowly decomposed by dilute acids. Besides small quantities of glycerophosphoric acid we have large quantities of free phosphoric acid split off

Clycerophosphoric acid, $C_3H_9PO_6$ = $\begin{matrix} CH_2.OH \\ CH.OH \\ CH_2\text{-}O\text{-} \\ OH\text{-} \quad PO \\ OH\text{-} \end{matrix}$ is a bibacis acid

with probably occurs in the animal fluids and tissues only as a cleavage product of lecithins. According to Willstatter and Ludecke, the glycerophosphoric acid split off from lecithins is optically active. Its barium and potassium salts are levorotatory and behave in certain regards differently from the corresponding slats of synthetically propared glycerophosphoric acid.

Choline (trimethyloxyethylammoniumhydroxide)

$$C_5H_{15}NO_2 = N\text{-}\begin{matrix} CH_2.CH_2(OH) \\ (CH_3)_3 \\ OH \end{matrix}$$

which occurs extensivly in the plant kingdom, is not identical with the base, NEURINE, propared by Leibreich as a decomposition product from the brain, which is considered as trimethylvinylammonium hydroxide, $C_5H_{13}NO$. Choline is a syrupy fluid readily miscible with absolute alchol. Hydrochloric acid gives a compound which is very soluble in water and alcohol, but insoluble in ehter, chloroform, and benzene. This compound forms a double combination with platinum

chloride which is soluble in water, insoluble in absolute alcohol and ether, chloroform and benzene. This compound is used in the detection and identification of this base. Choline also forms a crystalline double compound with mercuric chloride and with gold chloride. Choline is precipitated by potassium iodide and iodine (Gulewitsch), and potassium triiodide can be used for the quantative estimation of this base (Stanek). On heating the free base it decomposes into trimethylamine, ethylene oxide, and water.

Lecithin occurs, as Hoppe-Seyler has especially shown, widely diffused in the vegetable and animal kingdoms. Acording to the investigator it occurs also in many cases in loose combination with other bodies, such as is found in nearly all animal and vegetable cells thus far studied, and also in nearly all animal fluids. It is especially abundant in the brain, neaves, fish eggs, yolk of the egg, electrical organs of the Torpedo electricus, semen, and pus, and also in the muscles and blood-corpuscles, blood-plasma, lymph, milk, especially woman's milk, and bile. Lecithin is also found in different pathological tissues or liquids.

Siwertzow has determined the amount of lecithin in the human foetus and in children of various ages, and he finds that the quantity of lecithin is much greater in the organs (brain, liver, heart and muscles) of the ripe foetus as compared with the same organs of childern up to ten years of age. The child according to him has a certain store of lecithin when it comes into the world and this is consumed during the first months of its extra uterine life.

This wide distribution of the lecithens, as also the fact that they are primary cell constituents, gives great physiological importance to these substances. We have in lecithin, no doubt, a very important

material for the building up of the complicated phosphorized nuclein substances of the cell and cell nucleus. That the lecithins are a great importance in the development and growth of living organisms, in fact for the bioplastic processes in general, follows also from several investigations. The fact must not be overlooked that in the animal body we find besides the lecithins also other related phosphatides which have been little studied and which can be readily mistaken for lecithins.

Lecithin may be obtained in grains or warty masses composed of small crystalline plates by strongly cooling its solution in strong alcohol. In the dry state is has a waxy appearance, is plastic, but forms pulverizable masses when dried in vacuum, and is soluble in alcohol, especially on heating (to 40-50°)C; it is less soluble in ether. It is dissolved also by chloroform, carbon disuphide, benzene, and fatty oils. The solution of lecithin from egg-yolk is dextrorotatory (Ulpiani). The solution of lecithin in alcohol-ether or chloroform is precipitated by acetone. It swells in water to a so-called myelin forms (see Chapter XII). On warming this swollen mass or the concentrated alcoholic solution, decomposition takes place with the production of a brown color. On allowing the solution or the swollen mass to stand, decomposition takes place and the reaction becomes acid.

With the considerable water, lecithins give an emulsion or indeed a filterable colloidal solution, which is precipitated by salts with divalent cations, such as Ca, Mg, and others (W. Koch). This precipitate dissolves again in water after the removal from the solution of the electrolytes, and the formation of this precipitate can be prevented by the presence of salts of monovalent cations. We are here not dealing

with a chemical but rather with a physical precipitation reaction (Koch). In putrefaction lecithins yiled glycerophosphoric acid and choline; and latter further decomposes with the formation of methylamine, ammonia, carbon dioxide, and marsh gas (Hasebroek). If dry lecithin be heated it decomposes, takes fire, and (brüsn,?) leaving a phosphorized ash. On fusing with caustic alkali and saltpetre it yields alkali phosphates. Lecithins are easily carried down during the precipitation of other compounds such as the protein bodies and may therefore very greatly change the solubilities of the latter.

Lecithins combine with acids and bases. The compound with hydrochloric acid give with platinum chloride a double salt which is insoluble in alcohol, soluble in ether, and which contains 10.2 per cent platinum (for distearyl-lecithin). The cadmium-chloride compound which contains 3 molecules of lecithin and 4 molecules of cadmium chloride (Upliana) is difficultly soluble in alcohol, but dissolves in a mixture of carbon disulphide and ether or alcohol. A solution of lecithins in alcohol is not precipitated by lead acetate and ammonia.

Lecithin may be prepared tolerably pure from the yolk of the hen's egg by the following methods, as suggested by Hoppe-Seyler and Diaconow. The yolk, deprived of protein, is extracted with cold ether until all the yellow color is removed. Then the residue is extracted with alcohol at 50-60°C. After the evaporation of the alcoholic extract at 50-60°C., the syrupy matter is treated with ether and the insoluble residue dissolved in as little alcohol as possible. On cooling this filtered alcoholic solution to -5° to 110°C. the lecithin gradually separates in small granules. The ether, however, contains considerable of the lecithin. The ether is distilled off and the residue dissolved in chloroform and the lecithin precipitated from this solution by means of acetone (Altmann.)

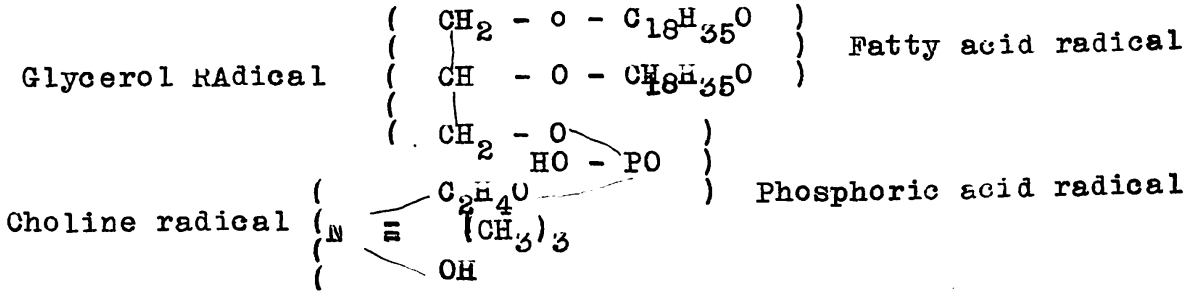
According to Gilson a new protion of lecithin may be obtained from the ether used in extracting the yolk by dissolving the residue after the evaporation of the ether in petroleum-ether and then shaking this solution with alcohol. The petroleum-ether takes the fat, while the lecithin remains dissolved in the alcohol and may be obtained therefrom rather easily by using the proper precautions, as described in the original publication.

Zuelzer's method is based upon the precipitability of the lecithin by acetone, and Bergell's method upon the preparation of the double salt of cadmium and its decomposition by ammonium carbonate. The preparations obtained by the different methods consist generally of a mixture of lecithins.

The detection and the quantitative estimation of lecithins in animal fluids or tissues is based on the solubility of the lecithins (at 50-60°C) in alcohol-ether, by which the phosphoric-acid or glycerophosphoric-acid salts which may be present at the same time are not dissolved. The alcohol-ether extract is evaporated, the residue dried and fused with soda and saltpetre. Phosphoric acid is formed from the lecithin, and it can be used in the detection and quantitative estimation. The distearyl-lecithin yields 8.798 per cent P_2O_5. This method is, however, not exactly corrrct, for it is possible that other phosphorized organic combinations, such as jecorin (see Chapter VIII) and protagon, may have passed into the alcohol-ether extract. In detecting lecithin the double compound of choline and platinum chloride must also be prepared. The residue of the evaporated alcohol-ether extract may be boiled for an hour with baryta-water, filtered, the excess of barium precipitated with CO_2, and filtered while hot.

The filtrate is concentrated to a syrupy consistency, extracted with absolute alcohol, and the filtrate precipitated with an alcoholic solution of platinum chloride. The precipitate after filtration may be dissolved in water and allowed to crystallize over siphuric acid. For the detection and estimation of lecithin we can make use of the method of heating with hydriodic acid as suggested by Koch. One methyl iodide group is split off at 240° and the two others at about 300°C.

The Lecithins are combinations of glycerol with fatty acids. Here only two hydroxyls are substituted by fatty acids in the triatomic glycerol, while the third is replaced by a phosphoric acid molecule which si also combined with the base, choline. The following formula gives an idea of the constitution of lecithin, also called disteryl-lecithin.



On saponification with alkalies, we obtain fatty acids, glycerol, phosphoric acid, and choline. Dilute acids have little action on lecithin. The fatty-acid component varies. We are acquainted with lecithins containing stearic, palmitic, and oleic acids. Even two different acids may participate in the constitution. We have not yet succeeded in preparing lecithin synthetically. As it is optically active, it must contain an asymmetric carbon atom. We are justified in making certain deductions regarding the method of grouping of the

glycerol and combined radicals, as indicated by R. Willstadter and Karl Ludecke. The following formulae are possible ones

CH_2-Φ Choline phosphate
*CH -O Fatty Acid
CH_2-O Fatty acid

I

CH_2-O Fatty Acid A.
*CH-O Choline phosphate
CH_2-O Fatty acid B

II

Formula II only contains an asymmetric carbon atom when the two fatty acids are different. The investigators mentioned decided in favor of formula I, because they succeeded in obtaining an optically active glycerophosphoric acid in phydrolysis. This is only possible when the molecule has the following grouping:

$$HO.CH_2\text{-}CH.OH\text{-}CH_2.O.PO_3H_2.$$

*

The base choline is of much interest. It is a quaternary-ammonium base, and has the following constitution:

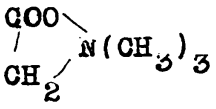
$$\begin{matrix} & CH_3 \\ & CH_3 \\ N & - CH_3 \\ & CH_2\text{-}CH_2OH \\ & OH \end{matrix}$$

It is, therefore, to be considered as trimethylhydroxyethylammonium hydroxide. Wurtz proved this by synthesis. He combined ethylene oxide, C_2H_4O, trimethylamine , $CH_3\text{-}N(CH_3)_2$, and water. Choline can also be derived from glycol, as shown by the following formula:

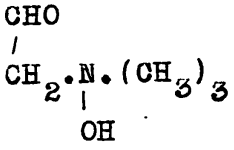
$$\begin{matrix} CH_2OH \\ | \\ CH_2\text{-}N\text{-}(CH_3)_3 \\ | \\ OH \end{matrix}$$

In aqueous solution choline breaks down into glycol and trimethylamine. It has also been found in a free state in plants. It is closely related to another base, also found in plants, and especially

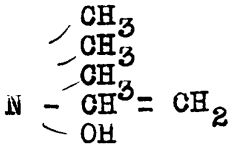
in sugar-beets, known as betaine, or oxyneurine. Its formula is:



It has been obtained from choline by oxidation. Other bases have been isolated from various plants, which in part have been given characteristic names; e.g., amanitine, from toad-stools; fagine, from buchu seeds, etc. They are, however, all identical with choline. In toad-stools (Amanila Muscaria), there is found besides choline, another base called muscarine, which is evidently an oxidation product of choline, and can also be obtained from it by oxidation. It is commonly considered to be an aldehyde, although its constitution has not yet been established positively:



Closely related to these is neurine, which has been isolated from the brain by Liebreich. Its composition is that of trimethylvinylammonium hydroxide:



The second component of lecithin, the glycero-phosphoric acid, is easily produced by uniting glycerol and phosphoric acid.

The lecithins are widely distributed in the plant and animal kingdoms. We could truly say that every cell contains lecithin. It occurs particularly in animal tissues, in the brain, nerves, fish-eggs, yolk of eggs, and plasma, and in spermatozoa. It is also found in the muscles and blood, in the lymph and leucocytes; in fact, in every

cell and in every organ. We find lecithin every widely distributed in the vegetable world, more especially in seeds. During germination the lecithin content increases.

In digestion, lexithin acts in an analogous manner to the fats; in fact, it resembles these very closely in every respect. It forms an emulsion with water. It partly resembles a colloid. Lecithin is decomposed by lipase into glycero-phosphorix acid, free fatty acids and choline; it is not certain that the decomposition of lecithin in the alimentary tract is complete, nor that unchanged lecithin can be directly absorbed. It is rather to be assumed that its components are separately turned over to the organism for further use.

The wide distribution of lecithin leads us to conclude justly that it is of great importance to the animal organism. We, however, know little about its function at present. From the constitution of it we can indeed assume that it acts as in intermediary body between various groups of compounds. We easily recognize its relation to the fats, from which it perhaps derives two components, the fatty acids and glycerol. On the other hand, lecithin evidently acts as a bridge to the very important nucleins. It is possible that lecithin plays a leading part in the internal metabolism of the cells. To a certain extent it represents the fat of the cells. Furthermore, it unites the inorganic foods with the organic ones. The nucleins possibly obtain their phosphoric acid from lecithin.

We do not know anything at present concerning the occurrence of lecithin in the organs. It may be there in the free state, or it may enter into numerous combinations. Many lecithides have been described, but as lecithin has the property of readily enclosing other substances, e.g.,

Albumin, all such claims should, for the moment, be regarded with considerable skepticism.

The following experiments may possibly give us some conception of the functions of lecithin, even if only indirectly. If we remove every trace of serum from the blook corpuscles by means of centrifugal machine, and careful washing with physiological sodium chloride solution, the corpuscles are not dissolved by the cobra poison of the Naja snake, when suspended in an isotonic sodium chloride solution. The process of dissolving the blook corpuscles in such a way is called hemolysis, and the poisons causing this are hemolytic. If the serum isnot separated from the blood corpuscles they immediately go into solution on adding cobra poison; i.e., the hemoglobin diffuses from the blook corpuscles into the surrounding medium. We can show the influence of serum in a better way by taking thoroughly-washed blood corpuscles, suspending them in a sodium chloride solution, and adding only one drop of serum to this, after having previously shown that cobrapoison alone had not caused hemolysis. S. Flexner andH. Noguchi, who first observed this fact, and noticed it also with other poisons (tetanustoxin, solanin, saponin, etc.), rightly concluded that some substance was undoubtedly present in serum which made it possible for the cobrapoison to act on the hemoglobin of the corposcles. P. Kyes then succeeded in showing that lecithin could be substituted in place of serum. Minute traces are sufficient to cause hemolysis. Lecithin alone, when used in small quantities, does not act hemolytically, but lecithin and the cobra poison together do so. This is not the place to dwell upon this

interesting biological phenomenon and its explanation. We must content ourselves with the knowledge that lecithin possesses the capacity of accelerating the activity of poisons. Many interesting questions are suggested by this fact. It is entirely possible that lecithin also acts as an accelerator in the animal cells, and even on the intracellular ferments. As a result of recent investigations we are forced to conclude that the ferments as a whole are not released from the cells in their active form, but that they require the influence of a second substance to develop their activity. With such a hypothesis we can easily explain the action of ferments in the cells.

To lecithin is ascribed a large influence in the construction of the cell walls, andalso in the resorption of the cells. What was said concerning the fat contents of cells is also applicable to this case. Lecithins act as solvents.

O. Hehner reviews all that has been previously contributed towards the estimation of phosphoric acid, either directly as phosphomolybdate, or indirectly by dissolving this precipitate in ammonia, precipitating with magnesia mixture, and weighing as magnesium phyrophosphate. The objection to weighing the dried phosphomolybdate precipitate is that it has been found to contain certain per centages of phosphoric andhydride v arying from 3.14 to 3.90. The explanation given by Lipowitz that this variation is due to $Mo\ O_3$ accompanying the precipitate in varying proportions is confirmed by the author who found further that the q uantity of $Mo\ O_3$ precipitated increased with the temperature at which the precipitation took place. Hence low temperatures (30-35°c.) should by employed. The molybcid solution should be prepared by the method of Fresenius by dissolving 1 part of molybdic acid in four parts of ammonia of 0.96 sp.gravity and pouring this solution slowly into 15 parts of nitric acid of 1.2 sp. gravity avoiding all rise of temperature. When the precipitate was thrown down by the means of the above molybdate solution in a solution mearly previously neutralized by addition of ammonia and at a low temperature it was found to be free from molybdic acid. It was found that it was soluble in 21,186 parts of wa ter, in 8,117 parts of streng alcohol, and 13,515 of dilute alcohol: hence this precipitate may be washed with dilute alcohol without sensible loss, and this is to be preferred to wate, because it does not cause the precipitate to pass through a good filter. Direct experiment shows that no one of these three liquids disdolved the precipitate sensibly when used for washing.

The method finally adopted for getting the precipitate in a form suited for weighing was as follows. The phosphate was precipitated with the precautions already mentioned, and also avoiding too great concentration of the solution, which encourages the simultaneous precipitation of moybdic acid. The precipitate was washed with dilute alcohol, leaving as much as possible of the dilute alcohol, in the beaker, the precipitate was dissolved from the beaker and filtered by ammonia solution, the solution was then evaporated to dryness on the water-bath, the evaporation to dryness being several times repeated after the successible addition of small quantities of water. The precipitate then consisted of ammonium phosphate and acid ammonium molybdate. It can be dried at 100° and weighed a constant weight being quickly obtained, since all ammonia which ordinarily escapes through the drying process has been removed by repeated evaporations. The weigh of the precipitate divided by 28.5 gives the weight of phosphorous anhydried present.

Any alcoholic solution of lecithin gives characteristic precipitation with cadiumn and platinic chlorides. On heating the precipitate with excess of baryta water, lecithin is gradually connected into fatty acids, glycerophosphoric acid, and choline. On the removal of the excess of barium oxide with carbon dioxide, the barium salts of the fatty acids remain undissolved, whilst the other products pass into solution. (Identification of Lecithin, Riedel. (Chemistry Centr. 1906 1. 772-773, from Zent.20 ,92-93.)

On evaporating and extracting with alcohol, the choline dissolves and may be identified by its platini chclride. The glycerophosphate may be oxidized with nitric acid and the phosphoric acid identified with ammonium molybdate. The barium salts of the fatty acids may be decomposed by heating with dilute HCL and the fatty acids extracted with ether. On agritating with dilute sodium hydroxide, the acids may be removed, and any cholesterol exphytosteral recovered from the solution. The amount of lecithin may be calculated from the quantity of phosphorus found, as 100 parts of lecithin equals 3.94 parts of phosphorous.

Estimation of Phosphoric Acid as Ammonium Phosphomolybdate- R. Finkener (Deut. Chemistry. Ges. Ber. 10,1638)

It is found that hydrochloric and nitric acids hinder or delay the formation of the yellow precipitate, and that the molybdic acid solution and ammonium salts hasten or bring about its action. Hydrochloric acid acts in the solution more energetically than nitric acid, and ammonium nitrate than ammonium chloride. The precipitate contains phosphoric and molybdic acid in the ratio of 1 P_2O_5 : 24 MoO_3. In the precipitation of the phosphoric acid, the quantity of the free nitric acid must always be greater than is necessary to preclude the possibility of a precipitate arising in the presence of the phosphoric acid, and a considerable quantity of ammonium nitrate can be dissolved inthe solution in order to assist the separation of the precipitate.

A mixture is recommended of 37 cc. of molybdic acid solution ,9cc. of nitric, and 40 grams of ammonium nitrate. This remained clear for 24 hours. In such a mixture to which 0.01 gram P_2O_5 was added, a very preceptible precipitate was formed, after 24 hours.

Except in very extraordinary cases the precipitation may be effected in 22 hours, by 1. adding to the solution so much molybdic acid solution that the latter is equal to 4 times the volume of the phosphoric solution and at the most is decomposed by the phosphoric acid up to two thirds of its quantity: and 2. by dissolving in the solution 25 grams of ammonium nitrate for every 100cc. of the mixture. A 20% ammonium nitrate solution is recommended for washing the precipitate, and in the first washings, this must be mixed with 1/3 of its volume of nitric acid, which prevents the separation of a difficulty soluble crystalline compound. The washing is complete when the filtrate is no longer immediately colored by potassium ferrocyanide. After removing the greater part of the nitrate with water the precipitate is removed from the filter to a weighed crucible, by washing. That adhering to the filteris dissolved off with warm dilute ammonia, evaporated, treated with excess of nitric acid and the solution quickly poured into the crucible.. The whole is evaporated and the ammonium nitrate driven off by gently heating over the wire gauze. The residue is hygroscopic, and must be quickly weighed after cooling in the exsicater. The precipitate contains 3.794 % $P_2 O_5$.

In Zeits Chemistry 100,16, Stolba[57] gives a method of determining ammonia-magnesic orthophosphate alkalimetrically instead of igniting and weighing. It is supposed that all under circumstances the phosphoric acid is separated in the form of the above named salt and that the tincture of cochineal is used as an indicator.

(57) On Stolbas Method for Alkalimetric Estimation of Phosphoric Acid F. Mohr. Zeits Chemistry. 16,326-328.

The following additional observations are made by the author in regard to Stolba accounts. The double salt mentioned consists of P_2O_5. $2MgO(NH_4)_2O$ if imagined in the anhydrous state, it contains 1 atom of phosphoric and 3 atoms of real base. By saturating 2 atoms of acid with base, phosphoric acid with 1 atom of base is left in the neutral state, and the smallest excess of acid occasions the acid reaction. The double salt known as microcosmic salt, consists of P_2O_5. NA_2O . $(NH_4)_2O$ r 9 aq, reacts alkaline and also may be measured alkalimetrically. 1 gram requires 4.8 cc. N HCl. It contains according to the formula $\frac{71.36}{209.36}$ 0.3408 grams phosphoric acid: Therefore 1 cc. normal acid $= \frac{0.3408}{4.8} = .0071$ gram P_2O_5, That is almost $\frac{1}{1000}$ of the atomic weight of phosphoric acid. By precipitating 1 gram microcosmic salt withthe magnesia mixture and titrating the washed precipitate, according to Stolba, 9cc. of normal acid., that is twice the above quantity was used. This is easily explained by the fact that the sodium salt contains 1 atom of basic water, which is alkalimetrically inactive, while the magnesium salt really contains three atoms of base. In the first case 1 atom of base and in the second two atoms are saturated. By heating the sidium salt in a platinum capsule until it melts to a glass, $P_2O_5.Na_2O$ is left, which, when dissolved in water is practically neutral. If however the ordinary sodic phosphate with two atoms of soda and one atom of basic water is measured alkalimetrically, it requires before and after ignition the same quantity of acid, because the atom of basic water remains inactive and the two atoms of soda are still present.

Estimation of Lecithin C. Virchow. (Chemistry Zeit., 1911,35, 913-914.

In Virchow's method of estimation lecithin one gram of lecithin substance is boiled three times in succession with ten cc. of absolute alcohol, and the filtrate and washings, measured, about 40 or 60 cc. distilled off. After removing the last traces of alcohol by blowing, the weighed residue is dissolved in 10cc. of absolute ether, which is then poured through an asbestos filter tube. The residue is washed three times with ether, then the ether is distilled off, and weighed . The residue treated with 3-4 cc. fuming HNO_3; the solution is transfered to a platinum dish and the flask rinsed three times in succession with 2cc. fuming nitric acid. After evaporating the acid on the water bath, the residue is mixed with 1 gram dry Na_2Co_3, using a platinum spatula. Five grams of the usual potassium nitrate-sodium carbonate mixture are now added, and the whole is heated to fusion four or five minutes. The fusion contains the phosphorous of lecithin as phosphoric acid, which is then estimated by the usual magnesia process.

A. Kitchin states that with certain precautions the uranium phosphate method is quite as accurate as the magnesium process and possess certain advantages over the latter. The estimation can be conducted in the presence of lime, etc., and the precipitate of uranic phoshpate is almost completely soluble in water containing ammonium acetate and free acetic acid. The principal precautions to take are to have a sufficient amount, of ammonium acetate and not too much free acetic acid. The precipitate should be dried and ignited strongly, until the filter is consumed.

A little HNO_3 should then be added and the ignition repeated gently. If the ignition be carried too far, the uranic phosphate is partly reduced, and a second evaporation with HNO_3 is necessary

Phosphoric acid may be determined volumetrically by use of silver nitrate.[59] The experiments which show that the reaction between silver nitrate and di-sodium phosphate is most conveniently expressed by the equation: $3Na_2HPO_4 + 6AgNO_3 = 2Ag_3PO_4 + 6NaNO_3 + H_3PO_4$. The volumetric method based on this is conducted as follows. The solution of phosphoric acid neutralized to phenalphthalein with NaOH (free from cholride) is treated with excess N/10 silver nitrate and well shaken. Zinc oxide is now added until the solution is newtral to litmus paper. The solution is filtered and the excess of silver determined in an aliquot portion of the filtrate by Valhord's method. It is necessary to add at last 3% excess of acetic acid, while a great excess of zinc oxide is to be avoided as it will precipitate some silver especially if allowed to stand in contact with the solution. For this reason it is wise to filter as soon as possible. In this way results have been obtained with sodium and ammonium phosphates deviating from the gravimetric figures by only about three parts per 1000.

A new process for estimation of sulphur and phosphorous is used in whichthe substance is burnt in combustion tube open at both ends, a stream of oxygen being passed through and products of combustion being made to traverse a layer of pure gramulated quicklime, make by carefully igniting calcium nitrate. This salt, is best prepared by calciming marble and dissolving in pure HNO_3, a little being left undissolved, so that the liquid has an alkaline reaction. In this way traces of alumina and ferric oxide

(59) (Phosphoric Acid; Volume Det. of J. Rosin J. American Chemistry Soc.1911, 33,1099-1104.)

are prevented from passing into a solution, whilst by adding two volumes of a mixture of ether (one Volume) and alcohol (two volumes)and leaving the solution to stand twelve hours, any phosphate and sulfate of calcium present are separated. The quicklime formed by finally igniting the purified salt is pulverized until the larger lumps are about five millimeters in diameter, the finer portions being removed by a sieve the holes of which are 1 millimeter in diameter.

In order to avoid the formation of metapherphates,(60) solid substances when burnt should be mixed with three times their bulk of marble quicklime; the magnesia method of estimation the phosphate produced is much less convenient than the uranium process, whether worked gravimetrically or volumetercally, whilst the absence of iron and alumina in most cases renders this method quite accurate.

J. Macagno states that the phospho-molybdic precipitate obtained in the course of Sonnedscheinz method is dissolved in ammonia, the solution is acidulated, and metallic zinc is added. The reduced molybdic acid is then oxidized by a titrated solution of per manganete.(61) The phosphoric acid calculated from the molybdic acid found, the original molybdic precipitate being presumed to contain 90.74 % molybdic acid. The variations from this proportion are stated to have but little effect on the result. Test experiments are given, the greatest error being 0.5 % of the phosphoric acid present.

(New Process for Estimation of Sulphur and Phosphorous in Organic Bodies. G. Brugelmaun Zeits Chemistry and Chen. 15, 1-27)

(60) (Volume Method of Phosphoric Acid Gazz. Chemistry ital. 4, 567.)

(61)

Neumann's[62] method for estimation of phosphorus in organic matter is based on the oxidation of the material with a mixture of concentrated nitric and sulphuric acids, precipitation of the phosphorus as ammonium p phosphomolybdate, and titration of the latter after removal of ammonia, with with standard sodium hydroxide solution, a study of this method has been made with special reference to the influence of the sulphuric acid in the precipitation of the phosphomolybdate.

It has been found that the ammonium phosphomolybdate contains sulphate, and excess of molybdic acid, and no nitric acid , and that its composition may vary with the concentration of the reagents in the solution, the different factors recorded by different observers for the titration of the precipitate with alkali hydroxide are thus explained. The composition of the precipitate for a certain set of conditions was found to be: $4(NH_4)3\ PO_4, 12\ MoO_3$, $(NH_4)\ 2\ SO_4$, NoO_3 .

Among the colorimetric determinations of phosphoric acid, the one recommended by Ponget and Chonchak is especially good. For the preparation of the reagent use the acid sodium molybdate of commerce, $Na_6\ Mo_7\ O_{24}22H_2O$, not the normal salt should be used. To insure the right composition of the reagent, however, the following method of preparation is recommended.

Two solutions A. and B. are prepared, A by dissolving 95 grams of molybdic acid and 30 grams of dry sodium carbonate in 500-600 cc. hot water, cooling adding 200cc. pure HNo_3 of 36 % , filtering and making up to 1 liter, and B. by dissolving two grams neutral sulphate of strychnine in 90cc. hot water, cooling and diluting to 100cc.

[62] (Precipitation of Phosphorus as Ammonium Phosphomolybdate in Presence of Sulphuric Acid-K. G. Falk and K. Sugiura J. Amer, Chem. Soc, 1915,37,1507-1515.

One CC. of B. is mixed with 10cc. of A. shortly before use and the mixture filtered. To obtain the maximum of accuracy with this method two tests should be carried out with 0.2 and 0.3 milligrams of H_3PO_4 respectively, to serve as standards, the one most resembling in tint the actual experimental solution being used for calorimetric determination. Mono-potassium phosphate is recommended for preparation of standard solution of phosphoric acid.(63)

In a new amethod defised by Bay(64) for the estimation of phosphorous in organic matter the substance is burned with magnesium and Na_2CO_3 in "bayouth" tube, product dissolved in dilute acetic acid, and phosphate titrated against solution containing 40 grams uramicin nitrate per liter, $K_4Fe(CN)_6$ used as indicator . Estimation of phosphorous or arsenic in o organic substance is made by preparing a solution by dissolving MgO in HNO_3, of specific gravity 1.38, so that 100cc. of liquids contains 10 grams magnesian substance. If solid it is immersed in the reagent contained in porcelain dish and the mass evaporated to dryness. Gradually and finally to red heat. If the carbon does not burn off readily a second treatment with HNO_3 is necessary. The residue is now dissolved in dilute HCl, and the phosphorous or arsenic is precipitated as magnesium ammonium phosphate or arsenate by adding ammonia.

(63) Phosphoric Acid Colormeteric Det. J- J. Panget and D. Chouchak. Bullitin Soc. China. 1911,9, 649-657.)

(64) New Method of Estimating Pure Organic Compounds I. Bay (Compound revd; 1908,146,804-815.)

For the estimation of lecithin in small amounts of blood, the method consists in the extraction of the phosphatides from blood or serum with warm alcohol-ether, and the determination of their amount by the precipitation of phosphoric acid after washing as the silver salt ,or as the silver-ammonium salt. The phosphate is precipitated by silver nitrate in faintly alkaline solution in the presence of ammonium salts, and the amount of the precipitation measured by the nephelometer. The method has an accuracy of about 2%.

In an experiment performed by J. Merking[65] a brain was submitted to fractional extraction with acetone ,light petrolium, benzene absolute alcohol,85% and ether and the phosphouous determined in the estracts. The experiments led to no method for quantitatively separating the lecithin. Attempts were then made to quantitatively precipitate the lecithin from various solutions in organic solvents by meansof pure acetone, and to which various acids or salts had been added. It was found tnat the egg-lecithin could be quantitatively precipitated from ethereal solution by acetone, if to the latter, a few drops of cold saturated alcohol solution of $Mg\ Cl_2$ had been added. From other solvents and by other methods tried(acetone solutions of tartaric acids etc.) the precipitation was incpmplete.

A method employed by Schiffers for the preparation of lecithin emulsions and determination of their concentration was the use of a weighed amount of lecithin dissolved in the smallest amount of toluene,

(65) (The Method of Lecithin Estimation J. MerkingBio. Chemistry, Zeits. 1909,73, 262-269.)

Enough water was added to give the desired concentration, the toluene driven off by a rapid stream of hydrogen after removal of the toluene, the emulsion is centrifuged and, if necessary, filtered through collon. The suspension so obtained will last two weeks. As not all of the added lecithin is emulsified, the exact concentration is determined by titration of the emulsion. 10cc. are mixed with 10cc. of solution ($K_2 Cr_2 O_7$ 5 grams 38% HCl, 300cc; H_2O to 1 L) in a stoppered bottle and heated at 90° for 6 hours. After cooling 10 cc. of 5 % KI solution are added. This mixture is allowed to stand two hours , and the free I is then titrated with 0.04 N $Na_2S_2O_3$.

(66)

The reason (67) that lecithin cannot be extracted completely with ether is that it is in the colloidad state and is absorbed by the colloidal albumin. This is borne out by the fact that the use of hot alcohol is not necessary for completing the extraction, cold alcohol being sufficient. By extracting with ether and cold alcohol in succession the whole of the lecithin is removed except a very small amount present as phosphatide insoluble in cold alcohol. Extraction of lecithin by ether followed by alcohol is satisfactory provided it is done in the cold. The use of a mixture of ether and alcohol is not reliable in presence of phosphoric a acid; in this case the alcoholic extract should be treated with chloroform and the estimation carried out on this latter extract.

(66) (A simple Method for Preparation of Lecithin Emulsion and Determination of Their Concentration. J. C. Schiffers Ansterdam. Bio. Chem. Z. 40, 187-192.)

(67) (Lecithin (Determination of lecithin in Foods) R. Cohn Z. Chem. 1911, 17, 208-217.)

In spite of the strictures of Hoppe-Seyler, Loew[68] maintained hes assertion that lecithin is not contained in yeast. Hoppe-Seyler approves to have proved its presence and calculated its amount by estimating the phosphoric acid contained in the ethereal extract of yeast.

Neither lecithin nor glyceri-phosphoric acid can be detected in the fatty extract of yeast prepared by a mixture of absolute alcohol and ether. The small quantity of residue obtained contains a considerable quantity of monopotassic phosphate. After removal of this by baric chloride and ammonia, a mere trace of phosphoric acid is found on evaporation and ignition.

According to Collisons'[69] investigations lecithin can by accurately determined by extracting with anhydrous alcohol and anhydrous Et_2O, evaporating the solvents and drying the resulting extracts taking up with anhydridrous Et_2O and determining phosphorous. Strictly anhydrous reagents are necessary. The most satisfactory method found was that in which the combined alcohol and ether extract of tissue are analyzed for phosphorous without previous treatment with ether and filtration, provided reagents are free from water.

Whenever the usual ammonium molybdate method[70] for the determination of phosphorous is employed, to ensure complete precipitation of very minute quantities of phosphoric acid, the ratio of ammonium nolybdate to H_3PO_4 should be 200:1 . Free HCl should first be neutralized with ammonium.

(68) (Detection of Lecithin. O. Loew. P flugers. Arch of Phys. 79,342-6)

(69) (A Brief Investigation on Estimation of Lecithin R. C. Collison. Wooster, J Biol. Chemistry 11, 217-29)

(70) (Estimation of Lecithin R. C. Collison J. Biol. Chemistry 1912,11,217-20)

(Phosphomolybdate Reaction-C. Reichard (Chem Zeit 1903,27, 833-835)

Tartaric, citric and oxalic acids, retard the precipitation although they do not the yellow phosphomolybdate when already formed.

Precipitation in the cold of alcohol lecithin by means of alcoholic NH_4MoO_4 solution acidified with HNO_3 causes the formation of two compounds which differ in regard to the excess of ether of the reacting compounds; thus 10 MoO_3 three molecules lecithin, and 2 MoO_3 1 molecules lecithin. Watery NH_4MoO_4 with alcoholic lecithin solutions in which the lecithin was in great excess gave a compound 5 $(NH_4)_6 Mo_7 O_{24})$ 1 volume lecithin. The first reaction gives a quantitative removal of the lecithin from the solution.

The test for lecithin described by Casanova is not practicable as, the ethereal solution does not mix with the ammonium molybdate solution. It is recommended that a small portion of the substance should by mixed with ammonium molybdate solution, then formed on the surface of concentrated H_2SO_4. If lecithin is present, a blue coloration is obtained immediately.

A review of the physico-chemical investigations of lecithin and the cholesterol show that a 1% alcoholic solution of lecithin is not precipitated by salts of the alkali metals, alkali earth metals cause very slow precipitation. $ZnCl_2$ and $CdCl_2$ five immediate precipitation up to dilutions of 0.01 N beyond which it is very slow. $HgCl_2$ slight precipitation at consentration between 0.2N and 0.05 N. $ZnCl_2$ and $CaCl_2$ slight precipitation at consentration of 0.05 N or less, $FeCl_3$ large precipitation up to 0.002N.

(72) (Molybdate Combinations of Lecithin R. Ehreufeld. Lab. Roysl Tech. H. S. Brum Z. Physiol. Chemistry. 56-(89-98)

(73) (Testing Lecithin. Seidler (Chemistry Zents. 1911,11,1895. from Apoth. Za Zeit. 1911,26,1912-913)

(74) (The Physico. Chemical Investigation of Lecithin and Cholesterol O. Porges and E. Neubauer. Z.Chemistry Ind. Kollvide, 5,193-7-9

Acids give slight precipitation up to 0.0001 N. sugar and mastic give more. An optimun concentration for the precipitate by Fe Cl_3 indicates the action to be one between unlike charged colloids, and theoretical considerations.

The accuracy of Nermamis' method for estimation of phosphorous was modified. The ususl titration of the phosphomolybdate precipitate by boiling off the NH_3 beforetitirating the excess of NaOH with 0.1 N HNO_3. Wales finds that neither Neuman's nor any one of the other modifications give correct results. The amount of $P2O_5$ calcalated from acidimetric titration is in every case too high, the average increasing with the amount of P2 05 present (with 11.25 Mg P_2 O_5present the average is about 1.8%, with 22.3 Mg about 3.4% with 44.5 Mg about 13.2%). Analysis of the yellow precipitate gave 12.75 Mo O_3 instead of the 12 MoO_3 usually written in the formula. The error due to this excess of MoO_3 carried down with the precipitate does not depend on the rate of precipitate and the mother liquor. The error connot be reduced by a lowering of the temperature of the precipitate since this leads to incomplete precipitation.

In the method of phosphorus estimation in lecithin by Freundler[77] two or three grams of lecithin are heated with 50 cc. of fuming nitric acid in a 500 cc. flask on a water bath. After two or three hours the reaction stopped: 25-30 cc. water are added, amd 25-30 grams of powdered permangam-

(76) The accuracy of Nermamis' method for Estimation of Phosphorus. Univ. Sydney. J. Proc. Regular. Soc. N. S. wales, 48, Part 1. 73-93.)

(77) Estimation of Phosphorus in Lecithin P.FReundler. Bull. Soc. Chima. 1912,4, 11, 1041-1043.

ate in portions of one gram at a time. This is allowed to stand . When the oxidation is complete th solutions diluted to 150-200 cc. the manganese dioxide dissolved with sodium nitrate and the whole evaporated to a syrup to expel the excess of acid. The phosphorus is then precipitated, without it being necessary to filter the liquid, with ammonium molybdate in the presence of ammonium nitrate, and estimated in the usual way.

According to Vedch's method the phosphomolybdate precipitate is titrated with N/6 KoH solution. If a much more dilute solution of KOH is used, more accurate results are obtained. The volume of solution when ready for precipitation should not be more than 20 cc. It has been found that 0.5 grams of ammoni n nitrate should be added and for 1 milligram P_2O_5 about 1.5cc. of molybdate solution or 1cc. for quantities less then 0.8 milligrams. Precipitation should be effected at 55°, the mixture kept at this temperature for 1 hour afterwards left for two hours before filtering. KOH used for titrating should not be stronger than 0.02N.

The Pemberton-Kilgore method, which consists in precipitating the phosphoric acid with molybdic acid and titrating the yellow precipitate thus formed, has been submitted to a critical examination by the author, mainly with the object of eliminating certain sources of error in the process. The phosphate solution should contain about 0.02 grams of P_2O_5 per 100cc. and this quantity requires about 15 grams NH_4No_3 and 30cc. of 5 % molybdic acid solution containing 7% free HNO_3 for precipitation.

(78)Titrimetric Estimation of Phosphorus in Small Amounts. L. T. Bowser American Chemistry J. 1911.45,230-237.

(79)The Pemberton-Kilgore Method for Estimation of H_3PO_4P. S. Hibbard J. Jnd and Eng. Chemistry 1913,5,998-1009.

When the yellow precipitate is contaminated from any cause, it may be purified by re-precipitation. Ignition with magnesium oxide is recommended for the removal of organic substances from a phosphate, previous to the estimation of phosphoric acid, and the use of silver phosphate suggested for standardizing the NaOH employed for titration of the yellow precipitate.

An account of various methods imployed for the estimation of phosphorus in animal tissue with the discussion on the difficulty (owing to the rapidity of sudden chemical change) of determining the relative proportions in organic and inorganic combinations at the time of death, is given by A. O. Whither.

The conclusions drawn are: 1, That the determination of organic phosphorus by the barium method gives high results with uncoagulated extracts, owing to the barium phosphate passing through the filter;

2, That at the boiling temperature, water has very little hydrolyz ing action on organic compounds of phosphorus in animal tissue.

3, That enzymes and bacteria have a greater hydrolytic action on organic phosphorus compounds than boiling.

4. That coagulation of the proteins by boiling alears the solution, giving more complete precipitation, and also arrests the actionof enzymes and bacteria.

(80) Estimation of inorganic Phosphorus in Animal Tissues, A. C. Whittier J. Ind. Eng. Chemistry, 1011,3,248-250.

Phosphorus acid may be determined volumetrically according to the method of A. Neuman.[81] The following changes in the method are suggested. In the washing, 20cc. of the acid mixture should be added immediately and during the continuation of the process only concentrated H_2SO_4 added. The precipitate should take place in a volume of about 250 cc. containing 15% NH No , employing ammonium molybdate in not too great an excess(for 10-25 milligrams of phosphorus use about 4 grams). In titration a small excess (0.5-1cc. N/2 acid) should by added, the CO_2 boiled off and the mixture titrated back to the neutral point with N/2 alkali. With small amounts of phosphorus only 10cc. of acid mixture need be employed and the precipitation should be made in a volume of about 50cc. The method with these modifications is very accurate down to 1 milligram.

In precipitating N_3PO_4 by the ordinary magnesia mixture the precipitation is contaminated with a small quantity of basic magnesium sulphate. Heintz recommended that the precipitate should be partly washed then re-dissolved in HCl and re-precipitated by ammonia, and the washing finished. If magnesium chloride is used as the precipitant, and the liqued free from sulphates this second precipitation is unnecessary.

The orginial method consisted in precipitating the solution of the phosphate, containing no free acid but acetic, with a standard solution of iron, $K_4Fe(CN)_6$ being used as indicator.[82] An objection to this method is that the precipitate itself will produce blue coloration with indicator.

(81) Concerning the Alkalimetric Determination of H_3PO_5 according to A. Numan. J. P. Gregerson. Pharm Dest. Univ. Copenhagen. 2. Phosiol. Chem. 53,453-63.

(82) Note on the Estimation of H_3PO_5. An Explanation. W. Heintz(Zeits Chemistry,13, 14-161.

The author[83] substitutes for $K_4 Fe(CN)_6$ potassium sulphoyanate. The test experiments show that the results are as near the truth as those given by the uranim method.

When phosphoric[84] acid is directly precipitated by magnesia mixture in the presence of ammonium citrate from solutions containing organic matter, the results are low. The organic matter as a rule is incompletely destroyed by evaporating with nitric acid, and the substance should be heated at dull redness in contact with air. Immediate precipitation with ammoniun molybdate avoids the error arising from the presence of organic matter. Since NH_4NO_3 is easily soluble in alcohol, Rohen[85] weighs NH_4 phsphomolybdate direct precipitation is made by Worps' method, filtered in a Tolise asbestos filter tube, with suction, washed with Worp's washing solution till the filtrate does not react with $K_4Fe\ CY_6$. To remove NH_4NO_3 he washes with 70% warm alcohol , once with absolute alcohol and finally with a few cc. ether, then dries at 110-20° and weighs as $(NH_4)_3\ PO_4\ 12MoO_3$.

The colorimetric method of Pouget[86] depends on the fact that phosphomolybdic acid forms an insoluble precipitate with alkaloids. A reagent is smade up as follows; 10cc. of 15% $Na_2Mo\ O_4$. 25cc. pure HNO_3, and 1cc. strycknine sulfate saturated in cold water. This reagent turns yellow on standing due to HNO_3 in strycknine.

(83)Modifications of Lelbings'. Vol. Process for Estimation of H_3PO_4 W. W. Shaddart. (Pharm; J Trans. 3. 5,197.

(84)Phosphates from Algeria. Phos. Rock at Bougie having Comp. Compt.rend 1895,121,443-445.

(85)Direct Determination of H_3PO_4 as Ammonium Phosphomolybdate. E. Rahen Kiel, Z. anal. Chemistry 47,546.

(86)Calormetric Determination of Phosphoric Acid. J. Pouget Bull. Soc Chen. 5,194.

3cc. of this reagent are used to obtain the precipitate with a phosphate solution. The sensitiveness is such that 0.005 milligrams in 100cc will give an indication with the above reagent. The reaction is not influences by SiO_2, and the various oxides. The phosphorus can be estimated in iron ores where iron is 1200 times as much as P_2O_5.

By the method of phosphoric acid determination by estimation of phosphoric acid, by Moeser and Frank(87) about 0.5 grams of native phosphate is heated to boiling with 4-6 cc. strong sulphuric acid in a round bottomed flask for ten to fifty minutes. When cold, the mass is extracted 30-40cc of 95% alcohol which completely dissolves the phosphoric acid. In order to render the solution more filterable, 2cc. of 10% KOH are added, which causes a precipitate of potassium sulphate.

From the filtrate(after diluting this with an equal volume of water) the phosphoric acid is precipitated by adding slight excess of ammonia and then, after heating to boiling, magnesium mixture.

Manganese, if present in more than traces, interfers with the process. In such cases a precipitate of the phosphoric acid as the ammonium manganese compound is proposed.

While agreeing with Ogilvie (Chemistry News 31.274) that accurate estimations of phosphoric acid can not be made by the magnesia method in presence of a notable quantity of some salts of ammonia, the Parnell(88) differs from the opinion that an accurate determination cannot be made if large excess of magnesia is used. The "ammoniacal solution of magnesia" must be added slowly with constant stirring in the presence of ammonium

(87) Estimation of Phos. Acid. L. Moeser and G. Jrank Zeits Chem. 1913,52, 346-349.

(88) On the Estimation of H_3PO_4E. W. Parnell. Chem News.32,222.

chloride and precipitation occurs on cooling, showing that the precipitate thus produced is not pure. On ignition, part of the phosphoric acid os lost by volatilization, and the residue is relatively rich in magnesium and poor in phosphoric acid, the errors approximately compensating one another. It is therefore unadvisable to re-dissolve and re-precipitate after ignition.

Action of zinc on solybdernn trioxide in H_2SO_4has been examined.(89) To prevent oxidation by the air, the reduced molybdernn solution was brought into contact immediately with excess of an oxidizing agent, for this is sensibly reduced by the hydrogen evolved. By using a solution of iron alum, the molybdic acid is found to be accurately reduced to the sesquioxide, $MO_2\ O_3$. Addition of phosphoric acid to the ferric solution makes the end point in the titration of the reduced iron with permanganate quite easy to recognize. It is shown that the method may be applied to the estimation of phosphorus after precipitation, as ammonium phosphomolybdate.

In the Marie(90) method of estimating phosphorus in organic compounds, the substance is oxidized by HNO_3 and $KMnO_4$, the H_3PO_4 precipitated with ammonium molybdate, the precipitate washed free from manganese, redissolved in ammonia, and precipitated with magnesia mixture. The ammonim magnesim phosphate should be washed until the filtrate gives no coloration with excess of HCl, a small quantity of ammonim thiocynate, and fragments of zinc.

(89)The Behavior of Molybdic Acid in the Zinc Reductor D. L. Randall American J. Sci., 1907,4, 24,313-16.

(90)Estimation of Phosphorus in Organic CompoundsC. H. Marie. Compt. rend. 1899, 129, 766-769.

15-20 cc. of concentrated HNO_3 are used for each gram of substance, heated on a water-bath and from 5-6 grams of finely powdered potassium permangnate are added in successive small quantities until liquid remains red several minutes. Even compounds difficult to oxidize by Carius' method are readily dealt with in this way.

Another method by Lange[91] for the estimation of nitrogen and phosphoric acid in organic substances consists in taking ten grams of the substance heated with fifty cc. concentrated sulphuric acid and 0.5-1 g gram $CuSO_4$ in a half-leter flask; when the reaction is complete, the flask is filled to the mark, fifty cc. of the solution is mixed with 100cc. Marchen citrate solution and 25cc. magnesia mixture for the estimation of phosphoric acid.

A method is described for the gravimetric estimation of quantities of phosphorus as low as 0.1 milligram.[92] It is a modification of Ibbotson and Brearleys method for estimation for phosphorus in steel. After preliminary oxidation to phosphoric acid the phosphorus is precipitated under certain conditions as ammonium phosphomolybdate. The molybdate in this is then estimated.

Molybdic acid[93] containing is a very sensitive reagent for detection of traces of H_3PO_4; it will detect 0.01 milligrams of P_2O_5 in 10cc. of solution while the limit for molybdic acid containing HNO_3 is about 0.1 milligram P_2O_5 in 10cc.

(91) Estimation of Nitrogen and Phosphoric Acid in Organic Substances. O. Lange-Chemistry Zeit. 12,1587-1588.

(92) The Gavimetric Estimation of Minute Quantities of Phosphorus. Henry Staneky Raper, (biochem J. 1914,8,649-655.)

(93) Estimation of H_3PO_4 particularly in Superphosphate. Zeits Chemistry 1912,56,465-487

To determine whether a method is trustworthy it is necessary to know the solubility of the precipitate and the influence of concentration, temperature, pressure of other substances, etc. The purity of the precipitate also requires investigation. In the precipitation of phosphoric acid with molybdic acid and titration of the precipitation with NaOH solution, the presence of ammonia interfers with the evaporating point when phenalphthalein is used as indicator. It is therefore recommended that the phosphoric acid should be precipitated as potassium phospho-molybdate and precipitate washed with 10% KNO_3 solution containing free HNO_3 (N/100 strength). Small quantity of acid remains in the filter can be estimated and corredtion made.

On the addition of Na_2CO_3 to phospho-molybdemium residues, molybdic acid is readily dissolved. Any metallic oxides precipitable by Na_2CO_3 are filtered off, and magnesia mixture added to the filtsate as long as a precipitation takes place. The ammonia-magnesium phosphate removed sulphuretted hydrogen is passed through the alkaline filtrate. On subseq uently acidulating with hydrochloric acid, molybdic sulphate is precipitated, and can be worked up as usual into ammonium molybdate either by roosting or evaporation with HNO_3 previous to dissolving in ammonia and crystallization.

The following modification is recommended in the determination of phosphoric acid. The phosphoric acid is precipitated in the usual way as ammonium phospharmolybdate, and the precipitate after washing, is treated with water and a measured quantity of N/2 KOH until it is dissolved.

(94) Critical Elaboration of Quantitative Preparation Methods Exemplified by a Method for Est. of H_3PO_4.H. Heicherchain J. Ind. Eng Chem. 1918.10} 426-429.

Fifty cc. of formaldehyde solution which has been treated with 5-6 drops phenolphthalein indicator and titrated until a faint red coloration is produced are now added in order to convert the liberated ammoninia into hexamethylene tetranize and the excess potassium hydroxide is titrated with N/2acid.(95)

In a new method for determining phosphorus in organic matter by Bay(96) the substance to be examined is burned in a tube with carbonates of Na and Mg, and then estracted with dilute CH_3COOH. The phosphorus in the solution thus obtained is determined by titration with a standard solution of uranim nitrate, using $K_4Fe(CN)_6$ as indicator.

In the analysis of citrate,(97) insoluble phosphoric acid, the sample is dissolved as for the estimation of insoluble phosphoric acid, and the solution diluted to a definite volume. An aliqunot portion of the solution is then treated with an excess of ammonia the precipitate formed is collestrol, washed , dissolved in HNO_3 and phosphoric acid estimated in this solution. The ratio between the citrate-insoluble P_2O_5 and the P_2O_5 precipitated by ammonia is 1:1.5. If therefore, the P_2O_5 precipitated by ammonia is divided by 1.5, the quantity of citrate insoluble P O is found. The results obtained by the method agree well with those obtained by the usual method.

(95) Determination of Phosphoric Acid by Numans' method. I. Bang. Brochen. Zeits. 1911,32,443-444.

(96) A New Method for Determination of Phosphorus in Organic Matter. Bay. Compt. rend. 146,1814, April 15.

(97) New Method for Citrate-Insoluble H_3PO_4 C. H. Gerst. (J. Eng and Ind. chemistry 1916,8,251-253)

In the detection[93] of phosphoric acid in acid solution with alkali molybdate solution and the slightly alkaline solution of the molybdate, containing glue, is run into the solution of the phosphate containing definite amounts of NH_4NO_3 and HNO_3 , till after boiling, further addition produces no precipitate. The latter first appears flocculent and contains NH_4, phosphomolybdate and glue, but upon boiling, the ordinary gramular precipitate is formed. The solutions are standardized by means of pure KH_2PO_4 and effect due to acidity is determined. For each set of solutions,100,000 analyses since 1888.

In estimation of phosphorus in organic matter the method[99] of combustion in a current of oxygen, heated with mixture of Na_2CO_3 and KNO_3 , or else boiled with both sulfuric acid and ammonium nitrate, has proven very successful.

In the determination of phosphoric acid to is difficult to remove all NH_3 as required by Neumans'[100] method. This can readily be overcome by addition of CH_2O after re-dissolving molybdic precipitate in 0.5 N, KOH. The solution is then titrated with 0.5N H_2SO_4 .

Phosphoric acid has been estimated by the use of ammonium citrate solution.[101]

(98)Detection of Phosphoric Acid in Acid Solution with Alkale Molybdate Solution and Glue. A. Grete. Turich. Ber. 42,1306.

(99) Estimation of Sulfur and Phosphorus in Organic Material. H. C. Shennan J. American Chemistry Soc. 1902,24,1100-1109.

(100) Phosphorus Detection according to Neumans'. 2 Bang Univ. Lund.Bio. Chemistry Z. 32,443-4.

(101) Preparation of Ammonium Citrate Solution and the Estimation of Insoluble H_3PO_4 P. Me.G. Shuy. J. Ind. and Eng. Chemistry 1947,9,40-45.

Neutral ammonium citrate solution may be prepared by dissolving 1814.37 grams of citric acid in 6961cc. of water and 1960cc. of 28 % ammonia , the water and ammonia being measured at 23°. The insoluble phosphoric acid in acid phosphate may be estimated with practically identical results, whether or not the weighed portion has been washed previously with water, and preliminary washing of water with samples containing cyannide does not appear to be necessary. It may be important to use a neutral ammonium citrate solution in the case of ground tankage, meat grams, fish, and similar materials not strongly acidified.

In Albert Neumans' method(102) for the determination of phosphoric acid in metabolism studies, the material is decomposed by heating with sulphuric acid. The H_3PO_4 is precipitated by $(NH_4)_2Mo\ O_4$ solution and allowed to stand from 12 -18 hours at a temperature of from 50-60°. The yellow precipitate is filtered by suction, washed with water, dissolved in excess of standard NaOH, boiled to expel NH_3 and this excessof alkali, titrated with standard HCL using phenolphtholein as indicator.

The results obtained by the use of sodium citrate solution is the estimation of citrate soluble phosphoric acid as proposed by Bosworth,(103) do not agree with those yielded with normal ammonium citrate solution unless a relatively concentrated solution of sodium citrate (500grams per liter) is employed. More favorable figures are obtained when N/10 citric acid is used as a substitute for ammonium citrate.

The official process for assay of P O in the Boiling point might be improved by the substitution of MgO for PBO at present used.

(102) Comparison of Neutral Ammonium Citrate With Sodium Citrate. N/10 Citric Acid,P Rudindk, W. B. Derby and W. L. Latshew.J Ind. Eng. Chem. 1914,6,486-487.

(103) H_3PO_4 and $(NH_4)_2(PO_4)_3$T. E. Wallis. Pharm J. 85,137.

The purity of $(NH_4)_2HPO_4$ can be rapidly and correctly determined by ignition with MgO. Di-ammonium phosphate of the purity demanded by the boiling point can be prepared; it does not redden blue litmus, and the inclusion of a statement to that effect would exclude many commercial samples deficient in NH_3.(104)

In the method of M. Benoit(105) for estimation of phosphates, the test liquid is made with 68.5 grams crystallized neutral nitrate of bismuth, 200grams HNO_3 at 1.55 and distilled water to bring volume to 1000cc. Each cc. of precipitate a centigram of P_2O_5. A solution of the phosphate in HNO_3 and water is brought to the boiling point, and the bismuth solution added drop by drop, allowing the precipitate of bismuth phosphate to subside after each addition.

When precipitated in the presence of citrate, $NH_4 MOO_4$ is of constant composition and can be weighed on tared filter paper. The facts 0.0374 is used to calculate the phosphoric anhydride.

(104)Estimation of Phosphates. M. Benoit. J. Pharm. Chim. 4. 21,388-393.

(105)DEtermination of $H_3 PO_6$ as Ammonium Phosphomolybdate. H. Pellet. Bull. Assoc. Ahin. Sucr. Dest., 24,525-528.

Some eight years ago and again very recently, Barbieri*, has reported results of experiments which he claims prove the non-existance of lecithin. His arguments are as follows:

"The fatty matter of egg yolk can be separated in a state of purity by the aid of neutral solvents. The nitrogen containing bodies can be removed by simple dialysis, or by repeated washing with distilled water in the presence of a little alcohol. The fat yields, on hydrolysis nothing but glycerin and fatty acids. Glycerolphysphoric acid can not be obtained by treating the egg yolk with a neutral solvent. It appears only after hydrolysis. The Phosphoris appears only in the form of metallic (K, Na, Ca, or Mg) slats of phosphoric acid and is entirely dialyzable. Egg yolk contains no ~~free~~ tract of choline, a supposed biological choline being a product of either the degradation of the ovochromin or of putrefaction."

From these results it would appear that the compound ordinarilly called lecithin is a mixture of fats, phosphates, and dialyzable nitrogenous substances. Such a mixture sould be capable of some separation by ordinary chemical means. Any method of rigorous purification such as that employed in the purification of lipoids, would certainly affect some change in the composition of this mixture.

Without criticizing the argument of Barbieri, some of which, e.g., (the statement that the glycerolphosphoric acid may be formed during the process of hydrolysis from the glycerol of the fat and dilute

* Barbieri, N. A., Comp. rend., 1910, 151, 405;
Gaz., 1917, 47, 1-13; J. Chem. Soc. 112, I.,238.

phosphoric acid) certainly are open to criticism, the following argument is offered for the existance of lecithin.

The works of earlier workers seem to be sufficient to show that lecithin is a chemical substance, even tho analyses of the products from various sources (brains, heart, liver and eggs) did not agree very well. But if any doubt existed as regards the existance of lecithin it would seem that the recent work of Levene and West* proves that such an idea is not tenable. Not only has lecithin, as such, been isolated from the above mentioned sources, but deravities have been prepared and subjected to rigorous purification, always with the same result. The following facts may be mentioned.

Lecithin from various sources such as the promary, alcoholic, extract, the primary ethereal extract, the secondary alcoholic extract, or the fraction disolved in egg oil has been precipitated as the cadmium chloride salt, giving a product of very similar composition. This salt has been purified by crystalization from two parts of ethyl acetate and one part of 80% ethyl alcohol, or by extraction with ether and subsequent crystalization with little or no change in its composition. Furthermore, the salt may be decomposed with ammonium carbonate (Bergell) and the free lecithin again converted into its cadmium chloride salt; this salt will still have the same elementary composition.

* Levene, P. A., and West, C. J., J Biol, Chem., 1918, 33, 111; 34 (in press).

A more convincing proof of the chemical individuality of lecithin is found in the preparation of hydrolecithin. Lecithin (especially those samples which have been washed with water and acetone according to the directions of McLean) is very readily reduced with hydrogen and yields a crystaline tetrahydrolecithin, which may be obtained in a pure form by crystalization from methyl ethyl ketone, and once pure, may be repeatedly re-crystalized, without any change in composition from such solvents as methyl, ethyl ketone, alcohol, or ethyl acetate. If, as Barbieri claims, fats are present, they would remain in the methyl ethyl ketone liquors; our experience in the purification of cerebrosides indicates that this is one of the best solvents for the removal of fats.

We have also combined these two processes. Lecithin has been precipitated from alcoholic solutions by cadmium chloride, the salt decomposed with ammonium carbonate, the free lecithin washed with water and acetone and then reduced with hydrogen. In this way, Levene and West have obtained a chemically pure tetrahydrolecithin.

It is hard to believe that a mixture of choline, glycerides, and phosphates, such as Barbieri claims for lecithin, can be subjected to the above methods of treatment, and give in every instance, a body with identical chemical composition. It is easier to accept the chemical individuality of lecithin.

Lecithin is a regular constituent of the muscles, and it is quite possible that the fat which is difficult of extraction and which is rich in fatty acids depends in part on a decomposition of the lecithin.

The amount of lecithin is not considerable. In normal god heart, as free from fat as possible, Rubow found that the lecithin amounted to 7.5-8.5 per cent of the dry substance; from the strated muscle the amount of lecithin was rather constant, nalely, 5.08%. The ether extract of the heart of the dog contained 60-70% lecithin.

Lecithin is a normal constituent of the liver, the amounts to about 23.5 p. m. according to Noel-Patton. In starvation the lecithin, according to Noel-Patton, forms the greatest part of the ethereal extract, while with food rich in fat, on the contrary, it forms the smallest part.

Samples of lecithin of different origin give the following results on analysis:-

	N%	P%	Ratio N:P
* Distearyl lecithin (Calculated)	1.73	3.84	1:2.22
Com'l lecithin from yolk of egg	2.25	3.49	1:1.55
" " Purified	2.37	3.78	1:1.59
Egg Lecithin, prepared by Wintgen and Keller			
From ethereal extract	2.50	3.69	1:1.48
From alcoholic extract	2.51	3.57	1:1.52

*The liver is generally stated to be the organ where most phosphorus accumulates; the brain and spine of course, appear to be still more important.

* Comp. of Lecithins. N. Wintgen and O. Keller (Arch Pharm. 1905, 244-(3-11).

* Det. of Phosphorus - August Fisher (Pflugers Archiv. 1900, 97, 578-605)

The addition of inorganic phosphorus to the normal diet of the * rabbit or dog lowers the amount of nitrogen retained in the body, altho the nitrogen balance does not necessarily become a minus quantity. With phosphorus-poor food (edestin in case of rabbits; cracker meal, lard, starch, and egg albumin for dog) the addition of inorganic phosphorus decreases the digestibility of the nitrogen and the nitrogen balances are generally neglegable. Organic phosphorus (egg yolk) favors nitrogen metabolism, and increases the nitrogen and phosphorus retention, especially in the case of phosphorus-poor foods. The nitrogen and phosphorus balances do not run parallel in all cases, altho the tendency is in that direction. In no case was there a retention of the added phosphorus whether fed in the organic or inorganic form when given with a food containing a normal amount of phosphorus. Organic phosphorus was never found in the urin.

It is true that lecithin has a very significant biological importance. Sterilized milk was fed to two infants about eight months old for a period of five days. A second period followed during which free lecithin in the form of "Biocithin" equal in nitrogen content to the molk diet of the first period and equal in calorific value by the addition of butter was fed. The nitrogen absorption was 89.2 and 88.25 % in one child and 90.64 and 88.82% in the other; nitrogen retention slight; body weight sixty grams and sixty five grams in the one and plus forty grams

* Metabolism Expts with inorganic and organic phos. J. A. Leclerc and F. C. Cook. J. Biol, Chem. 2, 202-217 Bur. Chem. U. S. Dept. Agri.

and plus seventy grams in the other. The fat metabolism in the* biocithim period was not judged probably because infants do not utilize butter well. The phosphorus utilization was better in the lecithin feeding than in the milk feeding; the one child showed a utilization of thirty and forty five and two tenths percent in the two periods while the other was forty six and five tenths and fifty and five tenths percent.

Small quantities of lecithin (.05 to .10 gram$ administered to a dog scarcely altered the nitrogen and phosphorus metabolism when there was a small deficite in these substances. Larger doces (.5 to .75 gram) caused a sparring action which was small when the nitrogen and phosphorus ingested were insufficient, but was more marked when these elements were in excess of the body needs. A sparring action of the phosphorus was larger than the amount injected as lecithin, and the fact that the injection caused an increase of the nitrogen in the urin at the expense of the fœcal nitrogen, indicates that the lecithin stimulates the degration of the injected protein.**

** Influence of Lecithin on the Nitrogen and Phosphorus Balance. A. Patta (Chem.Zentr., 1912, ii 939-940 from arch Farm. Sperim. 1912, 13, 515-528.

*Significance of Lecithin in Metabolism of Infant. J & W. Cromheim. Berlin. Z Physikol diat. ther., 14; through Zentr. Biochem Biophys., 10, 993-4.

Six experiments on man on the metabdism of calcium, magnesium, and phosphorus and a study of the amounts of these mineral constituents in the typical American diet are reported by Mettler and Sinclair. Aside from nitrogen, the elements of building material which appear to require special attention in dietaries are calcium, iron and phosphorus. Of the various classes of phosphorus compounds found in food the organic combinations appear in general to be of greater mutritive value than the inorganic form, and it is probably for this reason that different experiments indicate quite different amounts of phosphorus as necessary for the maintenance of equilibrium in man. From the results here obtained it would appear that a healthy man, accustomed to ordinary full diet, required for the maintenance of his ordinary store of phosphorus compound about 1.5 gr. of phosphorus per day. Of the dietary study it is shown that not less than 3.5 grams of phosphoric acid should not be eaten, lest under nurishment follows. Experimental dietary studies have shown that it is entirely feasible to increase largely the calcium and phosphorus intake by making a more liberal use of milk or milk products in the dietary. This is probably the simple and more effective means of improving the dietary as regards calcium and phosphorus compounds.*

*Calcium, Magnesium and Phos. in Food and Nutrition. H.C.Sherman, A.J.Mettler, and J.E.Sinclair. U.S.Dept. Agri. Office Expt. Station Bull. 227, 70.

It has been discovered by Heffter that the amount of lecithin present bears a definite relation to the weight of the liver, that an alteration of food does not affect this, but that the propotions decreases during a long fast. Phosphorus poisoning is accompanied by a material decrease of 50% upwards in the qantity of lecithin in the liver and the decrease is the greater the more fatty the liver. Heffter considers that this is occasioned by the direct decomposition of the stored up lecithin.*

The fact that all lecithin can not be extracted with ether from commercial preparations of lecithin and egg yolk may be accredited to an adsorption of this substance by the albumin. The whole of the adsorbed lecithin may be extracted with cold ethyl alcohol; it is not necessary to employ hot alcohol, as subsequent treatment with this liquid only removes tracbs of other phosphatides. If the original solution has been heated, it is not always possible toextract the whole of the lecithin with ether and alcohol or other liquids. Lecithin may be estimated im preparations which contain added phosphoric or glycerolphosphoric acids by extraction first with ether and then with alcohol. The alcoholic product contains some of the added acid and must be re-extracted wiih chloroform to remove the soluable lecithin from these substances.**

*Lecithin in the liver. A. Heffter. Chem. Zentr. 1891 i 495.

** Lecithin. R. Cohn (Zeitsch. offentl. Chem. 1911, 17 203-217)

It is pointed out that hydrolecithin of an elementary composition fully harmonious with the theory may be, and generally is, impure, containing between ten and twenty percent of its nitrogen in the form of amino nitrogen. This finding has a great significance because of its bearing on the structure of cephalin. On the basis of recent work on the hydrolytic products of cephalin, a structural formula has been assigned which requires an elementary composition of C = 66.17, H = 10.17, N = 1.88, and P= 4.17. However, practically all of the analyses from Thudichum up to the present, give an average composition of C = 60 H = 9.3 N = 1.8, and P = 3.8. The elementary composition of lecithin, according to the accepted theory is C = 65.7, H = 10.79, N = 1.74, and P = 3.86. It is argued that if cephalin and lecithin both have the composition required for them by theory, then the mixture of the two should possess practically the same elementary composition as either one in the pure state. On the other hand, if lecithin possessed the same composition assumed by theoy and cephalin that found empirically, then a mixture containing eithy percent of one and twenty percent of the other should possess a carbon content of sixty four percent. Conversly, if a mixture of the two reduced substances possessed an elementary analysis of C = 65.3, H = 11.2 N= 1.75 and P = 3.85, as was actually found, it would justify the conclution that both lecithin and cephalin possess the composition assumed. Material analysized contained eighty percent hydrolecithin and twenty percent impurity and was found to yield on hydrolysis, besides choline, also the base aminoethanol. This was assumed to indicate that the twenty

percent of impurity consisted of hydrocephalin. If cephalin had the composition found by experiment and a substance consisting of eighty percent hydro lecithin and twenty percent of cephalin should have an elementary composition C= 64.56, H = 10.49, N = 1.75, and P = 3.84. These facts furnish evidence in favor of the prevailing theory of the molecular structure of lecithin and cephalin. *

In view of the work of Mott and Halliburton on the importance of recognizing choline as a sign of nervous break-down of nervous tissues, the theory is advanced that the splitting off of choline from lecithin is due to ferment action, but attempts to isolate the inzyme were unsuccessful. The enzyme is destroyed by heating and acts best in a slightly alkaline media. It comes into play during autolysis, but the yield of choline is small. During putrifaction, the yield is large. Pepsin and trypsn fail to act upon the lecithin of brain tissue, and inhibit autolysis. Lipase, however, is capable of splitting lecithin. Of the methods tried, heating lecithin with barium hydroxide was the only one which lead to a theoritical yield of choline.**

*Lecithin I Hydrolecithin and its bearing on the constitution of cephalin. P.A.Levene and C.J.West, Rochefeller Inst. J. Biol Chem. 33, 111-17 (1918); Proc. Soc. Exp. Biol Med. 15, 31-3 (1917).

** Production of Choline from Lecithin and Brain Tissue J. H. Coriat (Amer. J. Physiol. 1904, 12 353-364.

*Three experiments on human beings have shown the retention of nitrogen reduced by the action of lecithin on the organism, is accompanied by a diminution of the amound of sulphuric acid in the urin. This indicates that the administration o lecithin causes a retention of proteids. The diminution in nitrogen extreted during the lecithin period, is mainly due to a diminution of carbanide in the urin. The nitrogen increase is accompanied by retention of phosphoric acid.** The administration of lecithin in animals produces a retention of phosphoric acid which is normally utilized for the development of bone tissues and nervous tissues. The increase of lecithin in the nervous system is not directly due to the lecithin gain but to that synthetically formed in the animal itself.

W. Ludwig prepared nuddles containing one, two, four and no eggs per pound of flower. These were analysed in a fresh state and after drying at 102° and compared with ordinary commercial muddles. The amount of lecithin, phosphoric acid in the commercial nuddles varied from 0.025 to 0.053 percent or from 0.029 to 0.061 percent in the water free substance, while the other water nuddles pepared by the author contained 0.0248 percent and the egg nuddles as follows: One egg, 0.0454; two eggs, 0.0784; four eggs, 0.1504 percent respectively. When the commercial nuddles were previously

*The action of lecithin on animal metabolism. B. Slowtzoff. (Beitr. Chem. Physiol. Path., 1906, 8, 370-388.

** Influence of lecithin on the development of the Skeleton and nervous system. Alexander Desgrez and Aly Zaky. (Compt. Rend., 1902, 134,1166,1168.)

dried at 102° before extraction, the loss of lecithin phosphoric acid was from ten to forty percent while the freshly prepared nuddles showed only from zero to four percent loss. A similar loss in ether extract was noted on heating. Heating the samples or storing them for several months causes a decrease in lecithin phosphoric acid. The loss is very large in water nuddles and comparatively small in egg nuddles. The amount of lecithin is found by alcohol ether extraction and by extracting three times with hot absolute alcohol. *

The close agreement between phosphoric percentage of various samples of protagon prepareg by the most diverse methods is strong evidence in favor of the view that protagon is an individual substance of a well chemical defined composition. Even more conclusive evidence is afforded by the observations of Posner and Gief, that after ten times repeated crystalization, the protagom crystals separating out have the same phosphorus percent as the mother liquor. The view that protagon is a mixture of substances differing in their soluability and in their phosphorus content is not compatible with these results and cannot be accepted until the substances constituting the mixture have been isolated.**

*The effect of heat on the lecithin phosphoric acid of pastes by W. Ludwig. Erfurt Z. Nahr. Genussm, 15, 668-80 June 1.

** On the Phosphorus percentage of various samples of Protagon by A. C. Lockhead and W. Cramer Ph. D. D. Sc. Lec. on Physiol. Chem. Univ. of Edenburgh.

Stenitz showed that feeding with proteids containing phosphorus yields better results so far as putting on of phosphorus is concerned than feeding with phosphorus free proteids plus inorganic phosphate. It was considered desirable to repeat this. Edestin was used instead of the myosin employed by Stenitz. The experiments were made on dogs and Stenitz results are confirmed.*

Koch states that in the changes of phosphatic natrients in the human body that in general inorganic and non protein phosphorus is not utelized. It is possible however that inorganic phosphorus may be utilized if organic phosphorus is excluded from food along time.**

Cholesterin and lecithin extracted from blood corpuscles and suspended in saline requires a definite percent of ether for solution. The absolute volume of a blood suspension is the total volume minus the volume occupied by the corpuscles. If one compares the percent of ether required to lake blood corpuscles suspended in saline, it is found to be the same percent of ether required for the solution of the cholesterin and lecithin of the corpuscles suspended in the same absolute volume. The conclusion may be drawn therefore, that the solution of the lecithin and cholesterin from the corpuscles produces laking of the latter, since both processes require the same percent of ether. Quantitative analysis shows that a small proportion of cholesterin and lecithin is removed from the corpuscles during

* Metabolism with Edestin. Richard Liepziger (Pflugers Archiv. 1899,78, 402-422)

** Changes of phosphatic nutrients in the human body. By E. Koch. Bied. Zentr., 1908, 37, 858 from St. Petersburg Med. Woch., 1906, 400-403.

laking. It is believed that the cholesterin and lecithin removed during laking is extracted from the envelopes of the corpuscles occurs until their substances have been removed from the envelopes. When ether is added to a blood suspension , some of the ether is absorbed by the cholesterin, and lecithin, in the envelopes of the corpuscles.*

Lecithin is readily hydrolized by pancreatic and gastric steastin. This hydrolysis is called forth much more energeticly by pancreatic steapsin than by the liptolytic ferment of the stommach. Plant enzymes and especially that fromthe seed of Ricimuscommunis are likewise capable of splitting off fatty acids from lecithin. Lecithin is not attacked by the liptolytic ferment of blood or blood serum of several different classes of animals.**

A number of analyses of bone marrow for lecithin are given for various classes of animal and for man. The question of age appears to have a signific ient influence upon the lecithin ~~inf~~ content of bone marrow; that of young animals being very high.***

*** Lecithin content of bone marrow of man and animals. W. Glikin Tier physiol. Laudwirtschaftl Hochsch, Berlin Brochem. Z. 4, 235-243.

* Ether laking: A contribution to the study of laking agents that disolves lecithin and cholesterin. S. Peskind Am. J. Physiol. 12, 184-206.

** The Behavior of Lecithin to Liptolytic ferments. S. Schinnoff Sinonowski and N. Sieber. Z. Physiol. Chem, 49 50-64 Chem. Lab. Imperial Inst. Ex. Med St. Petersburg.

The administration by mouth of eighty milligrams per day per animal of egg lecithin beginning at four weeks after birth, leads to no deformation of the curve of growth, the only demonstratable defects of the administration consisting of a very slight uniform retardation of growth and a low degree of resistance to infection, both defects being not improperly attributable to the injurious action of excess of choline absorped from the alimentary tracts. The administration of four milligrams per day of lecithin derived from the anterior lobe of the pituitary body produces similar effects. Having regard to the comparatively small dose administered it is possible that these defects may in part have been due to admixtur of other and more potent substances with lecithin derived from this source and at all events to a peculiarity of lecithin derived from the anterior lobe of the pituitary body. The lack of effect of lecithin administered by mouth in comparision with its effects when administered by subcutaneously or to low organisms is probably attributable to the fact that lecithin is completely split during digestion and not absorbed to any appreciable extent as such.*

The administration of 100 milligrams per day of egg lecithin to the mother slightly retards the growth of suckling mice while one hundred milligrams of cholesterin per day causes a very marked retardation of growth between the nineth and twenty first day after brith. Robertson and Cutler were unable to decide whether these actions represented the direct effect of lecithin upon the growth of sucklings or only an indirect effect due to interference with milk supply.**

*Influence of lecithin upon the growth of white mice. By T. B. Robertson, Univ. Calif. J.Biol. Chem. 25, 647-661 (1916)

**The influence of the administration of egg lecithin and cholesterol to the mother, upon the growth of suckling mice. By. T.B.Robertson and Ethel Cutler, Univ. Calif. J. Biol. Chem 25, 663-667.

Lecithin has a very characteristic color action worth noting. For detection of lecithin the following test is proposed: The solution is heated for a long at 30° to 60°C. to expell any alcohol present, and then extracted with ether and the etherial solution concentrated, treated with two c. c. of ten percent ammonium molybdate and then covered with a layer of concentrated sulphuric acid. In the present of lecithin a cherry red color is produced changing gradually to a green-yellow and deep blue. Cholerterol andphytosterol to not interfere.*

Fresh eggs contain 15.35% lechithin. After the sixth day of development, the lecithin content begins to deminish and by the twentieth day is reduced to one half its former amount. The lecithin of the yolk appears to be a storage of food for the developing germ and is used in the development of the skeltel system, in the building of the phosphorus proteins, and for the liberation of energy thru oxidation of the fat radical.**

An interesting property of lecithin was observed whereby sterile lechithin emulsions were made without any antiseptic precautions. In one to a hundred emulsion of lecithin, typhoid bacilli were rapidly dissolved. The bacilli shrank, became granular and disappeared as in the Pfeiffer reaction. In one to a thousand emulsion this phenomenon occured in the course of thirty to sixty minutes. Practivcal application of these facts proved unsuccessful.

*Lecithin of egg: Char. Color action. C. Casanova Boel Chim. farn. 1911 50, 309-313. Chem Zentr. 1911 2, 231 .

** Quantitative changes of lecithin in developing organisms. By P.G.Mesermizky Russky Wratsch. 1907 No.9.(From Boichem. centr. 6, 784.)

R. Bassenge was able, however, to prepare a useful toxin by making suspensions of agar growths of bacillus typhi in lecithin emulsions.*

In agreement with results obtained by Bischof in 1867, it was found that when the organism was in equilibrium the feces and urin containing the same amount of phosphorus as that of food consumed. Koranuth's results also confirmed Marcuses conclusion as regards the completment of the utilization in the intestional canal of the phosphorus and casein, and that with the deposition of the nitrogen of casein there is co-incidentially, a deposition of phosphorus. In opposition to Marcuses, it was found that the phosphorus of casein is not more completely utilized than that of the other materials even Liebermann's nueclin, but this may be due to different experimental conditions.**

The nature of the phosphorus compound in the fat of feces is not fully understood. It has usually been assumed that this phosphorus exists in the form of lecithin and an approximate extimation of these bodies is obtained through a determination of the phosphorus in the fat. Long and Johnson have examined the fat from the feces of a number o individuals in normal health and have found great variation in the amount of phosphorus present. The limits observed in the phosphorus content of the fats from the feces of several individuals were .20% and 3.66 of P_2O_5. The last value torresponds to 40% Distearyl

* An interesting property of lecithin. R.Bassenge Univ. Berlin. Deut. Med. Wochschr. 34, 139.

** Behavior of phosphorus in feeding. Karl Koranuch.(Bied Centr. 1902,31 605-606.)

lecithin. Several previous examinations of the feces fat of the same individuals give similar higher results, but all this phosphorus could be considered belonging to a true lecithin, is doubtful.*

The liver of dogs wer extracted for lecithin and jecorin. It is noted that extraction of the liver with cold alcohol yields a substance richer in dextros and poorer in phosphorus than is obtained for hot alcohol. Poisoning with alcohol appears to lover the lecithin content of the liver, but leaves the jecorin content unaffected. **

In dogs on a vegetable diet, much of the phosphorus in food is not execrated in the urin; also a large percent of the phosphoric acid injected subcutaneously as sodium phosphate does not re-appear in the urin; in the goat none re-appears, whether it is give in the food or injected under the skin. During lactation in the goat the excretion of phosphorus by the intestines is diminished, but under other circumstances with the animal in phosphorus equilibrium, the absorption and extretion of phosphorus by the intestines are equal. In the dog, during lactation, the phosphorus in the urin is diminished. Goat's milk contains a higher proportion of phosphorus, but less of it is in organic combination, than in cows or human milk. The administration of calcium glycerlphosphate causes no raise in the phosphorus of the urin of the dog, or in the urin or milk of the goat.***

*Phosphorus content of feces fat. J.H.Long and W.A.Johnson J. A. Chem. Soc., 28 1499-1503 (1906). N.W.Univ. Chicago.

**Lecithin and Jecorin in the liver of normal dogs and those poisoned with alcohol. A. Baskoe. Chem. Lab. Inst. Ex. Med. St. Petersburg. Z. Physiol. Chem., 62 162-172.

***Metabolism of Phos. Diarnud Noel Paton, J. Crawford Dunlap, and R. S. Atchison. (J. Physiol 1900, 25, 212-224.)

The assimilation of lecithin was studied in two persons, one with an occluded bowel duct, the other with pancreatic obstruction. Shutting off bile from the intestinal tract results in a greatly lowered use of lecithin or its leimination thru feces. A similar but not as great, increase is noted in the absence of pancreatic juice. The total lecithin is not split by the pancreatic juice, their derivative then being absorbed; but rather is taken up as such.*

The effect of Na phosphate, phytin, and lecithin on the P_2O_5 content of the animal organism was studied. In the urin of the dogs the phosphoric acid the Na phosphate and lecithin appeared almost quant. as inorganic phosphates. Only 30% of the phytin changed in the geces. In man phytin was completely slpit in the disgestive tract, apparently by the intestional bacteria. Since in vitro, the phosphoric acid was split off when phytin was mixed the fecel material. A small amount of phosphoric acid from the phytin was retained in the organism, the rest appeared in the feces as phosphates. Inosite could not be detected in the human urine after feeding phytin.**

The alcohol chloroform extract from the kidney contains from thundred to five hundred times as much phosphorus as the extract from the depot fat. The phosphorus from these extracts was wholly organic in character. Protagon could not be detected even in four hundred grams of the fecel. The most probable compound containing the phosphorus or compounds of lecithin. The barium hydroxide platinic chloride for the separation of chlorine was employed with the following

*Digestion of lecithin during disturbances of the gastro-intestional canal. By. R. Ehrmann and E. Kaupse. Berl. Klin. Woshschr. 50, 1111.

** Phosphorus Metabolism in the animal organism. F. Rogisinski. Anz. Akad. Wiss Kraukau. B. 1910, 260-312.

results:

		Extract (Gms)	P (%)	Lecithin (Gm)	Lecithin in Extract Disteryl Lecithin.
I	(	0.4600	1.43	----	37.23
	(	0.4600	1.47	----	37.45
	(	1.5859	----	0.0650	34.50
2	(	0.6032	1.12	------	29.11
	(	0.6032	1.11	------	28.99
	(	2.15566	----	0.0711	27.40

Assuming that phosphorus content of extracts obtained depended upon presence of some form of lecithin, it has been calculated in the human of certain diseases, the percent of lecithin as follows:- Pneumonia, 6.29; T. B. 4.02; Moderately fat kidneys, 4.76; Beef kidney, 8.21; dog kidney, 7.95; rabbit kidney, 10.96.*

By the artificial digestion and nuclein and lecithin, and by experimenting on dogs with substances containing them, Bokay has ascertained that nuclein appears to be unacted upon by their albumin dissolving ferment of the pancreas, and that at least the greater part of the nuclein introduced into the intesting is not absorbed into the organisms. Lecithin, on the other hand, as was to be expected from its chemical constitution, (On the supposition that it is composed of neurine, phosphoglyceric acid, and fatty acid), is decomposed by the fat decomposing ferment of the pancreas, andone product of its decomposition phosphoglyceric acid, is re-absorbed.**

Young dogs fed on a diet poor in phosphorus, stop growing,

*The lecithin content of fatty extracts from the kidney. E. K. Dunham, Science. 26, 79,80; also proc. soc. exp. biol. med., 1, 39-41.

**Digestibility of nuclein and lecithin. A. Bokay. (Bied Centr. 1879, 112-114.)

wasteand dye*. This, however, is notwholly attributable to a lack of phosphorus. The absence of other unknown constituents of the diet, possibly of lipoid nature, seems to be a factor, as in Stetts experiments. Inorganic phosphate appears to be as advantageous for neutrition as phosphatides.*

The ffeding of lecithin to rabbits increased the content of this substance and of the glycerolphosphoric acid in the liver. The lecithin remains inthe liver some time fifteen days after the stoppage of the injestion. The injestion causes a slight increase in the urin of glycerolphosphoric acid, formic acid, but not choline. The ingested lecithin is mainly absorbed since only a very small incrased is noted in the feces.* *

A yellow color is exhibited by an emulsion of lecithin and water showing the presence of fat, which greatly interferes with the keeping quality of lecithin. Transparent lecithin contains solvent in large quantities; this is frequently not removed because its presence increases the weight of the lecithin and improves the appearance and keeping qualities. Lecithin contains three and eight tenths, to nine one hundredths percent of phosphorus and about 1.7% notrogen. The ratio of P:N is about 31:14 = 2.21. If these elements are present in a ratio differing materially from this, the lecithin is ether impure or adulterated with phosphates, or glycerolphosphates.***

*Phos. metabolism. VI The importance of Phos. in nutrition of growing dogs. Ernst Durlach. Gottingen. Arch. Exp. path. pharm. 71, 210-250. J. Chem. Soc. 104, 1, 311, 12.

**Concerning the deposition of lecithin and its conduct in the organisms. G. Frankhini. Biochem. Z. 6, 710-825.

***Purity of lecithin. M. Morigi - Boll Chem. Farn. 48, 753-756. through Chem, Zentr. 1909 to 2135.

Lecithin has been prepared from ox-heart and hydrolyzed by boiling with 4.5% HCl. About 12% of its nitrogen is in a form insoluble in water after hydrolysis. Of the soluble nitrogen about one half of it is in the form of choline and the other half in the form o amine-ethyl alcohoo The very small amount of ammonia in the hydrolyzed solution is ;robably a contamination. The amount of amine aced nitrogen was also very smalll. Heart lecithin has practically the same composition as does brain lecithin as far as its main constituents are concered so that it is possible that the two lecithins are the same compound. Dehydration by means of acetone was found to be the most staisfactory ethod of preparing the tissue for wok on the phosphatides. *

Egg lecithin has been used to prepare glycerophosphate by Bailly, and was found that this is a mixture of the calcium salts of the alpha and Beta acids. Egg lecithin is therefore a mixture of the two isometrides having the constitution $OH.C_2H_4NMe_3.OPO(OH)O.CH_2.CH(OR).CH_2OR$ and $OH.C_2H_4.NMe_3.O.PO(OH)O.CH(CH_2.OR)_2$ where R is a fatty acid residue, of which the second form pedominates. **

The secréton of P_2O_5 and CaO into the iintestine is increased by food which like milk, contain a great deal of P_2O_5 and CaO; if CaO is given with P_2O_5 rich food; and if P_2O_5 is given with CaO rich food; i.e., whenever P_2O_5 and CaO are present in the organism simultaneously. Secretion through the intestin walls, probably in the form of $CaHPO_4$ takes place in no small amount. In animals also the destribution of Phosphoric acid inurin and feces, within certain limits, is dependent upon food s, while as in man, the feces phosphorus

*Nitrogenous Hydrolysis Products of Heart lecithin G.G.Macarthur F.G. Norbury, and W.G. Karr (J. Amer. Chem. Soc. j. 1917 39, 768-777)

**Constitution of the Glycerophosphoric acid of lecithin. Bailly. Compt. rend. 1915, 160, 395-398.)

is increased when inorganic phosphoric acid and calcium are present together in the organisms.*

Lecithin when extracted from hens eggs and sheeps brain by the Roaf and Edie method and administered by subcutaneous injection or by way of the stommach into tad poles, sea urchins, rats, and guinne pigs produce no positive proof that it acted as a stimulant.**

The lecithin of the fowl's egg increase the appetite of animals which receive them either by the mouth or under the skin. These animals rapidly increase in weight. Urea, total urinary nirogen, and the coe - fficient of utilization of nitrogen are increased, but the phosphoric acid in the urin is diminished.***

Some feeding experiments in which goays received, in addition to straw, blood nuclein, starch and oil, the following substances as sources of phosphous ; phytin, lecithin, casein, nuclein, nucleic acid and disodium phosphate. The food was mixed with molasses to make it palatable.

The results show that there is no essential difference in the utilization of the different forms of phosphorus. The imperfect assimilation of the phosphoric acid, of crude foods, must therefore, be due to other causes.#

Utilization of Calcium andphosphoric acid compounds by the animal organism. C. Fingerling (Landw. Versuchstat., 1913, 79,80)

*A contribution to the question of phosphoric acid and calcium maetbalism in normal adults. F. Oeri, Med. Klin. Bade. Z. Klin Med. 67, 288-306.

** Effect of Lecithin on growth. A. J. Goldfarb. Arch. entwickl. Organ. 29, 255.

***Influence of Lecithin in the egg in nutritives exchanges. By Alexander Desgez and A. Zaky (Compt. Rend. 1901, 132, 1912-1514.

The lipoid phosphorus and iron of the blood in polycythaenia rubra was investigated by Glikin. 40 to 65 cc. of blood from a case polycythaemia rubra were ground with qarts sand, dried, and extracted with alcohol and chloroform. The dried extract was re-extracted with petroleum ether andphosphorus and iron determinations performed upon the dissolves substance. The P_2O_5 content calculated for one leter of blook was 1.765 and 2.291. gr; the Fe_2O_3, 1.786 and 2.069 gr. The phosphoris indicates the lecithin content much above normal, doubtless because the lecithin, being a constituent of the cells, increases in proportion with them. The iron anglyses show that 6% of the total iron in blood is contained in the lypoids.*

In lecithin and nucleic acid, and probably in other organic phosphoric acids preparations, the phosphoric acid as soon as its freed from combination, follows the same laws of excretion as the inorganic phosphate. When calcium is in the flood, it combines with that and is excreted through the intestines; the calcium is lacking, the phosphoric acid is excreted through the kidneys.#

A contribution to the qquestion of phosphoric acid and calcium metabolism in the adults. F. Oeri, Z.Klin. Md. 67, 307-18.

*Boil. Significance of lecithin. IV. The lipoid phos. and iron of the blood in polycythamia rubra. Megalosplencia. W. Glikin. AGr. Hockschr, Ber. Boil chem. Z. 22,460-3

A change was found in the chemical composition of bone marrow of paralytic expressed by a disappearance or decrease in the amount of lecithin normally present. This was accompanied by a corresponding loss in the iron content of the marrow fats.*

White rats which receive lecithin by either injection or feeding, gain in body weight more rapidly than those which do not receive it, the gain in the experimental rats being, on the average, 60% greater than in the normals and controls; the relative weight of the central nervous system in the lecithin rats is normal; the nervous system in the experimental rats contains the same proportion of solids and water as in the controls; this is another indication of the normal character of the growth; the relative area of the axis cylinder to its sheath in the nerve fibers of the experimental animals is approximately the same as that in the controls, showing that the peripheral nerves have also grown normally; the rats which received the lecithin show a greater power of resistance against diseases and the unfavorable changes in the surroundings; the present investigation confirms strongly the previous observations of Danirlewsky, Desgrey, and Zaky, and others who claim the physiological effect of the lecithin to be that of a stimulating agent for normal growth.**

*Biol. Important of lecithin. On the lecithin content in degeneration of the central nervous system. W. Glikin. Tierphysiol Inst. d. Land wirtschaftl Hochs Berlin. Biochem. Z. 19, 270-3

** The effect of lecithin on the growth of the White Rat. By Shinkishi Hatai, Am. J. Physiol. 10, 57-67

Experiments on the reduction of egg lecithin have been carried on by C. Paal and H. Oehme. Lecithin purriss, ex ovo in alcohol and colloidal, Pd. in water (the amounts of alcohol were so chosen, 90% alcohol) that on mixing both the lecithin and collcidal solution it remained in solution, absorbed 58.7, 59.4 cc hydrogen per gr. lecithin, yielding a hydrolecithin, microscopic qubes more difficultly soluable than lecithin itself, sinters 83-4°C, decomposes above 150°C, iodin number 313, boilcd two hours with three molecules barium hydroxide in methyl alcohol, it gave phospheric acid, glycerol, choline, and a mixture of fatty acids, which, judging from the melting point and analyses of fractions obtained by repeated crystalization consisted chiefly of stearic and palmitic acids, with an acid of lower molecular weight, posibli muristic, lauric, or caproic acid, as well as a small amount of unsaturated acid which had excaped reduction, probably oleic. These results indicate egg lecithin is not homogeneous and mush consist of at least two different lecithins.*

Growing dogs of the same size were fed with phosphorras-poor food containing phosphate and phosphatide. The removal of phosphorus from the diet manifested itself in the loss of weigh in sickness. The dogs fed with lecithin phosphorus appeared to outlive those with phosphate phosphorus. However, there remains the posibility that in the lecithin increase such substances related to oryzanine, or to vitamines, play a part.**

*Reduction of Egg lecithin. C. Paal and H. Oehme. Univ. of Leipzg. Ber. 46, 1297-1304.

** Importants of phosphorus in the food of growing dogs. VI Ernest Durbsch. Gottingen Arch Exp. Paph. Pharm. 71, 200-50

39

The work of Fingerling and Gregerson is confirmed that the animal body and does synthesize its organic phosphorus compounds from inorganic phosphates.*

Lecithin was prepared by MacLean from three different portions of eggs. The method adopted was essentially the same as that adopted in my former papers, and need not be repeated; in every case (with one exception) I used the lecithin obtained from the thereal extract of egg yolk, the usual precautions being used to exclude as far as possible air and light during the process of preparation.

In one set of eggs a curous state of affairs was noticed: These eggs (100 of them) seemed to be perfectly fresh and were all rather large, it being naturally thought that these would yield a larger yield of lecithin. On extraction, however, it was found that the eggxs showed quite an abnormal increase of fatty matter, but only a trifling amount of substances precipitated by acetone: after treatment with five consecutive protions of fresh ether, the extract still continued much fat, and the combined yioed of lecithin was so small that after purification the total amount yielded to only a few grams. On subsequent extraction with alcohol the lecithin was also very much below the normal average.

In this particular case it would seem as if there was a great increase of fatty matter at the expense of the lecithin: a curous point with regard to the lecithin was that it differed greatly in appearance from lecithin obtained from other eggs: it was from the beginning quite dark brown in appearance and not so plastic as

* The metabolism of organic phosphorus compounds. Their hydrolysis by the action of enzynes. R. H. Aderz. Plimmer (Bio. Chem. J. 1913, 7, 43-71)

is generally the case; despite that fect tha the greatest precaution was taken to prevent oxidation: in general it looked more like a specimen that had been exposed for some time to air then th freshly extracted material, which is usually precipitated as a plastic, more or less whitish, mass with a sight brown tinge. The amount of lecithin here obtained was so small that it was not made use of for this experiment.

The first sample purified gave the following figures on analysis: Comparison with heart muscle lecithin shows a marked similarity in elementary composition:*

	Egg Lecithin	Heart lecithin.
N (3 Expts.)	Av. 1.876%	1.87%
P 2 "	3.95	3.95
C 1 "	64.18	66.29
H 1 "	10.6	10.17
N:P	1.05:1	

*(Distribution of Nitrogen in Lecithin-Maclean 1915, 9,365. Bioch. Journ.)

At first attempts were made to estimate the amount of nitrogen obtained by Van Slykes methods (1912) using the unhydrolyzed method. These experiments were rendered difficult, partly on account of the frothing which resulted and partyl account of finding a suitable solvent for the lecithin. The solvent used was strong acetic acid but it was found tht after the preliminary shaking o the sodium nitrite and acetic acid that accordi g to Van Slykes instructions, the addition of acetic acid acted in such a way as to give a good deal of gazs which was not taken up by the permanganate absorbing, mixture. This introduced an error which had to be allowed for by control, but even then the results were not satisfactory. In spite of these

disadvantages the numbers obtained are very suggestive. On hydrolyzing the lecithin and using the liquid containing the soluble products of hydrolysis, no difficulty whatever wasexperienced. The cholin3 3stimated by hydrolysis with weak acid followed by the steps already described.

(On the nitrogen containing radicalof choline and other phosphatides - MacLean, 240, Vol. IV, Biochem. J.)

Observations show that alcohol is a more suitable solvent than ether for extracting lecithin from tissues. From this reason alcohol is generally used, the usual prodedure being to dry the tissues as quickly as possible, grind to a fine powder and extract thoroughly with various changes of the solvent, using a shking machine. On evaporating the alcohol from the combined exyracts the residue is taken up with ether, and the phosphatides precipitated with the addition of an excess of acetone to the theral solution. By repeating the process several times all acetone soluble bodies such as cholesterol and fatty acids are removed. The final residue is supposed to consist almost entirely of phosphatides and this is divided into "lecithin" and kephalin by fractionation with alcohol. The insoluble part is considered as kephalin the alcohol-soluble as "lecithin".

In every case, however, in which this method is used as a basis for the prepartion of phosphatides, the resulting productss are far from pure and the lecithin fraction often contains as much as 50% of an extraneous substance. This substance is soluble in water and the treatment of the lecithin fraction by means of acetone and water, it remains in the acetone water solution. On evaporation of this solution, the substance is obtained as a sticky syrupy mass which is

insoluble in alcohol and absolute ether but easily soluble in alcohol containing a trace of water. It is exceedingly soluble in water: on standing some days in aqueous solution gradually deposits small round white crystals. These crystals are insolublein cold water, but dissolve in boilding water to be re-recepitated on cooling water. After recrystallizing in this way several times, a substance or mixture of substances is obained which has a very high nitrogen content and widely belongs to the putine group.

In one case small white concentric crystals were isolated which had all the properties of carnine. After drying at 105°, analysis showed th t the substances contained 28.35% nitrogen. On heating, they changed color about 230° and leackened about 240° with silver nitrate or white flocculent precipitate was obtained which did not dissolve in ammonia or nitric acid. A precipitate was also given which lwad acetate and with mercu ric chloride, while neither neutral lead acetate nor merciric nitrate gave any result. In its properties and nitrogen contant this substance appears to be idential with and closely related to the basi c carnine found by weidel in American meat extract. In another sample another small amount of a substance which appeared to be impure hypoxanthine was isolated; it contained about 40% nitrogen. The mother liquor from which these substances separated is distinctly acid in reaction, and it is probably that these bodies are set free by a process of decompsotion and are not present in the free state of the original liquid. This is suggested by their extreme insolubility in cold water and the difficulty experienced in dissolving them in the mother liquor after separation has once taken place. The fact that complete separation

tak days or even weeks is also suggestive in this connection. Owing to the high nitrogen percent in these bodies it is obvious that the small quantity present in lecithin would materially influence the N:P ration.

When the separation of these bodies is complete, an absolutely straw colored liquid is obtained which remains clear for a long time.

On evaporating a sticky hard gum-like substance appears. This substance is soluble in alcohol containing a trace of water and appers to dissolve in solutions of lecithin. Further, like phosphatides in general it is precipitated by acetone, by cadmium chloride and by platinum chloride. The body appears to be a very complex nature and so far it has been impossible to determine its chemical constitution. Different specimens contain on an average about 6% nitrogen, and there is generally a small amount of phosphorus present, though the latter may not amount to more than a trace. When tested by Van Slykes method a considerable paryt of its nitrogen was found to be present in the amino form. On hydrolysis some specimens yielded a very small amount of fatty acids, but this was probably due to the contamination with lecithin, for a specimen has been obtained recently which has on hydrolysis with HCl, given no trace of fatty acids. It decolorized Fehlings solution on heating, but no precipitate occurred: on prolonging the boiling whoever, a dense precipitate occured.

Nitrogenous Impurity associated with Lecithin - MacLean (Bio chem. 1915, 9, 353Observ.

Since it has been supposed that the peculiarities in the behavior of cells to certain electroytes and organis ions are due to a cell membrane which is permeable to some and impermeable to others, and that this membrane is compsed of, or consists essentially of lecithins or of other lipoids, which donfer upon it these peculiar properties of selective permeability and impermeability, it was considered of some importance to prepare some membranes in the presence of parchment paper and coating its surfaces, and to test whether such a membrane showed similar permeabilities to those supposed by many authors to be shown as cells, and, is so, whether osmotic pressure would be developed when ions to which the membrane is not permeable or present in different concentrations on the two sides.

Both lecithin and lanoline membranes were prepared and tested and it was found that these were readily permeable and that no trace of osmotic pressure was develpped.

This demonstrates, in the first place, that a lipoid membrane, does not furnish any explanation of the osmotic properties of living cells, and, secondly, that the presence of absence of an inorganic ion in the cell, or variations in concentration of such an ion inside an outside of the cell, are not to be described to a barrier opposed by such a membrane, but that the explanation is rather to be sought in the properties of the cell constants themselves, for combining with or absorbing such constituents of its inorganic invironment.

The lecithin was prepared by the method described by Roaf and Edie and dissolved in the smallest portion of ether, as possible. The discs of parchment paper, cut to the sixe to fit the osnometer, dried at a 100 C. and cooked in a dessicator, were dipped in this strong lecithin solution, and the ether allowed to evaporate off; they were then dipped a second time and allowed to dry again; in this

manner, the parchment paper is thoroughly soaked with lecithin, becomes translucent, and is coated wi a thin layer of lecithin on both surfaces.*

Since the investigations of Diacanow and Streker,[1] it has been generally assumed that lecithin is a compound of fatty acids with glycerophosphoric acid and a base choline. This assumption is based on the assumption that on the results of elementary analysis combined with the fact that hydrolytic decomposition of the lecithin yields the above mentioned constituents. From this it is obvious that the total amount of nitrogen present is represented by the nitrogen of the choline radical and in this way a knowledge of the total amount of nitrogen present yielded by any pure lecithin present makes it easy to deduce the amount of choline ($C_5H_{15}NO_3$) actually present from a theoretically standpoint. Many experiments have been made in order to choline content of different lecithins, but in every case the results actually obtained fell far below the theoretical values. Thus Erlandsen[2] obtained from pure heart lecithin, which had been split up by boiling with barium hydrate, only about 42% of the actual amount, and Heffter[3] using lecithin extracted from the liver, obtained under similar conditions only 25% .**

The fact that lecithin has been shown to consist of two components throws some light on the difficulties experienced by many investigators when endeavoring to ascertain the nature of the fatty acids present. Theoretically only two acid radicals exist in the molecule, and separate and identification ought to be easy, but

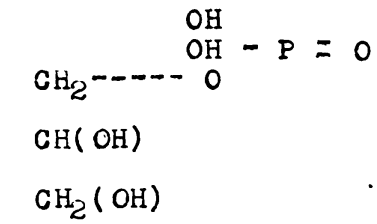
*Lecithin Membrances - Moore and Roaf, 1906 - 7, 269. Riochem. J.

** Phdrolysis and sponification of letithin (MacLean) 1908 9, 4, 38, 42, Biochem. Journ.

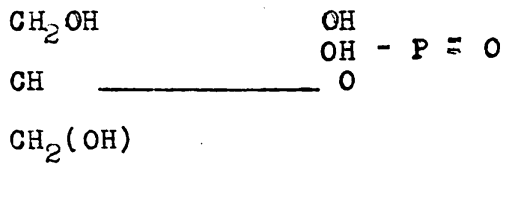
generally mixtures are obtained which appear to consist of more than two acids.

Glycerophosphoric acid. From the chemical standpoint this acid can exist in two modifications, the alpha and beta forms.

Alpha form (Unsymetrical)



Beta form (Symetrical)



The alpha form contains an asymetric carbon atom and is therefore optically active while the beta form is inactive.

After the discovery by Willstatter and Ludecke (1904) that the glycerophosphoric acid of egg lecithin was optically active, rotating plane of polarization of polarized light to the left, it was generally assumed that the acid present in lecithin as alpha glycerophosphoric acid, through Tutin and Hann disputed this and held that glycerophosphoric acid consisted of a mixture of the alpha and beta forms. Recently Grimbert and Bailly claim to have definitely shown that egg lecithin is a mixture of at least two isomers containing the symetrical and unsymetrical forms of glycerpohosphoric acid.

It is possible that the true lecithin part of "lecithin" containing all its nitrogen as choline, may have one form of this acid, while the kephalin part may have the other part. *

*Consiitution of lecithin - MacLean - 1915, 9, 376. Biochem. J.

Mac Lean has shown that the so-called acetone soluble phosphatide content of the heart of the ox is impure lecithin. The lecithin can separated from accompanying fats bythe addition of a small amount of electrolyte such as calcium chloride to the solution. The precipitate when purified has all the reactions of lecithin and is insoluble in acetone.+

++ Bickel states that commercial lecithin preparations were fed to a man, and the nitrigen and phosphorus balance being determined. The results showed that lecithin phosphorus was reabsorbed and deposited. Similar experiments indicated the P_2O_5 retention produced by feeding lecithin was not accompanied by a calcium and magnesium retention.+++ Lecithin fed to adults increased the assimilation og nitrogen and phosphorus, but not the digestibility of fat. Moderate quantities of lecithin appear to be desirable addition to the diet.++++ In insanity with depressant maniacal symptoms the nitrogen and phosphorus metabolism vary according to the acute or chronic stage of the disease; the elimination of nitrogen and phosphorus is increased in the initial stage of the dementia praecox and decreased in the chronic stage.+++++

+ So-Called Acetone Soluble Phosphatides. H. MacLean (Biochem. J, 1914) 8, 453-459.

++ Lecithin Metabolism, A. Bickel. Path. Inst., Univ. Berlin, Intern. Beitr, Path. Therap., 3, 11-19.

+++ Influence of Lecithin upon the Calcium and Magnesium Excretion. A. Loeb. Path. Inst. Berlin. Intern. Beitr. Path. Therap., 3, 255/251.

++++ Importance of Lecithin in the Metabolism of Adults. W. Cronheim. Zentr, exp, Med., 2, 121.

+++++ Balance of Lecithin, Phosphorus and Fats in Mental Diseases. H. Mizzi. Encephale, 1912, 245. Zentr. Berlin. Biochem. Biophys. 14, 340.

It is practically certain that some of the ductless glands contain phosphatides,as the work of Finger has proven.Light petroleum extracts relatively large quantities of phosphatides and fats from the pituitary,supra renal,pineal and thymus glands,and the corpus luteum than from the ordinary muscle tissue.The thyroid gland ,on the other hand,contains about the same proportions of these substances as the muscle.The conclusion is that the phosphatides are concerned in the operation of most ductless glands, but not in the elaboration of the escretion of the thyroid.+

Macro and microscopic examination of the skeleton of dogs showed that by feeding with phosphorus poor food,the growth of the bones is influenced in the sense of the decrease formation of brain substance.Since this food varies considerably from normal food,the view of Durlach must be modified to include the possibility that bone disease caused not only by a lack of phosphorus but also by other substances.++ There has been found to be an unequal distribution of phosphorus in the different organs of mammals,being more in the brain than in the kidney,which in turn contains more than the liver+++

++++ The phosphate metabolism is influenced by the proteins and calcium in the diet,and also by other factors,as was discovered by Goldfarb,who found that the influence of lecithin on the growth of tadpoles,had a slight influence on their growth but not marked.+++++

+Phosphatide in the Ductless Glands,F.Fenger(J.Biol.Chem.1916,27,303-7)
++The Influence of Phosphorus Poor Food Upon the Growth of Bone-GEO.S.Dresden.Arch.exp.Path.Pharm.,73,313-346.
+++Phosphorus and Sulphur in Foods.Balland.Lab.Cour.L'.Intend.Rev. intend.mil.,20,181-210.
++++The Metabolism of Calcium,Magnesium and Phosphoric Acid.Preliminary Communication.M.Kochmann.Pharm.Inst.Mine.Greifswald.Biochem. 2,27,85-86.
+++++Influence of Lecithin on Growth.A.J.Goldfarb.Lab.Biol.Zoo.Chem. Coll.Phys.and Surg.N.Y.City.Pr.Soc.Exp.Biol.Med.,June 22-1907.

Phosphorus is of much greater importance both for the growing and adult organism. We find phosphorus in the cells in the form of very important compounds, namely lecithin, the nucleins and nucleoalbumins. We know, furthermore that phosphorus combined with the alkaline earths forms one of the most important constituents of the human skeleton, and is also presetn in the same form in othe tissues. Phosphorus is present in milk, partly in organic combination, as in casein which belongs to the group of nucleoalbumins, and partly as inorganic salt. Milk also contains some lecithin. At present it is not known exactly how the phosphorus is distributed between these different compounds in the different kinds of milk. Apparently the amount of lecithin present is not very large.

There is no reasonable doubt that the living organsim can utilize phosphoric acid directly in the formation of lecithin. It is similarly possible that it forms a part of its nucleins from the latter substance. The fact that the animal organism can form lecithin from phosphateswithout difficulty is apparent from the experminents already cited of Miescher upon slamnn.

Phosphorus is especially important in the construction of nervous tissues. The brain of a new-bron infant weighs about 400 grams. This weight is doubled during the period of lactation. According to Schlossmann's computations, the bursling assimilates during this period for the building up of its central nervous system along about .75 gram of phosphorus. The skeleton reqires much more of this element. In fact, if we estimate the totalamount ofphosphorus required by the infant during the first year of its life, we shall find that it amounts to from

fifty to sixty grams. The amount of phosphorus required in the food is naturally even far greater, because in the above estimate it was not taken into consideration that phosphorus is constantly being eliminated in the form of phosphates. In one liter of human milk there is present 0.19 gram, and goat 'smilk 0.96 gram. Human milk, therefore, is deficient in phosphorus; it contains less than any of the other kinds of milk which have been analyzed. This is a remarkable fact, for we know that the human offspring is able to construct, while still nursing a nervous system which is but slightly developed. Compared to human milk, that of the above animals is extremely high in phosphorus. There must be some reason for this difference. Bunge, who oiced this fact in his analyses of different kinds of milk, compared the percentage composition of the ash with the rate of development of the species. It is to be assumed a priori, that an animal which develops rapidly will require more building material than one whose development is slower. If we compare the time required by the suckling to double its weight at birth with the amount of albumin and ash - perhaps the most essentail constituents for the formation of the tissues - contained in 100 parts of milk, it is evident at a glance that the amount of these increases in proportion as the development of the animal is rapid.

E X P E R I M E N T A L

The animals used in the research were three dogs, three cats and three guinea pigs. These animals were all healthy and in growing condition. The dogs used were all of about the same size, although one was somewhat smaller than the other two, all three, however, being young dogs. Two of the dogs were used for the experimental work proper while the other was used as a check on the first two. The diet ed the first two dogs consisted chiefly of calf brains as the source of its meat. In addition to the calf brains, was given bread scraps, vegetables of various kinds and water and milk to drink. Food scraps from the table were fed, which consisted chiefly of bread and vegetables. The third dog used in the experiment was fedexactly the same foods as the other two with the exception of the calf brains or foods of any kind which were unusually high in phosphorus and lecithin content. The conditions of living, care and treatment of all three dogs were exactly the same.

The cats used were all young healthy animals and were of about the same size and in the same state of development. A diet similar to that of the dogs was given the cats, and as in the case of the cats, two were used for the experimental work proper while one was used as a check , being grown under normal conditions and with no unusual amount of food rich in phosphorus and lecithin in its diet. The diet of the first two cats consisted chiefly of calf brains, milk, water, bread and a few vegetables. The control cat was fed exactly the same food with the exception of the calf brains. Some egg yolks were also fed to the two experimentalcats, although the calf brains furnished the chief source of phosphorus and lecithin. All three cats lived under exactly the same conditons, and care and treatment being just the same for all.

Three guinea pigs were also used in addition to the cats and dogs, and as mentioned above, two were used fo the experimental wo rk proper, while the other was employed as a control or check. The animals were young, healty and in a rapidly growing state. Their diet consisted chiefly of scraps of bread, vegetables, and cooked egg-yolks, with the exception of the control animal, whose diet lacked the egg-yolks, or other foods rich in phosphorus and lecithin. Egg yolks were fed in addition to the calf brains, as the guinea pigs did not seem to have as great desire for the calf brains as did the dogs and cats, although some calf-brains were eaten by the guinea pigs. All three guinea pigs were raised under similar conditions as to care, treatment, and surrounding conditions.

Calf brains and egg yolks were fed the animals three times each day for about seven months and a half, the feeding being started on September 19th, 1920 and ending on May 1, 1921. At the end of this time the animals were chloroformed and their brains immediately removed without destroying any of the brain tissues, being careful to keep the brain in tact and whole as much as possible. The brains were then finely minced, dried and analyzed for phosphorus and lecithin as described later. Fresh calf brains were bought each day, and about three pounew fed to each dog daily, while the cats received approximately two pounds each day. The amount of brains fed the guinea pigs was not more than half a pound each day, the lecithin rich food consisting principally of cooked eg yolks, of which they were feed

about three apiece each day.

Analysis of Calf Brains.

The calf brains used as the food rich in lecithin were analyzed at various times and the mean average of five different analyses considered as the per cent of phospnorus a dlecithin in them. Knowing this, it is possible to calculate how many grams or pounds of lecithin and phosphorus were fed daily to the animals. The results of the five separate analyses checked within a very close limit of error.

The method used for the analysis of the brains for determination was as follows:

Apparatus: - A Hpkins condenser was used to which a 250cc fat-extraction flask was connected. The flask was sealed from action of the air by using mercury in the sealing cup. From the return tube of the condenser a porcelain Gooch crucible of about 15 cc capacity was suspended by means of two platinum wires. The crucible hung within about an inch of the bottom of the flask. This apparatus was set up in triplicate and heated on an electric plate.

Procedure of Analysis of Calf Brains: - The calf brains were freed from blood by washing with cold distilled water. The brains were ground several times through a mincing machine. They were then weighed into an Erlenmyer flask (10 grams being used for analysis). The remaindor of the brain sample was put into a glass-stoppered 250cc volumetric flash and 60cc of absolute alcohol added. On starting the analysis, the material was heated in the Erlenmeyer flask to just below boiling $(78.4)^{\circ}$ with 60cc of ethyl alcohol for thirty minutes and then transfered to the Gooch crucible suspended by platinum wires from the return tube of the condenser. The bottom of

the Gooch crucible was covered with filter paper. The filtrate was allowed to drain into the 250cc flask belonging to the extraction apparatus, and the brain tissues were then extracted for eight hours in the extractor. 25cc more of alcohol was added during the process of extraction. At the end of eight hours the alcohol in the flask was gently evaporated and then 50cc of ether added. The extraction with ether continued for eight hours. The residue in the Gooch crucible was removed carefully by means of a fine spatula and chamel's hair brush. The residue after being transferred to a morter was gound up very fine, transfered again to the Gooch curcible and extracted six hours with alcohol, and four hours with ether. The alcohol and ether were then evaporated and treated as described below:

Emulsification and Precipitation of Lecithin:- The last traces of alcohol or ether were removed by gently heating from the alcohol ether residue which was ectracted by the alternate extractions of alcohol and ether extractions described above. Without removing the residue from the extraction flask, about 40cc of distilled water was added and allowed to stand about twenty four hours. Longer standing would endanger decomposition by bacteria. The brain material, after this preliminary softening emulsified very readily and was then transfered to a graduated 100cc flask with the addition of as little waser as possible. The addition of 1-2 cc diloriform and thorough shaking assisted very materially in removing all the material from the sides of the flask. A glass rod with rubber on the end also proved of great value. By the time that everything had been removed from the extraction flask there remained 90cc of liquid in the graduated flask. This was thoroughly shaken up, 1/2 cc. conc. hydrochloric acid and 2 - 4

cc. chloroform added, the whole was shaken up and made up to 100 cc graduation. The amount of acid added was left as low as possible in order to prevent danger of hydrolysis. With such tissues as muscle very rich in fat, the addition of at least 2 -3 cc. of acid is absolutely necessary for complete change of the supernatant liquid. The danger of hydrolysis of lecithin however, is rather slight. The complete settling process required about five to six days. The time of complete settling depends a great deal upon the complete evaporation of the alcohol; if the alcohol has not all been removed from the residue the time of complete pricpitation may be longer than two weeks. This is the main reason why the alcohol should be completely evaporated.The precipitate will hereafter be designated as the lipoid precipitate,as it contains akk the fat-like substances,such as cholesterin and cerebrin.In case of nerver tissues,such as the calf brains for example,it also contains the sukphur compound probably in combination with kephalin.The clear solutuion above the lipoid precipitate contains all the water soluble extractives,inorganic phosphates,phosphorus in simple organic combination ,and inorganic salts.Thus the lecithins are obtained practically free from all other phosphorus compounds.The precipiitate was washed free witha solution of 1 per cent hydrochloric acid to remove all soluble phosphates which adhere mechanically.

SEPARATION OF THE KEPHALINS.

The lipoid preciptates after having completely settled,the supernatant fkuid was carefully decanted through a 8 cm.,ashless filter paper,and the precipitate was washed by shaking in the flask with 10 cc of water containing 1%by volume of strong hydrochloric acid. The pre-

cipitate settled again in a few minutes and the wash fluid was also decanted through the filter. The precepitate in the flask was dissolved in hot alcohol, the solution transfered to a cleam 500cc long necked Jena flask, the glass stoppered flask, and the filter paper, through which the dilute acid solution had been originally filtered, thoroughly washed with successive portions of hot alcohol, and was finally rinsed with a small portion of ether to dissolve the last traces of kephalin. The volume of the solution was made up to 100cc with alcohol, the solution heated on the water bath to remove the small amount of ether added, and 5 cc of ~~whirling~~ a hot saturated solution of lead acetate added to the rapidly whirling solution. The flask was again placed on the water bath for about ten minutes, 1cc of 50% ammonium hydrate added, the whole shaken vigorously, and then allowedto remain on the water bath for five minutes longer. The flask was then set aside to cool. After twenty-four hours time, the clear supernatant liquid was decanted through a small ashless filter paper into a 500cc ~~Kjeldahl~~ long-necked Jena flask, and the precipitate washed with hot alcohol, and the alcohol washings combined with the filtrate. The precipitate while still hot on the filter paper was placed over the flask containing the main portion of the lead precipitate, 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 was carefully evaporated over a free flame without charring the organic matter in the flask. In this manner the necessity of bruning the filter paper was eliminated. The solution of lecithins in the other flask was evaporated to dryness on the water bath, the flask being turned on its

side as much as possible in order to allow to vapors to flow out through the neck readily.

OXIDATION AS DESCRIBED BY NEUMANN AND USED IN THIS EXPERIMENT.

PROCESS:

To each of the flasks containing the lecithin andkephalin residue respectively, 15cc of a mixture of sulphric acid, specific gravity of 1.84, with an equal volume of nitric acid, of specific gravity of 1.42, was add4d. The flask was placed on the wire gauze with the funnel steam extending barely into its mouth. It was then very carefully and slowly warmed with a Bunsen burner. It is at this stage of the analysis that special care must be taken at first, in order the reaction will noy become too violent and the flask crack as a result of too rapid heating. When the brown fumes had cleared away and the liquid had become only slightly colored from charring, the flame was removed, and furing nitric acid, of specific gravity 1.5, very carefully added drop by drop, and the process continued until the organic matter was completely destroyed, as was indicated by the failure of the clear, colorless, or bright yellow solution to become dark as a result of the charring of the organic matter when heated sufficiently high to cause the evolution of white fumes. Twenty minutes were required for the carrying out of the oxidation process. The time required for oxidation will vary with the organic matter to be destroyed. The advantage of using the fuming nitric acid andnot t e acid mixture lies in the fact that the amount of sulphuric acid is under control and is not added in amount sufficient to interfere later in molybadate precipitation.

ESTIMATION OF PHOSPHORUS

In the estimation of phosphorus in lecithin, the following reagents were employed:

1. Dilute sulphuric acid, made by adding 100 cc sulphuric acid, of specific gravity 1.84 to 200 cc of pure distilled water.

2. Amonium hydroxide solution, specific gravity of 0.90.

3. Nitric acid solution, specific gravity of 1.42

4. Cyrstalline ammonium nitrate solution, or a 60% solution.

5. Molybdate solution, made according to the formula of Olse, by dissdving 75 grams of crystalline ammonium molybdate in 500 cc of water, and puring this solution into dilute nitric acid (250cc nitric acid, specific gravity of 1.42 plus 250 cc water) in a bottle or beaker, with vigorous shaking. This was kept in a bath maintained about 65° C. for six days, until a portion heated to 70° gave no precipitate. The solution was filtered through into a glass-stoppered bottle.

6. Ammonium nitrate solution, 0.1 per cent.

7. Phenol-phthalein solution, used as an indicator, and made by dissolving one gram of the solid phenol-phthalein in 100 cc pure alcohol.

8. Half-normal solution of sodium hydroxide, standardized by titrating against half-normal oxalic acid solution containing 31.51 gms of special oxalic acid per liter, and also against a half normal solution of sulphuric acid.

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

In the preparation of all the above mentioned reagents,

only chemically pure chemicals were employed and pure distilled water always used.

PROCEDURE: The method is based on the acid character of the ammonium alkali hydroxide. $2(NH_4)_3 12MoO_3PO_4$ plus NaOH plus H_2O equals $(NH_4)_2MoO_4$ plus $23Na_2MoO_4$ plus 23 H_2O.

When cool, the solution obtained by oxidation of a lecithin residue or a kephalin precipitate in the manner above described was diluted by the careful addition of 50cc of distilled water, filtered under a 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 the 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, and combining the wash water with themain filtrate. The filtrate was carefully transferred from the filter flask to a 500 cc flask, and rinsing the filter flask several times with a little pure distilled water.

The filtered solution in the flask was neutralized with ammonia water, or specific gravity of 0.90, and acidified with a strong nitric acid, adding about 1 cc in excess. To this was added 50 grams of dry ammonium nitrate, or a volume of 60% solution containing that amount (65cc), and the volume was made up to 225cc. After heating to 75 degrees C, on the water bath, 25cc of freshly filtered molybdate solution was added, the flask was shaken well, replaced on the water-bath and kept at 65 degrees C. for six hours. After removing the flask from the water-bath, the solution over the yellow precipitate (ammonium phosphomolybdate) was decanted, and filtered under pressure through an ashless filter paper, supported by a cone of hardened filterpaper. The precipitate, flask, and filter were then washed with successive 20cc portions of the 0.1 per cent solution of ammonium nutrant until freed

from acid, as was indicated by the reaction towards phenol-phthalein of the last few drops falling from the funnel. after complete washing, the filter with the portion of the precipitate on it, was placed in the flask containing the other portion of the precipitate, about 150 cc of water was added, andt e flask then shaken to unfold the filter paper and distribute the precipitate more loosely. Half normal sodium hydroxide solution was now added from a burette until the whole of the precipitate dissolved on shaking, 5 cc in excess was then added, and the solution carefully heated over a free flame, protected by a gauze, andthen boiled until all the ammonia was driven off, as indicated by the reaction of the vapors to litmus paper, and likewise to the absence of the oder of the ammonia gass being given off. This required about 15 minutes. The flask was then allowed to stand until cool, or cooled quicker under a stream of cold water, eight drops of pehnolphthalein was added, and the excess sodium hydroxide titrated with the half normal solphuric acid, the total number of cubic centimeters of the sodium hydroxide solution added, minus the cubic centimeters of sulphuric acid added, gives the number of cubic centimeters of half normal sodium hydroxide required to dissolve the precipitate. This, multiplied by 0.553, the equivalent of 1cc of half normal sodium hydroxide solution in terms of phosphorus according ton Neumann, gives the amount of phosphorus found, which was 0.296%. This multiplied by the factor for lecithin, or kephalin, 25.75, assuming the molecular weight at approximately 800, gives the amount of lecithin or kephalin found, or 7.622% of lecithin in the brain of the calf.

Assuming that the dogs were each fed three pounds of calf brains, or forty eight ounces per day, the amount of phosphorus fed per day would be 7.622% of 48 ounces or 3.67776 ounces of phosphorus fed the dogs each day. The two cats diet of calf brains, which averaged two pounds per day would contain 7.622% of 32 ounces, or 2.43904 ounces of phosphorus per day. The guinea pigs averaged one-half pound of calf brains per day, or eight ounces, of which 6.622% is 0.60976 ounces of phosphorus. In addition to the calk brains, the guinea pigs were also fed the yolks of eggs which on analysis gave 6.6% of cephalin, 3% of lecithin, or 9.6% of the total phosphatides in egg yolk. The egg lecithin itself upon being analyzed gave 8.75% of phosphorus. The average egg-yolk weight ten to fifteen grams. Condiering twelve grams, as the mean average, it would contain 3% times twelve, or .36 grams of lecithin in one egg-yolk, or in terms of the total phosphatides, 9.6 times 12 grams or 11.52 gms of total phosphatides per egg yolk. Considering that each guinea pig was fed three egg yolks per day, the amount of lecithin per day was three times 11.52 grams or 34.56 grams per day each.

Analyses of Brains of Three Dogs, Three Cats, and Three Guinea Pigs.

The medium sized dogs were used as subjects for the determination of lecithin and phosphorus in their brains, using the method given above, i.e., the same method employed as in the analysis of the calf brains for the lecithin and phosphorus content. The dogs selected for this purpose were of the same size and as close in resemblance to those fed ad possible. They were killed by giving them chloroform. The brains of these animals were then removed as carefully as possible, especial care being taken not to injure any of the outside nerve tissues.

Three young healthy cats of practically the same size as those being fed were used to determine just what the lecithin and phosphorus content is for normal cats, living under normal conditions and eating no food which is not unusual high in lecithin and phosphorus. The cats were killed in the same manner in which the dogs were, namely; by the use of chloroform. New and sharp instruments were employed for the removal of the cats brains, exceeding care being used at all times not to injure any of the nerve tissues or any other part of the brain. The method employed for the analysis of the brains of the cats was the same as that employed for the analysis of the calf brains described above.

Three healthy and growing guinea pigs were next chloroformed and their brains removed with the same care and precision as in the case of the dogs and cats. These guinea pigs were, of course, of the same size and very nearly the same age, as those being fed on phosphorus and lecithin rich foods. They had been fed on an ordinary diet consisting chiefly of vegetables of different kinds. The method employed for the phosphorus and lecithin analysis was the same as that used for the determination in calf brains, dogs brains, and cats brains. The results of the above analyses will be given in the following pages.

Analysis of Egg Yolks:- Three egg yolks were next analyzed to determine the per cent of lecithin and phosphorus in the yolk of the egg. The eggs were broken and the whites separated from the yolks, the yolks being used for the analysis while the whites were throwr away. Care was taken to keep the yolk as completely separated from the whites as possible, and then the yolks all being kept separately, were put in glass beakers, where they were macerated and then dried by putting

on a glass plate and being fanned by an electric fan. After thorough drying, they were ground up very fine through a mincing machine, and then pulverized by means of a morter and pestle. Three seperate analyses were conducted, then grams being weighed out each time for the analysis. The results of the analyses checked exceedingly close. The same method used for the lecithin and phosphorus analysis of the calf brains was used for the egg yolk analyses. The results of the analyses will be given in a table following.

Following the complete extraction of the lecithin and phosphorus from yolks of the eggs by use of ether and ethyl alcohol, the residue in the Gooch crucible used for the extraction timble, was oxidized by the use of nitric acid (Neumanns method) and sulphuric acid, and then gently warmed with a Bunsen burber. Whem cool, the solution was treated as before, using the standard ammonium nitrate solution, and then after heating to 75°C, the molybdic acid solution (25cc) were added to ascertain as to whether any unoxidezed phosphorus remained in the residual egg-yolk left in the extraction crucible. The flask was gently heated for a while, and then allowed to stand for 24 hours, at the end of which time no precipitate whatever had formed. This was done to make sure that all the organic phosphorus in the egg yolk had been completely extracted with the previous treatment of alcohol and ether, and that none remained either unextracted or unoxidized.

Results of Analyses of Dogs, Cats, and Guinea Pigs Brains.

		Percent Lecithin	Percent Phosphorus
Dogs brain	(1st analysis	25.42	1.079
	(2d analysis	22.38	1.741
	(3rd analysis	23.9	1.41

Average of three analyses - % Lecithin - 23.9 % Phosphorus - 1.41

		Per Cent Lecithin	Per Cent Phosphorus.
cats brain	(1st Analysis	I3.76	0.59I
	(2nd "	I3.74	0.590
	(3rd "	I3.75	0.586

Average of three analyses % Lecithin - 13.74 % Phosphorus - 0.589

		Per Cent Lecithin	Per Cent Phosphorus.
Guinea Pig brains	(1st Analysis	I4.20	0.598
	(2nd "	I3.90	0.596
	(3rd "	I4.I0	0.507

Analyses of three brain samples Aver.% Lecithin-I4.07% % Phosphorus-0.507

Results of Analyses of Egg-yolks

	Percent of Lecithin	(Lecithin of Egg) Percent of Phosphorus
1st Analysis	.....3.I%	8.90%
2nd "	.....2.9%	8.75%
3rd "	.....3.0%	8.60%

Average of three Analyses.....3.0%(Lecithin) Egg Lecithin-8.75% Phos.

Average of Three Analyses - % Lecithin -

Kephalin	- 6.6%
Lecithin	3.0%
Total Phosphatides	9.6

For sake of cmparison with both lower and higher forms of animal life, a few figures are given below:

Analysis of Human Brain(Thudichum.)--Per cent Lecithin...3.75%(Dry method)
Per cent Phosphorus.4.00% " "
Per cent Carbon....66.75% " "
Per cent Hydrogen..I0.67% " "
Per cent Nitrogen...I.8I% " "

Analysis of Rabbit Brain--Per cent Lecithin..I2.4I%
Per Cent Phosphorus 0.532%

It is readily observed that the lower the form of animal life, the lower the per cent of lecithin and phosphorus seems to be. The figures given on the analysis of human brains by Thudichum as compared with the lower forms of animal life show this. In this research, it was shown that the dog, generally recognized as higher in the animal kingdom than the cat or guinea -pig, had more lecithin and phosphorus in its brain than did either the cat or guinea pig.

ANALYSIS OF BRAINS OF DOGS, CATS, AND GUINEA-PIGS USED IN EXPERIMENT.

The dogs, cats, and guinea-pigs after being fed foods rich in lecithin and phosphorus for seven and one- half months were then killed by the use of chloriform and their brains analyzed for the per cent of lecithin and phosphoruas according to the same method given above as in the analysis of calf brains. The results of these analysis are given below:-

	Per cent of Lecithin	Per cent Phosphorus.
Dogs Brains	(1st Dog 24.00%	I.49%
	(2nd Dog 24.10%	I.495%
	(3rd(Control Dog) 23.95%	I.41%

	Per cent Lecithin	Per cent Phosphorus.
Cats Brains	(1st Cat I3.75%	0.592%
	(2nd Cat I3.80%	0.594%
	(3rd(Control Cat) I3.74%	0.589%

	Per cent Lecithin	Per cent Phosphorus.
Guinea-Pigs Brains	(1st Guinea-Pig I4.10%	0.509%
	(2nd Guinea-Pig I4.12%	0.510%
	(3rd(Control Guinea-Pig) I4.07%	0.507%

The following table shows the results of feeding foods rich in the lecithin and phosphorus:

Ordinary Dogs Brain	Dogs Used in Experiment(Brain)
Per cent Lecithin... 23.9%	24.05%(Difference-0.05%)
Per cent Phosphorus. I.41%	I.492%(" -0.0825%)

Ordinary Cats Brain	Cats Used in Experiment(Brain)
Per Cent Lecithin... I3.74%	I3.78%(Difference--0.04%)
Per cent Phosphorus. 0.589%	0.593%(" --0.004%)

Ordinary Guinea-Pigs Brain	Guinea-Pigs Used in Experiment(Brain)
Per Cent Lecithin... I4.07%	I1.II% (Difference--0.04%)
Per cent Phosphorus. 0.507%	0.5095%(" --0.002%)

PREPARATION OF LECITHIN

Samples of lecithin were prepared in the laboratory, using a number of different methods for its preparation. As a source of obtaining the lecithin in a relatively pure state, egg-yolks were used. The objects of preparing it were (1) to gain a thorough understanding as to the best methods of preparing it, and second, to study the physics and chemical properties mentioned above.

The egg yolks were seprated from the white, and ground to a fine paste by using a mortar and pestle and a mincing machine, the pasty mass was then spread out on a large glass plate and dried at about 30° C, by using an electric fan as a means of furnishing the air current. The best results were obtained by treating with an excess of alcohol for a few minutes, and then filtering through a cloth and finally pressing the solid material into a hard mass. The dried mass was then minced very finely with a mortar and pestle and then with a mincing machine, obtaining a very fine dry powder.

The dried egg yolk was then thoroughly extracted six times with an excess of absolute alcohol, and various extracts were mixed and concentrated to small bulk under reduced pressure at 40 degrees C.

The residue was than taken up with a small volume of ether, in which much of it remained insoluble. To the mixture, wihout any attempt to filter, acetone was added in excess and the precipitate obtained separated. The precipitate was a second time mixed with ether pounded together by a pestle and precipitated with acetone and treated as before. This process was repeated three times, until all traces of acetone soluble bodies were removed.

The precipitate obtained above was rubbed up in a mortar with an excess of water, until a good emulsion was obtained; to the emulsion about one third its volume of acetone was then added. On the addition of the acetone a large amount o substance separated in the form of large white flakes and floated on the surface of the liquid. This was removed partly by means of a glass spatule, and partly by filtration. It was again emulsified and precipitated three times more. The precipitate obtained from the first emulsification contained more soluble matter-nitrogenous impurity but after the first treatment only traces of this impurity were found in the liquid.

The solid substance which separated was now dried by treating it with fresh additions of acetone. Finally as much acetone as possible was pressed out of the mass by means of a pestle and the whole taken up with ether, in which it was partly still insoluble, forming an opalescent mixture.

The ther mixture was centrifuged when a clear supernatant fluid and white precipitate obtained. The ethereal solution was decanted off and treated with an excess of acetone. The resulting precipitate was again taken up with ether when almost an clear solution was obtained. The centrifuging was repeated as before and the clear supernatant ethereal solution again precipitated with acetone. It was only necessary to centrifuge twice as the phosphatide obtained was soluble in ether.

The substance was now dissolved in alcohol, and the solution being rather cloudy and opaque was allowed to stand six hours until the insoluble substance (crude kephalin) settled upon the bottom of the flask. After separation of the alcohol-insoluble part by decantation the solution was filtered.

The solution was now evaporated under reduced pressure at 40 degrees C, the residue taken up with ether, and the phosphatide separated from the ethereal solution by acetone. The precipitate obtained gave a perfectly clear solution with alcohol and with ether. It was treated with acetone several times and dried in the dessicator over sulphuric acid. This substance had all the properties of lecithin. The samples obtained contained approximately 4 per cent of phosphorus, determined by means of Neumanns method in which 0.5990 gms required 43.7cc of n/2 NaOH=4.04 per cent phosphorus.

CONCLUSIONS AND DISCUSSION OF RESULTS

As stated in the introduction, the objects of this research were first, to confirm the results of Dr. Kock and others proving that there is a relationship between possession of superior intelligence and a high phosphorus content in the brain; and second, to ascertain, whether or not by feeding, it is possible to influence the phosphorus content of the brain.

The answer to the first of these questions is distinctly in the affirmative, namely, that there does exist a relationship between possession of superior intelligence and high phosphorus content. The analyses of the dog, cat, and guinea-pig brains as compared with previous analyses of human brains by Thudichum very distinctly prove this. Also in this research, the fact was proven that the phosphorus and lecithin content of the dog's brain is considerably higher than in the brain of either the cat or guinea-pig. This would be expected as the dog is an animal of much higher intellect than either the cat or guinea-pig. The figures onbtained for the phosphorus content of dog, cat, and guinea-pig brains show a somewhat relative degree of intellect in the lower animal kingdom.

Just as positive as is the answer to the first question of this research in the affirmative, just so positive and conclusive is the answer to the second question in the negative, namely; that by simply feeding animals foods rich in phosphorus and lecithin content, it is not possible to increase to any marked degree the per cent of these substances in their own brains. There seems to be no such concentration of lecithin and phosphorus in the brain possible.

In other words, every animal has a normal lecithin capacity and lecithin cannot be made to increase in concentration by any sort of feeding. Doubtless, if some experimentation could be carried on in the case of human individuals, that we sholud find that it is possible by mental exercise to increase the capacity of the brain for lecithin, which it will normally will take up from the food-stuffs available, but that brain power cannot be increased by feeding any sorts of foods whatever. The brain and nerves, heart and blood system, and the reproductive organs are three systems of the body cared for first, last, and all the time. The brain really has the power to draw from the body at any time, just what it needs for it own needs. Were it possible to increase the phosphorus content of the brain by feeding, or in case the brain really needed more phosphorus, it could easily draw from the bones or other places in the body where phosphorus might be stored. Undoubtedly the larger per cent of the lecithin and phosphorus fed the animals in this research, was either stored in the bones or possibly the liver, or else excreted through the urine and feces of the animals.

It is not to be understood that the brain power of an undernourished individual is equal to that of a well nourished one. The statement is simply that it is not possible to increase the lecithin content of the brain by feeding foods containing lecithin in high concentration. That is to say, the results indicate that the superstition regarding the influence of brain foods must be discarded along with the superstition regarding the influence of the zodiac and medieval faith in the powers of sorcery.

BIBLIOGRAPHY

Atom refraction of phosphorus and some of its organic compounds. By A. E. Arbuzov, and A. A. Iawov. J. Hess. Phys. Chem. Soc. 47, 2015-27 (1915)

Report on phosphoric acid A. J. Patton, Agri. Exp. Sta. East Lancing Mich. L. S. Walker, Agri. Ex. Sta. Amherst Mass. J. Assoc. Official Agri. Chemist 360-9 (1915)

Organic phosphorus compounds, R. Willstatter and E. Sommenfeld Ger., 288, 393, June 30, 1914.

Lecithin Content of different tissues. J. Cruckshank. J. Path. Bact. 19, 134-6.

Output of organic phosphorus in urin. G. C. Martinson Physiol. Lab. Univ. College, London. Biochen J., 4, 274-9

The lecithin. W. C. DeGraaff. Pharm. Weekbl., 45, 248-55 298, 308, 321, 330. (Feb. 29, March 14, 1921)

On the determination of the mean molecule metabolized and its variation in the animal organizm under the influence of inorganic compounds of phosphorus. By. A. Besgrez and J. Roen. Compt. Rend. Soc. Biol. 63 (33), 455-7

A study of the normal metabolism of the Guinea pig by L. Millard Smith and Howard D. Lewis. J. Am. Chem. Soc. 39, 2251 (1917)

A comparison of the brain of albino rat at birth with that of Fetal pig. M. Koch. J. Biol. Chem. Vol. 14, 267, 279 (1913)

Synthesis of lecithin. By Adolph Grun. Ber. 1913, 66, 125, 127

Esters and amides of the phosphoric acids. IV Reqction between ester of metaphosphoric acid and uni and multi-valent alcohol. Synehsis of glycerol-mono and di-phosphoric acid. Preparation of pure silver metaphosphate. By Kurt Langheld. F. Oppman. and E. Meyer. (Ber. 1912, 45, 3753-3760)

The influence of several organic alkali salts upon muscles, blood cells, protens and lecithin. By Rudalph Raber. Physiol. Inst. Univ. Kiel. Arch. Bes Physiol. 134, 311,-16.

Researches upon the phosphorus bases/ By A. W. Holfman, and A. Calhours. Abstracted from a peper red before the Royal Society, June 18th, 1858.

The existance of Lubrichs Prothegon in the brain. A. Gangee, and E. Blankenhorn (Proc. Roy. Soc. 29 151-156

A note on Protagon by A. Gangee (ibid, 30, 111.

Phosphates and phosphoric acid. Thermic and Bertholet and Lugurin, 1876 i 514. Comp. Rend LXXXI, 1011-1017 and 1077-1078

Chemistry of flesh IV proteids of beef flesh. P. F. Trowbridge and F. S. Grimsley. J. Amer. Chem. Soc. 1906, 28, 169-505.

On the micro-chemical detection of arsenic, antimony, and phosphorus. By. H. Sjollema Utrecht Che,. Week, Bred. 5. 11-5

Investigation on the elimation of Ca, Mg. and Phos. by starving animals. C. Wellman. Royal Hungarian Animal Physiology Exp. Sta. Budapest. Arch. Ges. Physiol. (Pfluger) 121, 508,-83

Titration of Phos. Acid. in presence of chlorides. By. V. Boulez. Chem. Stg. 32, 396 (Aug. 1918)

Some new constituents of the brain. G. Parus. J. Pr. Chem. (2) 24, 310-340.

Remarks on the paper "on some new brain derivates". E. Parcus, L. W. Thudichum. (J. pr. Chem. (2), 25, 29-41.

Extracts of brain and blood. S. Vincent and W. Cramer. Proc. physiol. Soc. 1903, X, XI; J. Physiol. 30

Lipoids of the brain ((A. New method for preparation of Galactosides and Sphingompyelin. O. Rosenheim and M. C. Tebb. Poc. physiol. Soc. 1910, 1-11 J. Physiol. 41.

Reseraches on brid and fish brains. By. Argiris Physiol. Inst. Berlin Z. Physiolchem. 87 288-295

Choline Content of lecithin from heat muscles. By. H. MacLeam Physiol. Inst. Berlin Z. Physiol. Che, 27, 296-302

Influence of Phos. Acid and Potash on Functions and structures of plants By. A. Counturier L. Engrais 22 301-2

Distribution of Phos. in food materials. Balland. J. Pharm. Chem (6), 25, 9-12.

Distribution of Pos. in the human body By. W. Koch. Fr. Am. Soc. Biol. Chem. 3, 35, ad Pr. Am. Physiol Soc. Am. J. Physiol 1919.

Elementary analysis of protein contain phos. M. Dennstedt, City Chem. Lab. Hamburg, Z. Phusiol. Chem. 52, 181-93

Quantitative estimation of extractive andprotein phos. W. Koch. Path. Lab. London Co. Asylum. J. Biol. Chem. 3, 189-64

Phos. acid and pos uable. phosphate for Fertilizer. Elect. smeltering and aluminum Co. Sived. 43, 411, Dec. 27, 1917

Phosphates P. Becquevort, Brit. 114, 277, Oct. 30, 1917.

Tech. Application of methelometry. P. A.Kober. J. Soc. Che. Ind. 27, 75. 67 (1918)

Intermediate metabolism in Exp. Phos. Poisoning. S. Issaac. Z. Physiol. Chem. 100, 1-33 (1917); J. Chem. Soc. 112, 1, 675.

Respiratory metabolism of the artificial perfused livers from dogs after Phos. poisoning. S. Isaac and A. Loeb. Z. Physiol. Chem. 100, 54-8 (1917); J. Chem. Soc. 112,1;768

The relation between chemical, physical, and electrical properties of nerves. (((Total ash sulfates and phosphates. NH Alcock and G. S. Lymde. Physiol. Lab. St. Marys' Hospt.; J. Physiol, 29, 402-10; thru chem. Centr. 1910, 1, 1271.

Phosphates H. S. Gale, et. al. U. S. Geol. Survey Bull. 403-4.

Detection of Phos. Acid and Mg. Amonium Phosphate. By. G. Jorgeusen. Z. anal chem. 50, 337, 43.

Volumetric estimation of Phos. Acid J.rosin; J. Am. chem. soc. 33 1099 1104

Report on organic and inorganic phos. in foods by H. S.Grimsley and T. L. Ross. Bull chem. 137, 142-4

Phos. compounds in food materials. A. Rising Svensk. Kem. Tidski. 22, 143-

The amount and partition of phos. in certain foods. W. Heubner and M. Reeb. Pharmakal. Inst. Univ. Strassburg. Arch. Exp. Pathol Pharmakol. Suppl. bd. 1908, 265-72.

Occurance of a monamino phospatide in egg yolk. H. McLean. Physiol Inst. Berlin; Z. Physiol.chem, 57, 304.

Cerebrosides of the brain (V and V. H. Thierfelder. Zeith. Physiol. Chem. 1914, 89, 236,-248, 247-250.; 91, 107-114

Quantitative analysis of mineral phos. and ashes by the CCl_4 method by. F. Jannasch and R. Leiste. J. Prakt. chem. 88, 273-92

Quantitative volatilization and separation of Phos. Acod. from metals of copper group and from aluminum, tin and alkalis. P. Prakt. Chm 88, 129-67

Galactosides of the brain II. preparation of phrenosin and kerasin by the pyrdine method. O. Rosenheim, King's College London, Biochem. J. 8, 110-120. 9, 604-610.

Oxidation of lecithin in presents of iron salts. O. Warburg and O. Meyerhoff. Z. Physiol. Chem. 85, 412-14

Lecithin degeneration; Pitchovguine - Nunv. Kezan. Compt. rend. soc. boil. 75, 294-6

Lecithin and Lecithin Salts.Laboschin.Pharm.Ztg.,59-63.

Determination of Phosphoric Acidin Foods.E.Werner-Z.Nahr. Genuss.,I5,73I-4.

Action of Lecithin on the WarmlBlooded Heart of Animals. N.W.Kazbelson.Pharm.Inst.Univ.Dorpat.Monograph.Dorpat,I9[illegible]0,I0I pp, thru Zentr.Biochem.Biophys.,II,570-2.

Chemical Analysis of Brain.W.Koch and S.A.Main.

Detection of inorganic and organic phosphorus in meats.-H.S.Grindley and E.L.Ross.J.Biol.Chem.,I9II,8,483-493.

Biological Importance of Phosphorus-M.S.Meslov.

Chemistry of the Brain.A.Weil.Z.ges.Neurologie.Physchiatrie,I9I3,2I; Chem.Zentr.,I9I3,I,I888.

Estimation of Phosphorus,hyposphoric and Phosphoric Acids in Mixtures.R.G.Van Name and W.J.Huff.(Amer.J.Sci.,I9I8,(iv),45,9I-I02)

The precipitation of phosphoric acid in state of ammonium phosphomolybdate.Estimationof phosphoric acid by a simple azotometric method. J.Clarens.(Compt.rend.I9I8,I66,259-262)

Estimation of Phosphoric Acid.Modification of Citrate Method. J.Goldfarb.(Zeitsch.anal.Chem.,I9I8,57,28-33).

Catalytic acceleration of the absorption of oxygen by lecithin with iron salts.Torsten Thumberg(Zeitsch.physiol.Chem.,I9I3,87,820; Otto Warburg(Ibid.,83-84).

Presence of Lecithin in Vegetables.C.E.Schlagdenhaufen and E.Reeb. (Compt.rend.,I902,I35,205-208).

New Method of Estimating Phosphatides.C.Caccio.(Arch farm.sper.sci. aff.,I9I7,24,23I-234;from Physiol.Abstr.,I9I8,3,309).

Estimation of Phosphoric Acid as Ammonium Phosphomolybdate. A.Villiers.(Bull.Soc.Chim.,I9I8(iv)2 3,305-6)

Gravimetric Estimation of Phosphates.W.R.Murmery(Analyst,I9I8,43,324)

Magnesium ammonium phosphate.K.Kule.Phys.Chem.Inst.Univ.Leipzig.Z. anal.Chem.,49,525-96)

The Purity of Commercial Lecithin Preparations.J.Nerking.Inst.Exp. Therapy.Dusseldork.Hyg.Rundschan,20,II6-8.

Cerebrosides of Brain Tissue.P.A.Levene and C.J.West.Rockefeller Inst.Proc.Inst.Exp.Biol.Med.I4,93-5(I9I7)

Synthesis of Lecithin.Answer to K.Langhekd.Ad.grun.Aussig.Ber.,46,I25-7.

The Water Soluble Lecithin Preparations.R.Cohn.Pharm.Ztg.68,406.

Metabolism of Lecithin and Choline. Aldo Patta, Univ. Pavia. Arch. Farmacol. 18, 284-98 (1918).

Is It Possible To Increase the Phosphatide Content in the Brain? E. Baltrowski. Berlin. Biochem. Z. 51, 407-428.

Technique of the Lecithin Determination. J. Nerking. Inst. f. Exp, Ther. Dusseldorf. Biochem. Z., 23, 267-9.

Narcosis and Lecithin. J. Nerking-Abt. Inst. Exp, Ther. Dusseldorf. Munch med. Wochshr. 55, 1733 (Aug. 18).

Distribution of Lecithin in the Animal Body. J. Nerking-Biochem. Abt. Inst. Exp, Ther. p. Dusseldorf, Munch, med, Wochshr., Biochem. Z. 410, 193-205

The Estimation of Phosphoric Avid as Magnesium Pyrophosphate. IV. D. Balareff (Zeitsch. anorg. Chem, m1918, 104, 53-56)

Composition of Gray and White Substances of the Brain-D. Petrowsky.

Alcoholysis of Brain. A. Rollett. Physiol. Inst.

Lipoids. (VI) A New Method for the Fractional Extraction of Brain Lipoids-Sigmund Fraenkel. Lab, d, Spieglerscher. Stiftung., Vienna; Biochem. Z., 19, 254-65.

Chemistry of the Senil Brain. Rudolf Allers. Z. Neurol. Psych., 5, 467-84 through Zentr. Biophys. Biochem., 12, 157.

Chemistry of the Brain.-N. A. Barbieri (Compt, rend, 1900, 131, 347-349)

Quantitative Analysis of the Brain and Spinal Cord-W. Koch. (Amer. J. Physiol. m1904, 11, 303-329.)

Biological Importance of Lecithin-J. W. Gliken. Animal-Physiol. Inst, H. S. Berlin. Biochem. Z., 7, 286-289.

Catalytic Action of Various Gases and Vapore on the Oxidation of Phosphorus-M. Centnerszwer (Zeit. physikal. Chem, m1898, 26, 1-46).

The Fatty Acids of the Egg Lecithin (Journ. Farm. Chem, 1903, XVIII. 102-110).

Influence of Lecithin Upon the Activity of Digestive Ferments. S. Kuttner. Chem. Lab., Inst. Exper. Med., St. Petersburg. Z. Physiol. Chem. m 50, 442-496).

Oxidation if Lecithin in Presence of Iron Salts-O. Marburg and O. Meyerhof (Zeitsch. physiol. Chem, m1913, 83, 412-414.

Influence of Lecithin on Metabolism. S. Yoshimoto. (Zeitsch, Physiol. Ch Chem, m1910, 64, 367-392)

Lecithin and Cultures of B. Diphtheriae. E. Calcaterra. Am. INst. Marag liano, 4, 233 -57; through Z. Immunitat. Ref. 4, 387.

The Determination of Phosphoric Acid as Phosphomolybdate-P.Christensen.(Z.anal.Chem.,47,529-45)

The Hydrolysis of Organic Phosphorus Compounds by Dilute Acid and by Dilute Alkali.-R.H.A.Plimmer.(*Biochem.J.,Vol7,72*).

The Composition of Lecithin Together With Observations on the Distribution of Phosphatides in the Tissues and Meghods for Their Extraction and Purification-.H:Mac Lean.(Biochem.J.,Vol. IX,331).

Determinati on of Phosphorus in Flesh-P.F.Trowbridge.(J.Ind.Eng. Chem.,Vol.I,675).

The Determination of Phosphoric Acid-By the Official Volumetric Mothod-F.B.Carpenter(J.Ind.and Eng.Chem.,Vol.II,159.)

Phosphorus in Flesh-P.F.Trowbridge and L.Stanley:(J.Ind.and Eng.Chem.,Vol.II,212).

Detection and Estimation of Very Small Quantities of Phosphoric Acid Especially in Potable Water.P.Medinger.(Chem.Zeit.1915,39, 781-782).

Factor to be Used for the Calculation of Phosphoric Acid in Neu-Mann's Method.The Factor as Influenced by the Water Used for Was hing the Yellow Precipitate.S.L.Jodidi and E.H.Kellogg(J.Frank. Inst.1915,180,349-367).

Estimation of Phosphoric Acid as Magnesium Pyrophosphate-H.Neubauer.(Zeit.Anorg.Chem.,2,45-50)

On Glycerophosphoric Acid and Its Salts as Obtained from the Phosphorized Constituents of the Brain-J.L.W.Thudichum and C.P.Kingzett.(J.Chem.Soc.Vol.,30,20)

Quantitative Estimation of Phosphorus and Silicon in Iron and Steel.A.E.Haswell(Dingl.polyt.J.237,314-316)

Estimation of Phosphoric Acid-B.Peitzsch and others.(Zeits.anal. Chem.1880,444-452).

Estimation of Small Quantities of Phosphoric Acid.-Koenig.(Land Versuchs.stat.,26,360-364).

Estimation of Phosphoric Acid as Magnesium Pyrophosphate-F.A. Gooch.(Am.Chem.J.Soc.Ind.,391-413)

Estimation of Phosphoric Acid by the Molybdate Process-O.Foerste (Chem.Zeit.,16,109).

Estimation of Phosphoric Acid as Magnesium Pyrophosphate,and the Influence of Reagents Employed in Separating the Acid from B Bases.-D.Lindo(Chem.News.,48,217-221)

Volumetric Estimation of Phosphoric Acid-E.Perrot.(Compt.rend. 93,495-496)

Estimation of Phosphoric Acid in the Ashes of Plants.E.v.Raumer. (Zeits.anal.Chem.,20,375).

New Method for Determining Phosphoric Acid-H.Pemberton(Chem.News 46,4-7).

Estimation of Phosphoric Acid by the Molybdic Method.-Stungel Tametake,and P.Wagener.(Zeits.anal.Chem.,21,353-368).

Relation of Phosphoric Acid to Nitrogen in the Urine During Feeding With Brain.-G.Politis(Zeits.f,Biol.,20,193-214).

New Method of Proximate Resolution of the Brain Substance-F.Baumstark(Zeit.physiol.Chem.,9,145-210).

Lecithin-E.Gilson(Zeit.physiol.Chem.,12,585-602).

Synthesis of Lecithin-F.Hundeshagen.(J.pr.Chem.(2)28,219-255).

A Volumetric Estimation of Phosphoric Acid.By J.Rosin.Vol.33, IO

On the Behavior of the Emulsions of Lecithin with Metallic Salts and Certain Non-Electrolytes.By J.H.Long and F.Gephart. Vol.XLX,S95.

A Study of the Phosphorus Content of Flesh.-A.D.Emmett and H. S Grindley.--Vol.XXVIII.P.25.

The Quantitative Estimation of Lecithins.-Waldemar Koch and Herbert S.Woods.(J.Biol.Chem.,I,203-212.)

Compilation of the Analyses of Agricultural Chemicals,Refuse Substances,Guanos,Phosphates and Animal Excretions.H.D.Haskins. 18th.Annal.Rept.Hatch.Expt.Sta.Mass.PP.156.

Observations of the Stability of Lecithins.J.H.Long.Vol.XXX,881

Study on a Method for the Quantitative Estimation of Certain Groups in Phospholipins.-Marie L.Foster.(J.Biol.Chem.,Vol20,403

The Quantitative Estimation of Extractive and Protein Phosphoru W.Koch.(J.Biol.Chem.,Vol3.159).

Phosphorus in Beef Animals.C. K. Francis and P.F.Crowbridge.(J.Bio Chem.,VolVII.P.481.)

The Determination of Inorganic and Organic Phosphorus in Meats. H.S.Grindley and E.L.Ross.(J.Biol.Chem.,Vol.VIII,P.483.

A Brief Investigation of the Estimation of Lecithin.-R.C.Colliso (J.Biol.Chem.,Vol.XI.P.217).

On Estimation of Phosphorus in Biological Material.-A.E.Taylor and C.W.Miller.(J.Biol.Chem.,Vol.XVIII.P.215).

On the Compounds Containing Phosphorus and Nitrogen-J.H.Gladstone,Ph.D.(Journ.Chem.Soc.Vol.II,P.121.

On the Precipitation of Small Quantities of Phosphoric Acid by Means of Ammonium Molybdate,Together With a Few Remarks on the Yellow Precipitate Containing Silico-Molybdic Acid.-E.Richeters. (J.Chem.Soc.,VolXXIV,P.157).

Volumetric Estimation of Phosphoric Acid by Means of Molybdic Ac A.Grete(Ber.,21,2762-2765).

Volumetric Determination of Phosphoric Acid-C.Schmidler.(Zeit. anal.Chem.,47,142-146).

Volumetric Estimation of Phosphoric Acid-J.Bongartz.(Arch.Pharm. (3),21,846-854).

On the Estimation of Phosphoric Acid-Chas,E.Munero(J.Chem.Soc., Vol.54,533).

On the Determinatio n of Phosphoric Acid-W.G.Williams (J.Chem. Soc.,Vol.53,383).

Estimation of Phosphoric Acid: Oxalic Acid Method as Compared with the Molybdate Method-D.Lindo(Chem.News,49,247-249).

Estimation of Phosphorus in Biological Material-A.E.Taylor and C.W.Miller(J.Biol.Chem., 1914,18,215-224).

Estimation of Inorganic Phosphoric Acid in the Presence of the Esters of Phosphoric Acid-Wolfgang Heubner(Biochem.Zeitsch., 1914,64,401-408).

Accuracy of Neumann's Method in the Estimation of Phosphorus-H.S.HalcroWardlow. (J.Roy.Soc.New South Wales,1914,84,73-93)

Phosphoric Acid Determinations by the Method of Igniton With Magnesium Nitrate and by that of Digestion of Acids.-B.L.Hartwell,A.W.Bosworth,and J.W.Kellogg.(J.Amer.Chem.Soc.,Vol.27,240.)

Colorimetric Determination of Phosphorus.T.E.Hewitt.(J.Am Chem. Soc.,Vol.27,121).

Expeditious Me thod for the Determination of Phosphoric Acid as Phosphomolybdate.-O.Hehner(Analyst,4,23).

Rapid and Exact Method for Detrmination of Phosphoric Acid.-S.W.Johnson and E.H.Jwnkins.(J.Amer.Chem.Soc.,Vol.I,352).

Method for the Determination of Phosphoric Acid.-Otto,Freiheer v.d.Pfordten.(VolXV..,p.1929).

Estimation of Phosphoric Acid in Fertilizers in Feertilizers. D.Lindo.(Chem.News.XLIX.247).

On the Existence of PhosphoricAcid in Ammonium Molybdate-M. Rupfferschlager.(Bull.Soc.Chem.XLI.172).

On the Oxidation of Phosphorus at a Low Temperature and the Alleged Decomposition of Phosphorus Anhydride by Sunlight.-R.Copper and V.B.Lewis.No.CCLIV,P.10.

Determination of Phosphoric Acid-A.Isbert and A.Stautzer. (Fres.Zeit.,26,583-587).

Note on the Determination of Phosphorus-E.K.Landis (Am_Chem.Soc. Vol.XV.480)

On th e Determination of Phosphoric Acid-H.Pemberton Jr., (Amer. Chem.Soc., Vol.I6,278).

Note on Pemberton's Method of Phosphoric Acid Determination as Compared with The Official Methods-W.C.Day and E.P.Bryant (Amer. Chem.Soc., I6,282).

On the Determination of Phosphoric Acid as Magnesium Pyrophosphate.-Hugo Neubauer. (Amer .Chem .Soc., Vol.I6,289).

On the Estimation of Phosphoric Acid-By Titration of the Yellow Precipitate-E.W.Kilgore. (Amer.Chem.Soc., VolI6,765).

The Determination of Phosphoric Acid by the Molybdate-Magnesia Method-G.W.Kilgore (Amer.Chem.Soc.Vol.I6,293).

Some Facts Observed in the Determination of Phosphoric Acid by t the Molybdic Acid Process-R.de Roodle (Amer.Chem.Soc., Vol.I7,43).

Determinatio/ of Phosphoric Acid-H.Pemberton, (Amer.Chem.Soc., Vol.I7,I78).

The Determination of Phosphoric Acid by the Molybdate -Magnesia Method and BY Volumetric Method-B.W.Kilgore (Amer.Chem.Soc., Vol. I7,94I).

On the Estimation of Phosphoric Acid by the Titration of the Ammonium Phosphomolybdate Precipitate With Standard Alkali-H.B.W.Kilgore (Amer .Chem.Soc., Vol.I8,950).

A Gravimetric Method of Estimating Phosphoric Acid as Ammo nium Phosphomolybdate-T.S.Gladding. (Amer.Chem.Soc., Vol.I8,23).

A Modified Ammonium Molybdate Solution-A.L.Winton (Amer.Chem.Soc Vol., I8,445).

The Proteids of the Egg-Yolk-T.B.Osborne and G.F.Campbell (J.Amer Chem.Soc., Vol.22,4I3).

The Protein Constituents of the Egg-White-T.B.Osborne and G.F. Campbell (J.Amer.Chem.Soc., Vol.22,422).

On the Determination of Phosphoric Acid as Phosphomolbdic Anhydride-H.C.Sherman and H.St.John Hyde (J.Amer.Chem.Soc., Vol. 22,652).

Phosphoric Acid-C.H.Jones (J.Amer.Chem.Soc., Vol.22,537).

Lecithin Estimation in Milk-N.A.Brodick-Pittard (Biochem.Z. 69,382-90) (I9I4).

The So- Called ''Acetone-Soluble''Phosphatides .-H.MacLean. (Biochem.J.8,453-9) I9I4.

Precipitating Phosophomolybdate-E.E.Mains. (Chem.Analyst, II,23. I9I4.

Biochemical Significance of Phosphorus .-H.Kincaid(Pharm.J.93,35 I9I4.)

An Improved Method for the Estimation of Inorganic Phosphoric Acid in Certain Tissues and Food Products.-R.H.Chapin and W.C. Tomick.(J.Biol.Chem.,20,97-II4)I9I5.

Factor to be Used for Calculating the Phosphoric Acid in Neumans Method-S.L.Jodidi.(J.Am.Chem.Soc.,37,I704-I0)I9I5.

Estimation of Phosphoric Acid by Means of Citric-Molybdic Acids Solutions-C.Mullerr.(Bul.Assoc.Chem.Sucr.Dist.,29,6I9-22)

The Influence of Phosphorus on the Respiratory Metabolism-O, Hirz.(Z.Biol.,60,I87-3I0).

Calcium and Phosphorus in the Growth of Later Infancy-O.Herbst. (Z.Kinderheilk.,7,I6I-92).

A Study of the Volumetric of Pemberton Method for Determining Phosphoric Acid With Some Experimants,Showing the Influence of Temperature and the Sulphuric Acid Radical on Results-P.McG. Shuey.-(J.Ind.Eng.Chem.,9,367-70)I9I7.

Some Suggestion Concerningt the Preparation of Ammonium Citrate Solution and the Determination of Insoluble Phosphoric Acid-P.McG.Shuey.(J.Ind.Eng.Chem.,9,I045)I9I7.

Determination of Phosphoric Acid and of Magnesium by Titration o Magnesium Ammonium Phosphate-Bayzil (J.Pharm.Chim.,I6,82I-4)I9I7

Volumetric Estimation of Phosphoric Acid -D.Balareff.(Z.anorg. allgem.Chem.,99,I84-6)I9I7.

The Precipitation of Phosphoric Acid as Ammonium Phosphomolybdate.Practica l Determination of Phosphoric Acid by a Simple Nitrometer Measurement.-J.Callarens.(Compt.rend.I66,259-62) I9I8.

Estimation of Phosphoric Acid.A Modifiacation of the Citrate Method.J.Grossfeld(Z.anal.Chem.,57,28-33)I9I8.

Estimation of Phosphoric Acid,Particularly in Superphosphate. G.Vortmann.(Z.anal.Chem.,56,465-87) I9I7.

Estimationof Phosphoric Acid as Magnesium Pyrophosphate.-D. Balareff.(Z.anorg.allgem.Chem.,I0I,229-36)I9I7.

The Determination of P_2O_5 as Ammonium Phosphomolybdate.-A.Viller (Bul.Soc.Chim.,23,305-6)I9I8.

Determination of Phosphoric Acid as Magnesium Pyrophosphate-III. Magnesium Tetrammonium diphosphate.-D.Balareff.(Z.anorg.Allgem. Chem.,I03,73-8)I9I8.

The Separation and Determination of Phosphoric Acid as Ammonium Magnesium Phosphate,-H.Lescoeur.(Bul.Soc.Ind.Nordfranc.,43,93-7) I9I4.

Estimation of Phosphoric Acids in Foods.E.Wormer.(Zeits.Untersuch.Nahr.Benussm.,1908,15,732-774).

On the Estimation of Phosphorus in the Presence of Other Salts. G.Estes.(Journ.Amer.Chem.Soc.,1909,31,247-250.)

Gravimetric Molybdate Method for the Determination of Phosphori Acid for the International Trade of Raw Phosphates.Ullmann. (Communicated to the Seventh International Congress of Applied Chemistry,1909:through J.Soc.Chem.Ind.,1909,28,616).

The Determination of Phosphoric Acid in the Mineral Phosphates. C.Jorgensen.(Analyst.Vol.34,392).

Estimation of Phosphorus in Organic Substances.A.Neumann.(Zeit. anal.Chem.,1909,48,612-617).

Precipitation of Ammonium Phosphomolybdate in the Presence of Organic Acids.G.Maderna.(Atti R.Accad.Lincei,1910,19,827-835: through Chem.Zebtralb.,1910,2,761.)

Estimation.C.Virchow.(Chem.Zeit.,1911,35,913-914).

Estimation of Lecithin in Oil.W.Fresenius and G.K.Mathison. (J.Ind.Eng.Chem.,1911,3,66-72).

Estimation of Phosphoric Acid as Magnesium-Ammonium Phosphate. G.Jorgensen.(Zeitsch.anal.Chem.,1911,50,337-343.)

Estimation of Phosphoric Acid by Neumanns Method.I.Bang. (Biochrm.Zeitsch.,1911,32,444:thru ChemZentrabl.,1911,II,307.)

Distribution of Phosphoric Acid in Milk.F.Bordas and F.Touplain (Ann.des Falsifications,1911,4,229-231).

Volumetric Estimation of Phosphoric Acid.J.Rosin.(J.Amer.Chem. Soc.,1911,33,1099-1104).

Note on the Gravimetric Estimation of Phosphorus in Milk, E.H.Miller.(Analyst,Vol.36.579).

Estimation of Phosphorus in Lecithin.P.Freundler.Bull.Soc.Chim. 1912,II,1041-1042.

Estimation of Phosphoric Acid.L.Moeser and G.Frank.(Zetitsch. Anal.Chem.,1913,52,346-349.)

Estimation of Phosphorus in Iron and Steel.P.Artmann and J.Preisingr.(Zeitsch.Angew.Chem.,1913,26,203-208.)

Rapid Method for the Determination of Phosphorus in Steel. H.Bhattacharyya.(J.Soc.Chem.Ind.,I9I3,32,738-739).

Volumetric Estimation of Phosphorus in Steel-ByMacagnoŕs Method .H.Wdowiszewski.(Chem.Zeit.,I9I3,37,I069-I07I(

Estimation of Lecithin in Medical Tablets.C.Virchow.(Chem. Zeit.,I9I2,36,906-907).

The Phosphomolybdate Estimation of Phosphoric Acid in Soils. By S.J.M.Ault,D.Sc.(Lond),Ph.D.,F.I.C.(Analyst 37,I30)

Estimation of Phosphoric Acid.By von Lorenz's Method.H.Neumann and F.Lucker.(Zeitsch.Anal.Chem.,I9I2,5I,I6I-I75.)

The Recovery of Ammonium Molybdate Used in the Phosphate Est imation.-By J.A.Prescott,B.Sc.(Analyst,40,390)

Colorimetric Estimation of Phosphoric Acid.-E.Riegler. (Bul.Acad.Sci.Roumanie,I9I4,2,272-279;through J.Chem.Soc., I9I5,I08,II,368-369).

Factor to be Used for the Calculation of the Phosph ric Acid in Neumann's Method.S.L.Jodidi.(J.Amer.Chem.Soc.,I9I5,37,I708-I

Precipitation of Phosphorus as Ammonium Phosphomolybdate in Presence of Sulphuric Acid.K.G.Falk and K.Sugiura.(J.Amer.Chem. Soc.,I9I5,37,I507-I5I5).

Estimation of Minute Quantities of Phosphorus.H.S.Raper.(Biochem.J.,I9I4,8,649-656.)

Nephelometric Estimation of Phosphorus.P.A.Kober and G.Egerer (J.Amer.Chem.Soc.,I9I5,37,2373-238I).

Volumetric Estimation of Phosphates in the Ash of Food Material B.Pfyl.(Arbeit.Kaiserl.Gesundhritsamte,I9I4,47,I-44.)

Estimation of Phosphoric Acid in the Presence of Phosphoric Acid Esters.W.Heubner.(Biochem.Zeitsch.,I9I4,64,40I-409.)

Study of the Pemberton-Kilgore Method for Determination of Phosphoric Acid.P.L.Hibbard.(J.Ind.And Eng.Chem.,I9I3,5,998-I009.)

Use of Sodium Citrate for Determinati n of Reverted Phosphoric Acid.W.Bosworth.(J.Ind.and Chem.Eng.,I9I4,6,227-228.)

Estimation of Phosphoric and (Alkaline) Phosphates by Alkalimetric Methods.J.H.Smith.(J.Soc.Chem.Ind.,I9I7,36,4I5-4I9.)

Lamberton Volumetric Method for Estimating Phosphoric Acid. J.McGahuey (J.Ind.and Eng.Chem., 1917,9,767-770).

Recovery of Ammonium Molybdate from the Filtrates Obtained in The Estimation of Phosphoric Acid. H.Kinder.(Stahl.u.Eisen, 1916,36,1094;through Chem.Zentr.,1917,i,,1917.,530.)

Estimation of Phosphorus Pentoxide After Citrate Digestion. C.C.Smith.(J.Ind.and Eng.Chem.,1916,8,1127-1128.)

Comparison of Methods for the Estimation of Phosphates in Soils W.C.Robinson.(J.Ind.and Eng.Chem.,1916,8,148-151.)

Estimation of Phosphorus in Plant Materials.A.W.Christie(J.Ind. and Eng.Chem.,1916,8,511.)

Constitution of Glycerophosphoric Acid of Lecithin.O.Bailly, (Comptes.Rend.,1915,160,395-398).

Methods of Estimation of Lecithin in Milk.N.A.Brodrick-Pitard. (Biochem.Zeitsch.,1914,67,382-390:through J.Chem.Soc.,1915,108, ii,293-294.)

Method for the Estimation of Inorganic Phosphorus in the Body Tissues and Fluids.A.Cistantino.(Chem.Zentr.,1915,ii,287:from Arch.Farmacol.sperim.,1915,19,307-316.)

Lecithin in Fats and Oils.Hermann Jackle.(Zeit.Nahr.Genussm., 1902,5,1061-1077.)

Lecithin in Supra-Renal Bodies.Leon Bernard,Bighart,and Henri Labbe.(Compt.rend.soc.biol.,55,120-122).

Lecithin in the Supra Renal Body of the Guinea Pig. Paul Mulon. (Compt.rend.soc.biol.,1903,55,82-83).

Phosphorus in Animal Tissues.A.L.Percival.(Compt.rend.,1902,135 1005-1007.)

Synthesis of Lecithin.By F.Hundeshagen.(J.Pr.Chem(2),28,219-55.

Existence of Free and Combined Lecithin in the Yolk of Eggs and In Animal and Vegetable Structures.Nicola Alberto Barbieri, (Gazz.Cheim.Ital.47,I,1-37,1917.)

The ''Reversion'' of Lecithin Phosphoric Acid R.Cohn.(Chem. Ztg.,37,581-3).

Investigation of Phosphorus Metabolism Of Growing Dogs.Alex. Lipschutz.(Pharm.Inst.Gottinger.Arch.Exp.Path.Pharm.,62,210-43)

Thecnique to be Followed in the Preparation of Lecithin and of Emulsions and Solutions of Lecithin for Hypodermic Use.-Nino Mondolfi.(Boll.Chim.Farm.,50,413-9.)

The Metabolism of Organic and Inorganic Compounds of Phosphorus. E.B.Forbes(Bull.No.6.Ohio Agri.Exp.Station).

The Determination of Inorganic and Organic Phosphorus In Meats. H.S.Grindley.and E.L.Ross.(Lab of Physiol.Chem.Uni.of Ill.Pub.No.29)

The Metabolism of Nitrogen,Sulphur,and Phosphorus in the Human Organism. H.C.Sherman,Ph.D.—Instr.in Anal.Chemistry,Columbia Univ.

A Study of the Metabolism and Physiological Effects of Certain phosphorus Compounds with Milch Cows.W.H.Jordan,E.B.Hart,and A.J.Patten.—Technical Bulletin No.I.

Calcium and Phosphorus Supply of Farm Feeds and their Relation to the Animals Requirements.—E.B.Hart,H.Steenbock and J.G.Fuller. Research Bulletin No.30. University of Wisconsin.

Calcium,Magnesium and Phosphorus in Food and Nutrition—H.C.Sherman, A.J.Mettler,and J.Edwin Sinclair.Dept.of Chemistry,Columbia Univ. Office of Exp.Stations.U.S.Dept.Agric.—Bulletin 227.

Futher Studies on the Chemical Composition of Normal and Ataxic Pigeons.—Mathilde Koch and Oscar Riddle.Reprinted from the Journ. of Comparative Neurology.Vol.3I,No.2.Dec.I9I9.

Contributions to the Chemical Differentiation of the Brain of the Albino Rat During Growth. W.Koch and M.L.Koch.Reprinted from the Journ.Biol.Chem.Vol.XV,No.3,Sept.I9I3.

Journ.Ind.and Eng.Chem.Vols.4,7,8,and 9.

Technical World.Vols.I9,20,2I,22, and 23.

Technical World Magazine.Vols.I0,I3,I4,I5,I6,I7,and I8.

Science.Vols.42,43,44,45,46,47,48,and 49.

Scientific Monthly.

Scientific American and Scientific American Supplement.

Engineering Record.

Engineering News.

Engineering News Record.

Engineering.

The Determination of Phosphoric Acid.Analyst, IX, 204.

The Determination of Phosphoric Acid by the Titration of the Yellow Precipitate With Standard Alkali.Journ.Frank Inst., I893, cxxxvi, 368-376.

The Determination of Phosphoric Acid by the Titration of of the Yellow Precipitate with Standard Alkali.H Pemberton, Jur. (Jour.Am.Chem.Soc., I893, 382).

A New Method for the Determination of Phosphoric Acid By Precipitation With Molybdenum.Meineke.Repert der Anal Chem. (85) IO, I53.

The Estimation of Phosphoric Acid.A.Smetham.F.C.S., Analyst.Vol.V.I07.

Estimation of Phosphoric Acid by Titration of the Molybdic Precipitate(E.W.Kilgore, Jour.Chem.Soc., I894, pp.765-772.)

Simplified Method For Determining Phosphoric Acid by Means of Molybdate Solution.J Hannamann.(Chem.Zeit., I895., xix., 553-4.

A Volumetric Method for the Estimation of Phosphoric Acid. Matteo Spico.(Gaz.Chim.Italiana, xxii., i.II7.

A Simplifiaction of the Molybdic Acid Process for Determining Phosphorus.Reis.Rep.Anal.Chem.5, 38I-389.

A Critical Examination of the Processes for the Estimation of Phosphoric Acid.C Meieeke.(Chem.Zeit., I896, xx., I08).

A Gravimatric Method of Estimating Phosphoric Acid as Ammonium Phospho-molybdate T.T.Gladding.(Journ.Amer.Chem.Soc., I896, xviii., 23-27).

Determination of Phosphoric Acid as Phospho-Molybdic Anhydride.Woy.(Chem.Zeit., xxi., 44I).

A New Method for the Determination of Phosphoric Acid.Woy. (Zeit.fur.offentl.Chemie., iii, 32I.On the Determination of Phosphoric Acid as Magnesium Pyro-phosphate.H.Lasne.(Bull.Soc.Chim. I897, xvii., 823-832.

On the Estimation of Phosphoric Acid as Phosphomolybdic Anhydride.H.Pellet.(Rev.Chim.Analyt.Appl., vol.v.(20), p.405.

Gravimatric Method of Estimating Phosphoric Acid as Ammonium PHosphomolybdate.T.S.Gladding(The American Fertilizer, I898, i)

Determination of Phosphoric Acid as Phospho-Molybdic Anhydride.J Hannaman.(Zeits.landw.Versuchsw.Oesterr., I900, iii., 53: through Che.Zeit.Reit., I900, 72.

The Determination of Phosphorus in Organic Compounds.C Marie. (Bull.Soc.Chem., I900, xxiii., 44, 45.

The Determination of Lecithin in Milk.F Bordas and S.de Raczkowski. (Ann.de Chim.Anal., I902, vii., 331-333.

The Analysis of Commercial Lecithins.Imbert and Merle.(Bull. de Pharm.Sud.Est., May, I902: Ann.de Chim.anal., I902, vii, 350-5I.

Notes on the Estimation of Phosphoric Acid Milk, etc.F Rieger. (Zeits.physiol.Chem., I90I, xxxiv., I09: through Chem.Zeit.Rep., I90I, 336.

Influence on Heating on the Lecith ins in Milk Bordas and S. de Raczowski.(Ann.de Chim.Anal., I903, viii., I68, I69.

The Determination of Phosphoric Acid by means of Ammonium Phosphomo ybdate G.P.Baxter.(Amer.Chem.Journ., xxviii., 298).

Determination of Sulphur and Phosphorus in Organic Materials. H.C.Sherman.(Journ.Amer.Chem.Soc., xxiv., II00.

Determination of Lecithin in Plants.E Schulze.(Chem.Zeit., I904, xxviii., 75I, 752.)The Iodiometric

The Iodiometric Determinati on of Phosphorus.E.Rupp.(Arch. Pharm., I903, ccxli., 32I-326: through Zeit fur Untersuch.der Nahr.und Genussmittel, I904, vii., I58.

Determination of Free Phosphorus.J Katz.(Oesterr.Chem.Zeit., I908.vi., 5I5.

Estimation of Phosphorus in Organic Substance by Means of Sodium Peroxide.F.Von Konek.(Zeit.f.angew.Chem., I904, xxvi, 886.

The Determination of Phosphoric Acid as Ammonium Phosphomolybdate.Second Paper.G.P.Baxter and R.C.Griffin (amer.Chem.Journ., I902, xxviii., 298.

Quantitative Estimation of Phosphorus in Solutions.A.C.Christamanos.(Zeits.anorg.Chem., xc., 305.

Determination of Phosphorus in Iron and Steel.L.Fricke.(Stahl u Eisen, I906, vol.xxvi., p.276: through Chem.Zeit.Rep., I906, xxx., 82.

The Lecithin Content of Various Milks.J Nerking and E.Haensel. (Biochem.Zeits., I908, I3, 348-353.)

On the Behavior of Lecithin Emulsions with Metallic Salts and Certain Non-Electrolytes.J.H.Long and F.Gephart.(Journ.Amer. Chem.Soc., I908, 30, 88I-895.)

(BIBLIOGRAPHY -CONTINUED)

BULLETINS AND PAMPHLETS USED AS READING REFERENCES

Studies from Rockefeller Institute for Medical Research.Vols.I-25, Vol.29.Vol.30.

Journ.of the Society of Chemical Industry.Vols.22,23,24,27,30,33.

Chem.and Met.Engineering.Vol.I9.July-December,I9I8.

Journal of the Franklin Institute.Vols.I80,I82,I83,I85,and I86.

Seventh International Congress of Applied Chemistry.Secs.4-5. Sec.8bII.Sec.6 8a.Sec.I-3.

Journ.of the Amer.Chem.Soc.Vols.29,30,33,36,37,38 and 39.

Journal of the Chemical Society.(Transactions and Abstracts) Vols.7I,75,77,79, and 9I.

Methods of Chemical Analysis.Bigelow,Willard Dell(Meat Extracts and Similar Preparations including studies of the methods of analysis employed)-W.D.Bigelow and F.C.Cook.-Washington Printing Office.I9I8.

Bulletin of Chemistry. U.S.Dept.of Agriculture.No.II4.F.C.Cook. Library U.S.Dept.of Agriculture.

Phosphorus,Determination-E.B.Hart and W.H.Andrews I.26I. N.Y.State.Bulletin.No.238-Aug.I903 pp.I79-I96.(E.S.R.vol .I5.p.496.

Phosphorus,Determination of-A.J.Patten and E.B.Hart.N.Y.State Bulletin.No.250.Mar.I904.pp.I69-76(E.S.R.Vol.I6.p.I8)

Phosphorus,Inorganic,Determination in Organic Substances.-E.B. Forbes et al.Ohio.Bull.No.2I5.Apr.I9I0.pp.459-489(E.S.R.Vol23,p.303)

Phosphorus-Cook,Frank Cummings.Metabolism of Organic and Inorganic and Feeding Expt.Using Phytin and Sodium Phosphates-F.C.Cook. Washington Gov't.Printing Office. U.S.Dept.Agr.Bur of Chem.Bull.I23.

Phosphorus Metabolism of Lambs Fed a Ration of Alfalfa,Hay,Corn and Linseed Oil-E.L.RossmM.H.Keith,and H.S.Grindley.U.S.Dept.Agr.Journ. of Agr.Research.Vol.IV.No.V.
Feeding of Lambs and Phosphorus Metabolism-Keith,Mary Helen and Grindley,Harry Schultz(Joint Author)(Library,U.S.Dept.of Agriculture.I.Ag 84J.Vol.IV.)

(Lecithin and Allied Substances The Lepins Hugh Mac Lean M. D. D-Sc Toregmars. Green and Co. 41 and 30 St. New York.)

(Lubuich-Liebigs Annalen, Volume C54. Page 29,1864.)

(Gauge Text book of Phosiol Chem. p. 428
" " " " " " Vol.1 London. Page 44.,1880)

(Baumstark. Zeitsskrift fur. Physiol.Chem. vol. 9,page 145,1888)

(Kossel and Freitag Ibid.; B.Volume. 31. page 86,1892.

(Ruppel. Zeutschrift fur. Biologie. Vol 31. Page 86,1892.

(Cramer Soc. Cit. Posser and Gies. Loc. Cit. p. 97,109)

(Posser and Gies, Loc.Cit.page 111.
" # " " " " 1107.)

(Bung. Zeitschrift fur Physiol Chemin.Vol 44. page 47,1905)

(Korner and Thierfelder, Zeitschriftfur Physiolo. Chemie. Cramer. Journal of Physiology Vol 31 page 31. 1904.)

(Threifelder, Zeitschrift fur Physiolog. Chemine. Poser and Gies J. of Biol. Chem. Vol. 1 page 59. 1905. Vol 43. page 21 1904)

(Estimation of Phosphorus in Organic Matter C. Garola. (Chemistry Zents. 1896,22,597,from piv. Chim. anal. appliq. 1896,269.)

(Phosphatides of the Stromate of Red-Blood corpuscles of Sheep and Man. M. Bugrer. and H. Beemer. Bio.Chemistry Zeits. 1913,56,446-456)

(Phosphoric Acid reaction with $(NH_4)_2 MoO_4$ and H_2O_2 V. Macri, Bull. Chem. farm 56,417,8.(1917)

(Organic Phosphorus compounds Soc. anon. Pour. Chem. A. Brele Brit., 104 , 579 October 24,16)

(Iso and Heteropoly Acids 15, Analytic and Synthetic Methods for Investigations of Heteropoly Acids, Authur Rosenheim and Johannes Isemike Z. Anorg. Allgem. Ahem, 101,215-24. 1917. J. Chem. Soc 114.2,48, of C. A. 562,12 1624.)

(Method of Analysis of Brain Lipoids. J. L. Smith and W. Main. J. Path. Bact.,17,609-18. A Modification of the Method Given in previous Papers.: CF. C. A. 7,2061.)

(Lecithin Content of Different Tissues. J. Cruckshank. J. Path. Bact. 18. -134-6. Using a Method Before Described J. Biol. 17,118, the Contents of Various Tissues Was Determined.)

(Notes of the Titration of Phosphoric Acid T. Schwcht. Z. angerv. Chem. 19 1708-1711. Vienenburg.)

(The Direct Oxidation of Phosphorus. E. P. Jungflersch. Col. rend., 145,325)

(Observation of the Formation of Lecithides. preston Kyes. Biochem Z. 8,42-46)

(Relation Between the Total Phosphoric Acid and that of the Lecithins in Different Varieties of Peas. P. Halosy. Univ. Kiloszoan. Biochen. Z.87;104-6.(1918)

(Chemical Stimulation of Cerebral Cortex. SS. Maxwell. Avi. J. Physiol. 47,283-5 L1918.-NET_4 cl,Unlike Other Substances Which Stimulate Medullated Nerve is also a Stimulant of Grey Matter.)

(The Velocity of Oxidation of Nitric Oxide. Eugene Wortgel Compp rend 1920 170,229-231. J. C. S. March 1920, No.639. 2. 163.)

(Fish as a Food and Its Effect on the Metabolism of Phosphorus, Ca and Mg. Bt Jr. Slovtaos. St. Petersburg. Verb. Ges. Russ. Argte. Abst. in Z. ges Physiol, in Path. Stoff week Sesl 4,669)

(Study of Phosphorus Content of Flesh A. D. Emmeth. and Harry S. Grindley J. American Chem. Soc., 1906, 2 8,24-63.)

(Methods of Estimation In Methytamine in Urm. Lecithin Metabolism. P. Cacua 7th Interu. Congr. Appl Chem. 1909,60-73; J. Chem. Soc. 100 2-550)

(Chemistry of Nerve 3. Chem. Composition of the Ventral and Dorsal Medullary Fibers of the Ox. G. Buglia. and d. Maestrun. Chen Zentr 1915,1 376,from Arch farm sperin, 1914,18,271-275)

(Swelling of Nerve Tissue 2. Investigation of Human Brain.Bauer and Ames. Arb. Neurol Inst. Wien 19,226-51 Zents Biochen Bioflys. k. 14,609-10)

(Fasting Metabolism of the Dog in Experimental Changes of the Condition of the Brain. Paul Froch. Inaug. Diss. Halle. 1912-23. Zents. Biochen 15,9.)

(Biol Inuf. of Phosphorus. M. S. Wastov. Petersburger Med w. 2. 38,98-102 M Reports extersive Research on Dogs Fed with Phosphorus.)

(A Reaction Distinguishing Phosphotein from Mucleprotein and the Distribution of Phosphoproteins in Tissues. R. H. Aders Phmimer. and F. H. Scott.Physiol Bab. Univ. A. Col. London. J. Chem. Soc. 93-4,1600-1921 (Oct)

(Influence of Sodium Chloride on the Chemical Composition of the Brain. 1. Nove. C. P. Flugers. Archiv., 48,320-335.)

(Influence of Lecithin on Nutritive Exchanges G. Carriere. Compt. rend. 1901 133,314-6.)

(On some Compounds in Which Phosphorus Appears to Exist in an Allotropic State. Arm. Gautici. Compt. rend, 135,49-52; 126,173-176)

(On Ammonium Molybdate. L. Kaemmerer.J. Pr. Chem 2. 6. 358-60.)

(Newtral Calcium Phosphate. E. Reichaidt. Zeits Chemistry and anal Chemll. 775-777.)

(Methods of Analyzing Natural Phosphates. C; Maine Compt. rend 120.7.430)

(Analysis of Commercial Amorphous Phosphorus R. Tesemius and E. Lucht. Zeitschr. Anal. Chem. 11.63, Chem.)

(Phosphorus Content of White andRed.-Striated Muscle. George Winfield J. Physiol. 1915,49,189,171-179.)

(Recovery of Ammonium Molybdate from the Filtrates Obtained in the Estimation of Phosphorus in Steel and Pig Iron. R. Friedrich. Chem Zeit. 1917,41,67)

(Structure of Lecithin Molecule-Willststher and Ludeke. Bev.37,3753-58.)

(Det. of Phopphorus-Lidholm (Z. Anagerv. Chem. 17,1452)

(" " " Christomanos(z. Anorg. Chem. 41,305.)

(Estimation of Small Quantities of Phosphoric Acid by the Official Vol. Method of the Cessaeration of Official Agriculture Chemists. T. P. Vertah. Bur. Chem. W. S. Dept. Agriculture. Bullitin.90)

(Phosphoric Acid precipitate of $\overline{NH}_2$ inPresence of Lime, Magnesia, Alumina, and Ferric oxide. J. Chem. Soc. Vol 32,1827, 478.Pellet. P. 518.)

(Estimation of Phosphoric Acid C. Mohr. Zeits Anal. Chem. 21,216)

(Det. of Pin. Pig Iron and iron ores (Durgel Polyt. J. c 25,158,161.)

(Caurus Iron Apr. 1877, Stockman Zeit. fur Anal. Chem 1877,174, C. Haltfof Zeit. fur Anal. Chem. 1877,174,189, Korschelt. Verband, des Ver. zur. Beford des. Gerverbfleisses, 1887,page 262-277)

(On Some Compounds of Phosphorus Containing Nitrogen Page 15, J. H. Gladbcure. Ph.D., F. R. S. J. Chem. Soc. Vol.22,1869)

(On the Influence of an Ammonium Salt in the Precipitation of Phosphoric Acid by Ammonium Molybdate. J. Konig. Zeitschrift fur. Anal. Chem. 10,305.)

(Calc. of Phosphoric Acid Estimation 19 Mictche. Zeit. Anagl. Chem. 1888,288)

(Volume Est. of Phosphoric Acid G. C. Caldwell Chem. News. 486,61-62)

(Volume Est of Sulphuric Acid and Phosphoric Acid. J. T. White. Chem News. 57,165-166,187)

(volume Est. of Phosphoric Acid. M Spica. Gazzetta. 22,1,117-123.)

(Process for Determination of Phosphoric Acid F. Jean 9.Compp rend. 1, 38, 1305-1306)

(Factor to be Used for the Calculatio of the Phosphoric In Neuman Method J. and L. Jodidd, J. AM. Chem. soc. 1914,37,1708-1910)

(Estimation of Uranium and Phosphorus. H. D. Newton and J. L. Hughes. J. Amer, Chem Soc 1915, 37,1711-1715)

(Determination of Phosphoric Acid G. Jorgensen Lab. Sterm. Kopenhagen Z Chem. 24,542-4)

(A New Method for Grav.of Volume Determination of Phosphorus, As, S, Cl Br_2 and I_2 in organic Substances, and in Vegetable and Animal substances, as well as for Ale . of Sen. Cod Gas. G. Buzelman Zeitschr and Chem. 1611-2.)

(Estimation of Phosphorus in the Form of Ammonium-Phosphomolybdic Salt. kern Chem. News. 35,1)

(Estimation of Phosphorus and Arsinic by Ammonin Molybdate-champion and Pellet. Bull. Sox. Chin. 2. 37,6.)

(Estimation of Phosphoric Acid by the Citro-marium Method. D. Crisps and R. W. Tuinzing Laudiv. Versucks. Slat 1916,88,131-141)

(Estimation of Citrate Soluble Phosphoric Acid by Iron Citrate Method. M.Popp Chem. Zeuts., 1966,I 29; from Zeitsch landiv. Vers.Wesen. Oesterr. 1915,18 18,392-394)

(Use of Sodium Citrate for Estimation of Revected Phosphoric Acid . Alfred W. Bosworth. J. Ind Eng Chem. 1914,6,227-228)

(Simple and Rapid Method for Estimation of Phosphoric Acid in Bakery Materials. and Similar Products. L. Sohel Chem. Zeuts. 1914, 1 73, from Schwiez-Woch chem. Pharm., 1913, 51 667-679)

(Recovery of Ammonium Molybdate from the Filtrates Obtained in the Estimation of Phosphoric Acid. H. Kinder. Shahl Eisen 1916,36,1094, from Chem. Zuts. 1917 1,530.)

(Oxid. of Phosphorus .W.P. Jorissen and N. H. Van Reesena Chem. Wiekblad 1909,6,931-938)

(Experiments on Effect of Oxygen Towards Phosphorus. M. Centnessgwer.Chem Zentr., 1901. 2, 1022, from Koswos 1910,35, Radziszewske-Festhand.526-527)

(Determination of Phosphorus-J. Reset. Zeitsch. and. Chem. 1909,48,35-36)

(The Intro-molybdate Method for Determination of Phosphorusin Tissues. G. G. Nasmith and E. Fidlar J. Physiol 1908,37,278-284)

(Estimation of Phosphoric Acid in Organic Substances. F. Rieger. Zeit.Physiol. Chem. 1901,34,109-113)

(Estimation of Certain Groups in Phosphatides M. L. Foster. J. Biol. Chem. 1915,20,403-413)

(Factor to be Used for Calcalation of Phosphoric Acid by Meumans' Method. S. S. Jodds. J. Amer. Chem Soc. 1915,37,1708-10)

(Comparison of Grav. Methods Employed in Anal. Chem. for Estimation of Phosphoric acid with processes used in Teck Analysis. A. Korevaar Chem. Weekblad., 1915,12,814-18)

(Comparative Investigations on the Content of Amino-Acids in the Different Constituent Parts of the Nervous System 2. The Amino Acids of the Gray of White Substance of the Brain. Emil Abderholden and Arthur Weit. Zeitsch. Physiol Chem., 1913,83,425-440)

(Lecithin, lecithin-albumin, and their By-Products. Process of Obtaining. Out of Raw Material Containing Lecithin J. Hahermann and R. Ehrenfeld , Brum Austria Hungary U. S. Patent. 987,33, March 21, 1911, Sec. Fr. Patent 390 , 683 of 1908)

(Volume Estimation of Phosphates in Ashes of Food Stuffs. B. Pfgl. Arb. Kars Gesund. Amt. 1914,47, 1-44.)

(Use of Ammonium Citrate in Estimation of Phosphoric Acid . A. Quartaroli and A. Rogar. (Chem. Zentr., 1914, 11,263-264, from Stag. sperin.agrar. 1914,471 410-426)

(Lecithin, Existence of, C. J. West. Science 47, 389-90.1918)

(A Food Containing Lecithin. Sce Brit. 589,1914, C. A. 9,2567. U. S. 1. 150,791. Aug. 17, H. Martin)

(The Influence of Age ofthe Quantity and Chemical Distaibution of Phosphorus in the Nerves Ch. Dhere and H. Maurice. Compt. Rend. 148,1124-5.)

(Transformation in the Phosphorus Compounds in the Hen's Egg During Development. R. H. Aders Plimmer and F. H. Scott. Physio. Bal Univ. Col. London J. Physiol. 38,247-53.)

(Absorption and Assimilation of the Organic Phosphorus Compounds P. Marfors Arch. Exp. Path. Pharm. Suffl., 1908, Schmiedeberg Testachrifts.373-88. Chem Zents., 1908,2,2021)

(Narcosis and Lecithin J Nerking Abt Inst. Esp. Ther . Dusselforf. Munch Med. Wochshr. 55,1933, Aug. 18.-)

(Distribution of Lecithin in Animal Body J. Nerking. Biochem. Abt. Inst. Exp Therop. Dussilforf. Biochem Z. 10, 193,203.)

(Determination of Insoluble Phosphoric Acid J. B. Carpenter. J. Ind. Eng. Chem. 1 119)

(The Volume Determination of Phosphoric Acid Mono-alkali and De-alkale Phosphates. J. M. Wilkie. J. Soc. Chem. Ind. 28,689.)

(Contribution of the Analysis of Brain. Concerning the Particular Colesterol and Fatty Acid content. N Masuda. Tokio Biochen. Z. 25,161-4.)

(Chemisty Constituents of Brain. A. Rielander. Marburg. Zents. Phy. 22,377-80

(Neutralizing Properties of A Substance Isolated From Normal Brain. A. Marie Compt. rend. 150,1775-1777.)

(Patent) 199,591 sept 17,1904, Erist Zeigler. Chartrochemerburg Prep. Containing Lecithin)

(Examination for As., Sb,P. Phosphorous Acid and H_2SO_3, Hager. P 759.J. Chem Soc. Vol. 24.1871)

(Vol. Determination of Phosphoric Acid by Means of Uranum in the presence of Iron. C. Mohr (Zeits. Anal. Chem.,1880,150,153)

(New Alkalimetric Method for Estimation Phosphoric Acid (Arch. Pharm. 3 15,325-334)

(Estimation of Citrate Soluble Phosphoric Acid in Bosic Slag and Numeral Phosphates. P. Wagner. (Chem. Zeit.,1894,18,1153-1154)

(Estimation of Phopsphoric Acid in Phosphates. J. A. Mueller. Bull. Soc. chin. 1901,3, 25,1000-1002)

(Estimation of Phosphoric Acid with Silver Nitrate. T. Clark (J. Soc. Chem Ind. 7,211-312)

(Phosphorus-oxide. E. Baarman (Ber.,16,2146-2152)

(Conversion of Carbonic Oxide into Carbonic Anlydride by Nascent Oxygen- A. R. Leeds. (Chem. Univ. 48,25-26)

(Oxidation of Carbonic Oxide by air and Moist Phosphorus E. Barmann Ber. 17,283-286.)

(Moist Phosphorus, Air and Carbonic Oxide. A. R. Leeds. Chem. Views. 49,73)

(Conduct of Moist Phosphorus and Air Towards Carbonic Oxide. I. Remsen and E. H. Keiser. Chem. News. 48,199-201)

(Behavior of Moist Phosphorus Toward Carbonic Oxide I. Rensin and E. H. Keiser Ber., 17,83-92)

(Analysis of Molybdeur Compounds. H. Brearley. Chem. News, 1899,79,2-4,14-15)

(Estimation of Phosphoric Acid By. C. Schumann. J. Pr. Chem. 2.416)

(Estimation of Phosphorus-H Wattermann and A. Pelger. (Chem. Zents.,1898,18 981-982; from Forsch. Beu. Bebensm. Hgg. 4,241-258)

(Determination of Free Phosphorus P. Mukeyi. J. Asiat. Soc Bemgal. 1900,69 2. 97-101)

(Detection of Organic Matter in Anal. of Substances Centaining phosphorus, Arsenic or Metals. Gm Meillere J. Pharm. Chem., 1902, 4,6,15,97-99)

(Iso and Heteropoly Acids 15. Analytic and Synthetic. Methods for Quant. Investigation of Heteropoly Acids. A. Rosenheim and J. Jaenicke. Z. Amorg Allgem. Chem., 101,215-24. (1917) J. Chem. Soc 114, 2,48 Cf. C. A, 2. 552,12,1624)

(Organic Phosporus Compounds. Soc. Anon Pour Chin. A. Brit. 109,879.Oct 24, 1916)

(A New and Delicate Reagent for Phosphoric Acid and Phosphates. G. Deemiges Bull. Soc. Pharm. 50,195-8)

Determination of Phosphorus in Flesh P. F.Trowbridge. Lab. Agric. Chem Univ. of Mo. J. Ind. Eng Chemistry. 1,675-6)

(Estimation of Phosphoric Acid as Ammonca-Magnesic Phosphate. U. R. Ogilvie. Chem. News. 31,274,32,5,12.

(Estimation of Phosphorus in the Form of Ammonium Phosphomolybdic Salt. S. Kern, (Chem. News, 35,1) $Mg_2P_2O_7$ is weighed which contains 13,51 %of P.

(Volumetric Estimation of Arsenic Acid and Phosphoric Acid by Uranium Solution. G. Brugelmann Zeits Ahr. Anal. Chem. J. 16,22-24.)

(Ethology of Berihein with Especial Reference to Schaumanns Theory of Phospsous Starvation. V. Terumche. Tokio Sarkingakuzashi. Tokio,1910,No 179, thru Zents. Biochem. Broplys,11,719-20)

(Browolecithalbumin and Browmolecithin. P. Bergell Ger. 307,490,1918)

Determination of Phosphoric Acid in stargs and Natural Phosphates by the Use of Citric Acid. Lab. Etct. Liege. Bull. Soc. Chin. 23, 454-7.)

(Remark upon the Driest Titration of Phosphates by Alkalimetry. E. Pozzi Essot. Bull. Assoc. Cjima. Suer. Dist. 27,775-7)

(A Modification of the Volume of Molybdate Method for Estimation of Phosphoric Acid in Acid Phosphates or Commercial Fertilizers. R. Wilhams. Chem. Eng. 8,97-9.(September)

(

(Properties of Protein of Brain 2. A. Marie. Compt, rend, soc. Biol. 70 459-62. J. C. A. 4,2506)

(Estimation of Lecithin. C. J. West Science, 47,389-90.(1918)

(Phosphoric Acid, reaction with $(NH_4)_2MoO$ and H_2O_2, V. Veaeri, Bull. Chim. farm 56,417-8 (1913)

(The Phosphatides of Heart and Other Organs. H Mac. Lean. Brochen Zeits Chemistry, 1913,54,132-142)

(phosphomolybdic Compounds 3, Complex. Hybophosphormolybdermin-semipentoxide-molybdic Acids and their Salts. J Mawrow and N. Nikoloro Zeits Chem. Onorg. Chemistry., 1915,93,170-176)

(Method for Quant. Det. of H_3PO_4 in Murine and Alkaline Phosphate Solutions P. V. Lieherman. Brochem Z. 18,33-57: Then Chem. Zents., 1909,2,474.)

(Estimation fo $H_3PO_4$by Direct Weighing of $NH_4MoO_3PO_4$, N. von Lorery, Aeoten Chem. 14, 1-5: thru. J. Chem. Soc., 100 2, 1028)

(Notes on the Titration fc Phosphoric and Boric Acids, W. Blitz and E. Marcus Z. anorg. Chem. 77131-8.)

(The Reversion of Lecithin Phosphoric Acid. R. Cohn Chem. Ztg. 37,581-3.)

(Detection of Pin Lecithin; P Freundler. Bull. Soc Chin. 11, 1041-3.)

Wet Methods of Organic Analysis (Phosphorus). J. Messinger. Ber. 21,2910-2910)

(Determination of Phosphorous Acids by Numans Method J. M. Krasser. Z. Nahr Genussm 21 , 198)

(Titration of H_3PO_4. M Waglwaer. Utrecht. Pharm. Weakbled. 48,845-50)

(Caruauleon, A Glacerol-free.Phosphate Resembling Lecithin , With Galactose as a nucleus. E. K. Dewhauer and C. A. Jarabson. Z. Physiol. Chem. 64,302-15)

(Determination fo H_3PO_4 by means of Standard $AgNo_3$, J. M. Wilkie. J. Soc. Chem Ind. 29,704-6)

(Purity of Lecithin L. Carcaur. Bull. Chim farm 48,403)