Studies from the research laboratory of the Connecticut Agricultural Experiment Station : the nucleic acid of the wheat embryo / by Thomas B. Osborne and Isaac F. Harris.

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STUDIES FROM THE RESEARCH LABORATORY

OF THE

CONNECTICUT

AGRICULTURAL EXPERIMENT STATION

BY

THOMAS B. OSBORNE AND ISAAC F. HARRIS

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The Nucleic Acid of the Wheat Embryo

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THE NUCLEIC ACID OF THE EMBRYO OF WHEAT.

THE NUCLEIC ACID OF THE WHEAT EMBRYO.

By Thomas B. Osborne and Isaac F. Harris.

I. Introduction.

All organisms, whether animal or vegetable, begin life as single cells which develop by division, the single cell becoming two, or in some cases four, which grow and again divide, so that their number multiplies in rapidly increasing progression. This division is preceded by division of the nucleus, a small body within the mass of the cell, which has a different chemical constitution from the protoplasm contained in the other parts of the cell. The nucleus is evidently the center of the physiological processes of the cell and is present in all those which are capable of growth and development. It is, therefore, of the greatest importance for an intelligent study of life phenomena to have the most complete knowledge possible of the substances contained in the cell and especially of those forming the nucleus.

Investigations made during the past twenty-five years show that a large part of the nuclei of all the cells so far examined consists of one or more of a group of acids, with peculiar and characteristic properties, which have been named the nucleic acids. Although these acids have been the subject of much study, our knowledge of them is as yet very imperfect. The ultimate composition of no one of them has been established beyond a reasonable doubt, although that from salmon-milt appears to be fixed within narrow limits. Of the basicity of these acids and the details of their relations to other substances we have little definite information. The constitution of none has been established, although a number of their decomposition products have been obtained. It is true that Bang has recently described the constitution of a somewhat similar acid found in the pancreas, but this evidently belongs to a different group of substances.

Nucleic acid is usually found in nature combined with protein matter, and it is necessary, therefore, in continuing the studies of vegetable proteins, which have been carried on in this laboratory for years, to obtain a thorough knowledge of nucleic

acid and its protein compounds, the nucleins and nucleo-proteids. These latter are bodies of great physiological importance, about which much confusion and uncertainty still exists. They are described as compounds of nucleic acid with protein bodies, but whether they are salts or esters or some more intimate form of combination, is not set forth by any one. Whether the nucleo-proteids, obtained by the methods now employed, are to be considered as constituents of the tissues from which they are derived, or as products of the investigator's manipulation, is a question which is not yet established.

Sometime ago one of us[1] briefly described a nucleic acid found in relatively large amount in the embryo of wheat, and similar in properties and composition to the nucleic acids of animal and vegetable origin previously described.

So far as we are aware, this is the only nucleic acid which has yet been isolated from the higher orders of plants, the only other one heretofore obtained from a vegetable organism in a state of approximate purity having been found in yeast.

As the nucleic acid of wheat is distinctly different from the nucleic acids already described, we propose to call it tritico-nucleic acid.

We have recently made an extensive study of tritico-nucleic acid, have determined its decomposition products and have also obtained information which has enabled us to describe the probable constitution of its molecule. Although this investigation is not yet completed, we have thought best to publish the results obtained and hope that we may soon be able to supply the data now lacking.

Nucleic acid was first found in combination with protein matter and it was for a long time thought that these compounds formed a special class of phosphorized proteids which were called nucleins. Even after Miescher found the protamine compounds of nucleic acid which were free from protein, and had isolated the free acid from these, he still called the latter nuclein, although he fully recognized its acid properties. It was not until about ten years later that Altmann designated this substance nucleic acid, and distinguished sharply between this acid and its protein compounds—the nucleins.

[1] Osborne and Campbell, Report of this Station for 1899, p. 305, also Jour. Am. Chem. Soc. **22**, 379.

After the discovery of the nucleins most preparations of protein which contained a notable quantity of phosphorus were considered to belong to this group of bodies, but it was soon found that many yielded on hydrolysis one or more bases of the xanthin series, while others did not yield any of these. A distinction was, therefore, made between the two, the former being called the true nucleins, since they were characteristic products of tissues rich in nucleated cells, and the latter para- or pseudo-nucleins, being largely contained in substances free from nucleated cells, such as milk or the yolk of eggs.

The present state of our knowledge of nucleic acid and the confusion which still exists in regard to it are set forth in the following review of the literature:

II. Review of Literature of Nucleic Acid.

Miescher's[2] investigations of the constituents of cell nuclei led, in 1869, to the discovery in the nuclei of pus cells of a substance rich in phosphorus which showed reactions characteristic of proteins. This substance he called nuclein and regarded it as a special form of protein in which the phosphorus was firmly bound. Efforts to obtain preparations still richer than the original in phosphorus and, if possible, free from protein, led Miescher to examine nucleated cells of other kinds, and among them the sperm cells of salmon-milt. From these he got a compound of a new base, which he named protamine with what he then called a very "insoluble nuclein." This latter contained 9.8 per cent. of phosphorus but no sulphur, did not give the xanthoprotein or Millon's reactions and appeared to be a polybasic acid, free from protein, whose salt-like compound with protamine formed 96 per cent. of the dry matter of the spermatozoa heads of the salmon-milt. This phosphorus-containing body he recognized as different from one similarly obtained from hens' eggs. From it he separated a "guanin like complex" which gave the xanthin reaction with nitric acid and soda. Later he prepared its barium salt, from which he inferred that it is either a penta- or hexa-basic acid.

Somewhat later he gave its formula as $C_{22}H_{32}N_6P_2O_{16}$ and then described it as a tribasic acid.

[2] Die histochem. u. physiol. Arbeiten von H. Miescher. Leipzig, 1897. Briefe I-XV, 1869-1870.

Soon after Miescher's discovery, Hoppe-Seyler[3] found in yeast a body very similar to Miescher's nuclein, and at about the same time Plosz[4] found another in the nucleated red corpuscles of goose blood.

Kossel[5] obtained from yeast nuclein several preparations with a nearly constant content of phosphorus, 3.28 per cent. to 3.94 per cent., which he concluded were definite chemical compounds. One preparation, however, contained 6.2 per cent. of phosphorus and was manifestly a different body. On decomposition, this yeast nuclein yielded protein matter in two modifications according as the nuclein was boiled with water, when freshly precipitated, or was washed with alcohol. Hypoxanthin and sarkin resulted on decomposition, but were not obtained in definite proportions.

Having found that hypoxanthin was a product of decomposition of yeast nuclein, Kossel[6] undertook a study of the origin of this base in the organism.

From nuclein, prepared from cells by Miescher's method, he got 1.03 per cent. of hypoxanthin, and from nuclein from goose blood corpuscles 2.64 and 1.97 per cent.

The hypoxanthin was obtained from the nuclein by treatment with a weak acid and did not, therefore, result from a profound decomposition of the protein part of the nuclein.

Kossel[7] next showed that hypoxanthin was more widely distributed and present in greater amount in the various organs of animals and plants than had up to that time been supposed. From yeast, lycopodium spores, wheat-bran and many animal tissues, he got hypoxanthin by the same process that he used to get it from the nucleins.

In his work, "Die Nucleine und ihre Spaltungsprodukte," Strassburg, 1881, Kossel gave the substance of the preceding papers together with some additional observations. His conclusions were that the chemical relations of the nucleins to one another could not then be established; that some of them were complicated bodies, standing near the proteins, but that others were simpler; that some nucleins yield hypoxanthin on decom-

[3] Med. chem. Untersuch. 500, 1871.
[4] Ibid., 461.
[5] Zeit. f. physiol. Chem. 3, 284, 1879; and 4, 290, 1880.
[6] Zeit. f. physiol. Chem. 5, 152, 1881.
[7] Zeit. f. physiol. Chem. 5, 267, 1881.

position while others do not, and that in respect to the physiological role of these bodies we have scarcely any information.

He directs attention to the fact that nucleins are abundant in seeds and that destruction or solution of nuclein seems to occur in degenerating tissues.

Kossel[8] again investigated the nuclein from goose blood and stated that the nuclei of the red corpuscles when treated with hydrochloric acid shrunk in a striking manner, and that this was doubtless the same phenomenon which microscopists had observed when treating cell nuclei with acids. From this nuclein he isolated a peptone-like body which he named histon.

The wide distribution of nucleic acids in plants was soon after made probable by Schulze and Bosshard,[9] who found both hypoxanthin and guanin in extracts of many parts of numerous kinds of plants.

Kossel[10] next confirmed Miescher's earlier statement that the nuclein obtained from the yolk of hens' eggs yields none of the nitrogenous bases characteristic of the nucleins from cell nuclei, and also added that the entire yolks do not contain any of them. Consequently these complexes are formed during the development of the embryo, since in the latter they are easily detected. Two classes, therefore, of nucleins are to be recognized, those of the cell nuclei which contain these bases, and those of egg yolks and milk which do not.

From the extract of a large quantity of pancreas glands Kossel obtained a new base which he named adenin. He also found this base in yeast nuclein and in a large number of animal and vegetable tissues, and in especially large amount in tea leaves.*

In a later paper Kossel[11] stated that adenin was present in tissues rich in nucleated cells but was not found in those which, like muscle, were poor in such cells, and that in these it occurred loosely combined with protein and phosphoric acid. In this

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[8] Zeit. f. physiol. Chem. **8**, 511, 1884.
[9] Zeit. f. physiol. Chem. **9**, 420, 1885.
[10] Zeit. f. physiol. Chem. **10**, 248, 1886.
[11] Zeit. f. physiol. Chem. **12**, 241, 1888.

* It is to be noted that, since Kossel's discovery of adenin, hypoxanthin has rarely if ever been found among the decomposition products of nucleic acids or tissues containing them. It is probable that the "hypoxanthin" previously found was, in many cases, either adenin or resulted from it by oxidation.

paper he also gave an extended account of the properties of adenin and its compounds.

During the same year Liebermann[12] obtained reactions in solutions containing yeast nuclein which he considered to be characteristic of metaphosphoric acid. Since artificial compounds, made by precipitating egg albumen with metaphosphoric acid, behaved like the natural nucleins, Liebermann concluded that these latter were compounds of protein with metaphosphoric acid.

Liebermann[13] also obtained reactions of metaphosphoric acid in hydrochloric acid extracts of egg yolks. By precipitating a solution containing xanthin and egg albumin with metaphosphoric acid he obtained a product from which xanthin could be extracted with boiling water or with ammonia. Guanin is precipitated by metaphosphoric acid, and he therefore concluded that these bases, xanthin and guanin, were admixed with the nucleins and that hypoxanthin, which is not precipitated by metaphosphoric acid, when found in nuclein results from other substances, perhaps adenin or carnin.

Liebermann[14] obtained a barium salt from yeast nuclein, which he thought to be barium metaphosphate, as it contained approximately the required quantity of phosphorus, was precipitated from an acetic acid solution, and reacted for orthophosphoric acid only after boiling with acids.

Altmann,[15] who was the first to emphasize the distinction between the nucleins and nucleic acids, stated that *nucleic acids* were organic phosphorus compounds which can be split from the different *nucleins* and are characterized by a higher phosphorus content than that of the original *nuclein*. Like the nucleins, the nucleic acids are easily soluble in alkalies and ammonia but in contrast to the former are not precipitated by acetic acid from such solutions, but readily by hydrochloric or other mineral acid, by an excess of which, however, the nucleic acid is decomposed, the amount of change depending on the time of action and strength of the acid. Solutions containing the free nucleic acids precipitate proteins and proteoses, the resulting compounds having the properties of nucleins.

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[12] Ber. 21, 598, 1888.
[13] Centralbl. f. d. med. Wissensch. 210 u. 225, 1889.
[14] Plüger's Archiv. 47, 155.
[15] Archiv. f. Anat. u Physiol. physiol. Abth. 1889, p. 524.

Altmann then described his method for preparing nucleic acid by removing all of the protein that can be dissolved by pepsin, dissolving the residue in alkali, precipitating the protein by acetic acid and then separating it from the filtered solution by adding hydrochloric acid.

Altmann considered that the nucleic acids are easily decomposed by alkalies and that the free acids are less resistant to the action of pepsin than their protein compounds. The nucleic acids from yeast, thymus, egg yolk, and salmon-milt were prepared by his method and the supposedly pure acids found to contain respectively 9.44, 9.2, 7.9 and 9.6 per cent. of phosphorus. Since these preparations of nucleic acid manifested many and important differences from those products which had, up to that time, been called nuclein, Altmann considered it to be important to designate these last described products as nucleic acid.

In his opinion these nucleic acids are not identical but similar in their characters and do not contain metaphosphoric acid, as Liebermann suggested, but are organic phosphoric acids, which, however, are not identical with any of the known organic acids which form precipitates with protein substances.

Having found that solutions of fatty acids or of lecithin, under suitable conditions, precipitate egg albumin, Altmann concluded that it is not impossible that a component related to or derived from the fatty acids may be the cause of the protein precipitation caused by nucleic acids.

Malfatti[16] obtained artificial compounds of protein with metaphosphoric acid which he regarded as identical with Kossel's paranucleins. He also stated that guanin could be made to combine with this artificial nuclein so that it was afterwards separated with difficulty. The resulting product he considered to be a true nuclein and that it differed from the paranucleins only in containing the nuclein base. A similar attempt to introduce hypoxanthin failed.*

Kossel[17] next showed that the nucleic acids from yeast and thymus gland differ in composition, properties and decomposi-

[16] Zeit. f. physiol. Chem. 16, 68, 1892.

[17] Verhandlungen der physiol. Gesellsch. zu Berlin, 14 Oct., 1892.

* Guanin metaphosphate is insoluble in water, hypoxanthin metaphosphate is not, hence the apparent combination of guanin and the failure of the experiment with hypoxanthin.

tion products. Analysis of the thymus nucleic acid led to the formula $C_{30}H_{52}N_9P_3O_{17}$, which he thought should probably be doubled. This formula agreed closely with that obtained by Miescher for the nucleic acid of the salmon-milt, namely $C_{29}H_{49}N_9P_3O_{22}$.

Kossel further found that yeast nucleic acid on decomposition gave evidence of a reducing carbohydrate, yielded two phenylhydrazine compounds, one melting at 204-205° the other at 150°, and also much furfurol when distilled with acids.

By treating yeast nucleic acid with alkali, at the room temperature, the organic parts were gradually split from the phosphorus-containing part, and products resulted which were very rich in phosphorus. The first of these, *plasminic acid,* differed from nucleic acid in its ready solubility in water or aqueous hydrochloric acid. Its solution precipitated protein bodies, yielded the nuclein bases by hydrolysis, but contained no carbohydrate group, this latter being easily separated by the action of alkalies. It contained another nitrogenous group, which, on boiling with acids, yielded its nitrogen in the form of ammonia.

Its analysis corresponded to the formula $C_{15}H_{28}N_6P_6O_{30}$, which contains twice as much phosphorus as the formula which Kossel then assigned to nucleic acid. From this he concluded that nucleic acid must contain at least six atoms of phosphorus. Together with this plasminic acid and, as he thought, from it, there resulted another acid, which contained less oxygen than phosphoric acid, probably an anhydride form of phosphoric acid, since its properties corresponded to those of metaphosphoric acid. With this latter, however, it was not identical, since essential differences existed between them. Kossel, therefore, concluded that in the nucleic acids a nucleus must be present which results from the union of several atoms of phosphoric acid with the separation of water. The formation of plasminic acid can thus be easily explained by the successive splitting off of the carbon and nitrogen-containing groups from the phosphorus groups.

The next year A. Kossel,[18] in conjunction with A. Neumann, gave the results of their investigations of the thymus nucleic acid, which they called adenylic acid, as adenin was the only

[18] Verhandlungen der physiol. Gesellsch, zu Berlin, 8 Dec., 1893.

purin body found among its decomposition products. Together with adenylic acid they found in the thymus gland another acid which was more easily soluble in water and formed salts that gelatinized on cooling. Both were precipitated by hydrochloric acid and when boiled for a short time with water yielded a third acid and also adenin or an adenin-containing complex.

To this third acid they gave a provisional formula, $C_{15}H_{23}N_3P_2O_{12}$, the true formula being undoubtedly several times greater than this. They called this acid paranucleic acid, since it precipitated protein.

They further showed that the nuclein bases were organically combined in the nucleic acid and that in neutral and weakly alkaline solutions the nucleic acid was more stable than in acid solutions.

Their paranucleic acid when boiled for a longer time with water was converted into thyminic acid which did not precipitate protein. On decomposing thyminic acid with strong sulphuric acid (30 per cent.) thymin and orthophosphoric acid were produced.

Thymin separated from its purified solutions in large, white double-refracting crystals, slightly soluble in cold, much more soluble in hot water, and by boiling alcohol was gradually dissolved.

Above 250° it melted, and sublimed in crystals on cooling. It had neither pronounced acid or basic properties, was precipitated by mercuric nitrate but not by phosphotungstic acid, was precipitated by silver nitrate and a little ammonia, and also by mercuric chloride when neutralized by sodium hydroxide.

They stated that the nucleic acids of yeast and spleen also contained thymin.

H. Kossel[19] described experiments tried in conjunction with A. Kossel on the bactericidal properties of nucleic acid. He called attention to the fact that several investigators had observed that aqueous extracts of tissues rich in nucleated cells had bactericidal properties, but that it had not been shown to what constituent of the extract this property belonged.

He found that thymus nucleic acid had a pronounced bactericidal action decidedly greater than could be attributed simply

[19] Verhandlungen der physiol. Ges. zu Berlin, 8 Dec., 1893.

to the *acidity* of its solutions. Since different bacteria behaved very differently towards nucleic acid, they concluded that the bactericidal action of the nucleic acid was connected with its power to combine with protein.

Although the details of the preceding experiments on these nucleic acids were promised in a future paper, many of them, especially those relating to the nucleic acid of yeast, have not as yet appeared. The two papers next noticed contained such of these details as have been thus far published. In the first of these Kossel and Neumann[20] state that nucleic acids are to be regarded as constituents of young cells of vegetable or animal origin which are capable of growth, and that these acids occur in the cell nuclei either free or combined with protein.

On boiling the free nucleic acids with water, the relative proportion of the nuclein bases split off was not the same, Inoko getting from the nucleic acid from bull's semen 6 per cent. of xanthin, 2 per cent. hypoxanthin and 0.7 per cent. adenin, while they found only adenin in this acid from calves' thymus. From these facts they concluded that there are different nucleic acids and suggest that each may contain but a single one of these bases.

On boiling adenylic acid with water, another acid results which contains no adenin, is not precipitated by hydrochloric acid, but forms insoluble compounds with protein bodies. This they think may be paranucleic acid, though this was not proved. A second acid is also formed, thyminic acid, which is free from adenin, soluble in water and does *not* precipitate protein solutions. On heating this acid with 30 per cent. sulphuric acid for one hour, it is decomposed and a substance which they named thymin results, as already stated in a previous paper.

In the nuclei of the leucocytes of calves' thymus, Lilienfeldt[21] found a body which he named nucleohiston, and which was a compound of a peculiar protein substance, histon, with pronounced basic proporties and leuconuclein, which latter yielded, like other nucleins, both protein and nucleic acid when decomposed by acids. The nucleic acid yielded by decomposition the nuclein bases adenin and hypoxanthin.

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[20] Ber. 26, 2753, 1893.
[21] Zeit. f. physiol. Chem. 18, 473, 1894.

Kossel and Neumann,[22] in a paper appearing a year later than their preceding, gave the method by which the thymus nucleic acid was prepared in quantity as a white amorphous powder, entirely free from protein.

They decomposed this acid by:

1. Boiling with 30 per cent. sulphuric acid for one hour.
2. Heating with 5 per cent. sulphuric acid at 115°.
3. Heating with 20 per cent. sulphuric acid at 150°.
4. Heating with water at 170-180° for two hours.

By process 3, adenin was completely destroyed and the following products resulted: thymin, cytosin, laevulinic acid, ammonia, phosphoric acid and formic acid. They concluded from their experiments that adenin cannot be the source of the thymin. They also obtained thymin from the nucleic acid of bull's semen, having the same composition as that from thymus nucleic acid. They state in this paper that the composition of thymin given in the preceding paper was incorrect and that its true composition is $C_5H_6N_2O_2$, with which formula their determinations of its molecular weight by the boiling point method agreed.

About 8 per cent. of thymin was obtained from the thymus nucleic acid. By treating this nucleic acid with 20 per cent. sulphuric acid at 150° or with water at 170°, they obtained about 2 per cent. of another base in well formed crystals for which they gave the provisional formula $C_{21}H_{30}N_{16}O_4$ and proposed to call it cytosin. Laevulinic acid was extracted from the products of hydrolysis by ether, from which the presence of a carbohydrate group yielding a hexose was inferred. The distillate first passing over from the products of hydrolysis also contained formic acid.

At about this time Hammarsten[23] obtained a nucleo-proteid from mammary glands, pancreas and liver, which on boiling with acids yielded a reducing substance and guanin. Three different preparations of this substance from the pancreas had the same composition. He regarded this as a body standing near to the nucleins, since it yielded nuclein on digestion, but differing from them in yielding a reducing body.

This reducing substance, obtained as a syrup, reacted like the

[22] Ber. 27, 2215, 1894.
[23] Zeit. f. physiol. Chem. 19, 19, 1895.

pentoses and yielded an osazone with a melting point similar to that of a pentose, but not that of any of the pentoses then known.

In concluding this paper, Hammarsten proposed the following designations for the various phosphorus-containing proteid substances:

Nucleins are those substances which are compounds of protein and nucleic acid, insoluble on pepsin digestion and which yield xanthin bases when decomposed.

Psuedo-nucleins are those nuclein-like substances which form when various proteid bodies are digested with pepsin, but yield no xanthin bases on decomposition.

Nucleo-albumins are those phosphorus-containing protein bodies which, like milk casein, are not compound proteins, and on digestion yield pseudo-nuclein.

Nucleo-proteids are those substances which yield true nuclein on pepsin digestion and xanthin bases when further decomposed.

He objects to Kossel's term paranucleins, because it probably includes many unrelated substances.

M. Tichomiroff[24] next made the observation that the tox-albumin, ricin, and the poisons excreted by the tetanus and diphtheria bacteria were precipitated from their solutions unchanged by nucleic acid, while from the culture fluids of streptococcus and the cholera bacteria precipitates resulted, which had no poisonous properties.

Kossel and Neumann,[25] continuing their investigations, found that thymo-nucleic acid does not yield two acids which are free from nuclein bases as before stated, but only thyminic acid, which results when the nuclein bases are split off from nucleic acid by heating with water, no other decomposition products being then formed. They also found that thymus nucleic acid, which they formerly supposed to contain only adenin and therefore called adenylic acid, in fact contains guanin also. By cautiously heating nucleic acid with water, until the filtered solution gives no precipitate with hydrochloric acid nor a precipitate of barium phosphate with baryta, the nuclein bases are split off and thyminic acid is formed. By neutralizing with baryta and adding alcohol to the filtrate from the precipitate that formed barium thyminate is precipitated, which has the following composition: $C_{10}H_{23}N_3P_2O_{12}Ba$.

[24] Zeit. f. physiol. Chem. **21**, 90, 1895.
[25] Zeit. f. physiol. Chem. **22**, 74, 1896.

Thyminic acid is easily soluble in water, is not precipitated by mineral acids, and gives a precipitate in acetic acid solutions of albumin, which dissolves readily on adding hydrochloric acid.

They confirm their previous statement that the nuclein bases are not in salt-like combination, as might be supposed, but are organically bound.

Schmiedeberg[26] next worked up the notes and material which Miescher had collected through many years and left unfinished at his death. Miescher had found that in acid solutions the nuclein bases are easily detached from the nucleic acid of salmon-milt, and that in working with such solutions the temperature must be kept as near 0° as possible.

The method of preparing nucleic acid from salmon-milt is given in detail and the formula $C_{40}H_{54}N_{14}O_{17},2P_2O_5$ as the result of several analyses.

A sample of the nucleic acid prepared by Altmann from yeast was likewise analyzed and its formula given as $C_{40}H_{59}N_{16}O_{22}$, $2P_2O_5$. Schmiedeberg considered that this preparation was an acid ammonium salt containing two molecules of ammonia, so that the formula for the free acid would be $C_{40}H_{53}N_{14}O_{22},2P_2O_5$. Nuclein bases were recognized among the decomposition products of the salmo-nucleic acid, but were not identified. By heating with hydrochloric acid, a substance having the same properties as Kossel's thymin was obtained, which Schmiedeberg named nucleosin. The nucleic acid formed about 60 per cent. of the dried heads of the spermatozoa, in which it existed in combination with protamine as a salt.

Since Schmiedeberg's nucleosin had the same properties and composition as thymin, Kossel[27] prepared thymin from sturgeon's spermatozoa, and found it to be identical with that from calves' thymus and with Schmiedeberg's nucleosin.

Milroy[28] next compared the behavior of natural with artificial nucleins, obtained by precipitating thymo-nucleic acid with syntonin, deuteroalbumose and "Witte's peptone." The artificial contained approximately the same proportion of phosphorus as the natural.

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[26] Miescher and Schmiedeberg, Archiv. f. expt. Path. u. Pharm. 37, 100, 1896.

[27] Zeit. f. physiol. Chem. 22, 188, 1896.

[28] Zeit. f. physiol. Chem. 22, 307, 1896.

As Kossel and Neumann's method for obtaining nucleic acid from the thymus yielded no nucleic acid when applied to the artificial nucleins, Milroy concluded that the union between the protein and nucleic acid in these was a firm one, similar to that which exists in the pancreas nuclein.

The acidity of the original nucleic acid appeared to be partly neutralized in the artificial nucleins, but experiments with indicators did not give uniform results.

After digestion with pepsin the natural, as well as the artificial, nucleins, except that from "Witte's peptone," left residues containing the same proportion of phosphorus as the original substance, hence the components of the nuclein must have been dissolved in the same proportion as they existed in the nuclein.

Less than one-tenth of the phosphorus dissolved by pepsin digestion could be precipitated by magnesia mixture, except in the case of nuclein from duck and goose blood, of which 42.8 and 15.7 per cent. respectively was precipitated thereby. These solutions, with the exception of that from the pancreas proteid, contained an acid, which precipitated proteids from acetic acid solution. The residue left, after digestion with trypsin, contained a much smaller percentage of phosphorus than did the nuclein before digestion, and a larger proportion of orthophosphoric acid was found in the solution than after pepsin digestion.

Digestion with dilute sodium carbonate solutions, *without trypsin,* gave essentially the same results as the latter, but the action was slower.

The proteid-precipitating acid in the trypsin solution was not metaphosphoric acid since its acid solution could be boiled for a long time without increasing the amount of orthophosphoric acid.

A comparison of the syntonin compound of thyminic acid with the paranuclein of egg yolk showed a similarity between the two, but these cannot be identical, because the proteid-precipitating acids split from them are different.

The year following, Mathews[29] found that the spermatozoa heads of the invertebrate sea urchin contained nucleic acid which had the same proportion of phosphorus and nitrogen as that of the salmo-nucleic acid. It was, however, not united with protamine, but with arbacin, a substance which resembled

[29] Zeit. f. physiol. Chem. 23, 399, 1897.

histon, and from which it differed only in not being precipitated by ammonia. Mathews also found that the heads of herring spermatozoa consisted almost entirely of a compound of protamine and nucleic acid, having the same composition as that found in salmon-milt, $C_{30}H_{57}N_{17}O_6$–$C_{40}H_{54}N_{14}P_4O_{27}$. The semen from the boar and bull he found free from protamine.

Noll[30] found that laevulinic acid was produced in relatively large quantity by the hydrolysis of nucleic acid from sturgeon's sperm. This nucleic acid contained the same amount of phosphorus as that from the salmon-sperm.

Ruppel[31] soon after found in tubercle bacilli a nucleic acid which contained 9.42 per cent. of phosphorus and showed the characteristic properties of acids of this class. This substance he named tuberculinic acid.

Bang[32] next described the acid which in combination with protein formed Hammarsten's pancreas nucleo-proteid. This he named guanylic acid, since when hydrolyzed it yielded guanin as the only purin base. The free acid, as well as its potassium salt, was much more soluble in hot than in cold water, and was purified by repeated precipitation from its solution in hot water. Its composition corresponded to the formula $C_{22}H_{34}N_{10}P_2O_{17}$.

By hydrolysis it yielded about 30 per cent. of pentose, assuming the pentose to have the same copper-reducing power as dextrose; about 35 per cent. of guanin; a very small quantity of ammonia, probably arising from slight decomposition of guanin; phosphoric acid and possibly other unknown products.

This acid contained no thymin nor iron and was nearly ash-free. The ratio of phosphorus to nitrogen was 1:5, whereas in the acids previously investigated this ratio was nearer 1:3—a difference which he attributed to fundamental differences in the structure of these acids.

In a paper which appeared the same year, A. Neumann[33] defined the nucleic acids as phosphoglyco-compounds containing in their molecules the alloxure bodies.

He found that nucleic acid, prepared according to the then current methods, is a mixture of three acids, which he designated

[30] Zeit. f. physiol. Chem. **25**, 430, 1898.
[31] Zeit. f. physiol. Chem. **26**, 231, 1898.
[32] Zeit. f. physiol. Chem. **26**, 133, 1898.
[33] Archiv. f. Anat. u. Physiol. physiol. Abth. p. 374, 1898.

nucleic acid *a* and *b* and nucleothyminic acid. To this fact he ascribes the lack of agreement between analyses of different preparations.

By a method which he described later he obtained these three acids separately, the acid *a* or *b* resulting according to the time of preparation. The chief difference between these acids is that the 5 per cent. salt solution of *a* gelatinizes while that of *b* does not, but in other respects their properties are nearly alike and similar to those of nucleic acid, as earlier described. By hydrolysis of either of these acids, nucleothyminic acid is produced, which still contains the alloxure and carbohydrate groups as well as phosphoric acid, and can be precipitated by hydrochloric acid. Nucleothyminic acid is fairly easily dissolved by water. Of the alloxure bodies it appeared to contain only those of the hypoxanthin group, since the xanthin reaction was obtained only feebly with the purin bodies separated from this acid. Analyses of the acid *b* and the nucleothyminic acid were stated by Neumann to agree well, but were not given.

These three nucleic acids all yielded the same decomposition products that Kossel and Neumann obtained from the thymus nucleic acid, as already described.

When fed to a dog, about four-fifths of these acids were eliminated by the kidneys, the remainder passing off with the faeces.

Nucleic acid given per os, or nucleothyminic acid subcutaneously injected, within a few hours caused a strong hyperleucocytosis, which was not preceded by a hypoleucocytosis.

In a second paper, published the next year, A. Neumann[34] described the method of preparing the three nucleic acids just described.

One kilogram of pure thymus gland was boiled in weak acetic acid, then chopped as fine as possible and brought into two liters of boiling water, made alkaline with 100 cc. of 33 per cent. sodium hydroxide, and 200 grams of sodium acetate were added. After heating on the water bath, for one-half hour, if the acid *a* is to be obtained, or for two hours if acid *b* is desired, the solution is neutralized with 100 cc. of 50 per cent. acetic acid, filtered and concentrated to 500-1000 cc. It is then precipitated, at about 40°, by an equal volume of alcohol and allowed to stand

[34] Archiv. f. Anat. und Physiol. physiol. Abth. suppl., Bd. 552, 1899.

till clear and cold. The liquid is then decanted and the sodium salt collected on a linen filter and redissolved in 500 cc. of water. After heating on the water bath until the insoluble matter separates, leaving the solution clear, it is filtered and the solution precipitated by pouring into alcohol, containing a little sodium acetate. The precipitate is then dissolved in water and reprecipitated by dilute hydrochloric acid. The products thus obtained are very difficultly soluble in water, acid *a* more so than acid *b*. When treated with sodium acetate solution ensues, that of *a* being gelatinous. Neumann considers the sodium salts of these acids to be very stable when heated, while the free acid is much more easily decomposed.

By heating the free acid to 100° with water, it is rapidly decomposed, but if heated to 60° the nucleothyminic acid results, which is separated from its solution by pouring into three times its volume of alcohol containing 15 cc. of concentrated hydrochloric acid per liter.

Neumann thinks that these three acids can be obtained from all tissues containing nucleated cells, capable of development, and states that he has found them in the thymus, spleen, pancreas and bull's testicles.

Schmiedeberg,[35] continuing the investigation set forth in his preceding paper, found that the preparations previously analyzed all contained some protamine which could be replaced by copper. He also found, in agreement with Kossel and Neumann, that nucleic acid is stable in neutral and alkaline solutions, if the latter are not too strong, and that, when freshly precipitated, it is dissolved by potassium acetate. Analysis of a number of copper salts prepared by Schmiedeberg led to the formula, for the free acid: $C_{40}H_{56}N_{14}O_{16}2P_2O_5$. The differences between this and the formula first given, he explains by the fact that the former preparations were acid sodium salts as shown by a determination of sodium in one of them. This alkali had been reckoned as oxygen and therefore the formula previously given for yeast nucleic acid was incorrect.

On decomposition, the salmo-nucleic acid yielded guanin and adenin, not more than one "atom" of each being present. These bases he considers to be certainly in salt-like combination with

[35] Archiv. f. Expt. Pathol. u. Pharm. 43, 57, 1899.

the acid and not organically combined as Kossel had stated them to be in thymo-nucleic acid.

By subtracting the formulas of adenin and guanin from that of the nucleic acid, Schmiedeberg obtained figures which he thought represented the formula of an acid, which he named nucleotinphosphoric acid. The nuclein bases, he states, cannot be completely separated from this acid without profoundly decomposing it, and when this happens the decomposition products are precipitated with the bases and removed from them with such difficulty that accurate determinations can scarcely be made. Nucleotinphosphoric acid is characterized by the tenacity with which it retains bases in combination.

He finds all its phosphorus to be present in a form yielding orthophosphoric acid on treatment with acid, and on heating with strong hydrochloric acid, it yields a black substance, which he called a phosphorized melanin. The following formula is suggested for nucleotinphosphoric acid: $C_{30}H_{38}N_4O_9$; $(OP(OH)_2)_4$. The phosphorus-free substance that remains, after splitting off the phosphoric acid, may be called nucleotin, although, as he says, the different atomic groups may be severally united to the phosphoric acid.

Herlant[36] next obtained nucleic acid by Schmiedeberg's method from unripe salmon-milt and assigned to it the same formula as that given by Schmiedeberg. From calves' thymus he made three preparations of copper salts, the analyses of which led him to assume that the formula of the free acid was $C_{40}H_{58}N_{14}P_4O_{26}$.

The purin bases obtained from this acid were guanin and adenin.

From yeast he got preparations of two copper salts for which he gave the following formulas:

$$C_{36}H_{50\frac{8}{10}}Cu_{4\frac{6}{10}}N_{14}O_{28}, 2P_2O_5 \text{ and } C_{36}H_{48}Cu_2N_{14}O_{20}2P_2O_5.$$

These formulas he thinks show no satisfactory agreement, probably because during the preparation of the substance analyzed, some of the carbohydrate group which this nucleic acid was found to contain was split off.

A. Kossel[37] proposed a classification of nucleins and paranucleins as follows:

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[36] Archiv. f. Expt. Path. u. Pharm. 44, 148, 1900.
[37] In Liebreich's Encyklopaedie, Bd. III.

A, *nucleins;* Under this name are included the nucleic acids and their compounds with protein. These yield nuclein bases on splitting. The nucleic acids are divided into, *a,* thymonucleic acids, which yield on decomposition thymin and other products; *b,* inosinic and guanylic acids which yield no thymin; *c,* plasminic acids, splitting products not sufficiently known.

B, *paranucleins,* phosphorus-containing protein compounds which yield no nuclein bases.

Ascoli[38] obtained from yeast, preparations of an acid which contained from 16 to 27 per cent. of phosphorus, gave both nuclein bases and phosphoric acid on decomposition with acids, and yielded furfurol on distillation. These preparations he regarded as mixtures, which, however, probably contained plasminic acid. Kossel, as already noted, obtained from yeast-nucleic acid a product rich in phosphorus, which he designated plasminic acid, and Ascoli's preparations were therefore supposedly likewise derived from the nucleic acid of the yeast.

The investigation of the properties of this acid led Ascoli to conclude that it contained phosphorus in the form of a hexametaphosphoric acid. A phenylhydrazine and a strychnine salt were made, having a composition similar to the metaphosphates of these bases, yet the differences found between the analytical figures and the calculated compositions were considerable.

Ascoli[39] then examined the leuconuclein from leucocytes and also milk casein, but was unable to obtain any evidence of metaphosphoric acid by the methods employed in his last cited investigation of the plasminic acid from yeast.

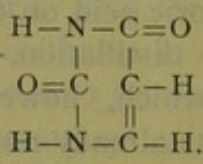
Soon after this Osborne and Campbell[40] found a nucleic acid in the wheat embryo whose analysis corresponded to the formula $C_{21}H_{31}N_8P_2O_{15}$. This yielded, on boiling with acids, both guanin and adenin, together with phosphoric acid and other decomposition products.

Continuing his investigations of yeast, Ascoli,[41] by Jones' method for getting thymin from the thymus gland, obtained from yeast nuclein a body which behaved with silver nitrate

[38] Zeit. f. physiol. Chem. 28, 426, 1899.
[39] Zeit. f. physiol. Chem. 31, 156, 1900.
[40] Report of this Station for 1899; also Journal Amer. Chem. Soc. 22, 379, 1900.
[41] Zeit. f. physiol. Chem. 31, 161, 1900.

like thymin and was not precipitated by phosphotungstic acid. It crystallized in rosettes of needles, and did not sublime undecomposed as easily as thymin, but yielded copious red vapor and a gummy reddish deposit. Analysis of the recrystallized product led to the formula $C_4H_4N_2O_2$, and a molecular weight estimation by the boiling point method gave 110 instead of 112, calculated.

This is the formula of uracyl, and Steudel[42] considers that there could be little doubt but that this is, in fact, Behrend's hypothetical uracyl, that is 2.6 dioxypyrimidin.



Levene[43] next proposed the following method for preparing nucleic acid. The tissues containing it are digested with 5 per cent. sodium hydroxide or 8 per cent. ammonia solution for about two hours, then the cold solution is nearly neutralized with acetic acid, picric acid added to acid reaction, an excess of acetic acid added, the solution filtered and the nucleic acid precipitated by alcohol,

Bang[44] continued his investigation of guanylic acid from the pancreas and prepared a silver salt, from the analysis of which he concluded that this acid is pentabasic and has the formula $C_{44}H_{70}N_{20}P_4O_{34}$.

The amount of ammonia obtained by distilling this acid with magnesia was only 0.11 per cent. and he definitely concludes that this is derived from the guanin, since experiments with this latter substance yielded about the same amount.

He also concludes from his experiments that this acid contains four molecules of guanin and three of sugar.

The remaining decomposition product, which he had not previously recognized, he found to be glycerin, of which not less than 12 per cent. is probably present. From the results of this investigation he concludes that guanylic acid probably has the following structure:

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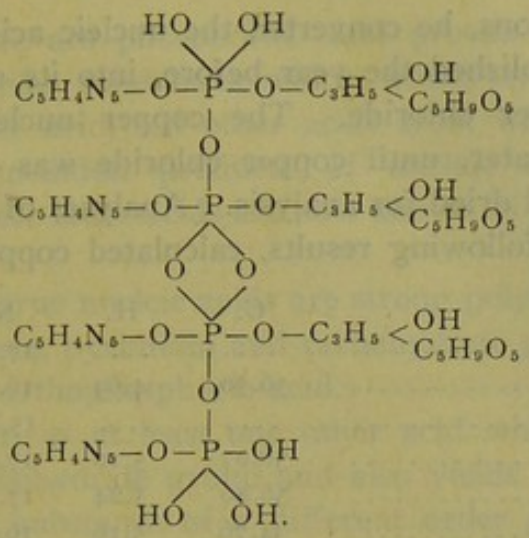
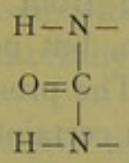
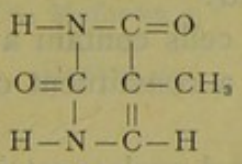
[42] Zeit. f. physiol. Chem. 32, 244, 1901.
[43] Jour. Am. Chem. Soc. 22, 329, 1900.
[44] Zeit. f. physiol. Chem. 31, 412, 1900.



H. Steudel[45] found that thymin by oxydation with barium permanganate yields urea, from which it is evident that one side of the pyrimidin ring is formed by the group



By chlorinating thymin, dichlorthymin is obtained, from which it appears that there must be an oxygen atom near to a hydrogen atom. Since the resulting body would be the same, whether the oxygen atom was in the position 4 or 6 he assigns it to 6. The methyl group cannot have the position 4 since thymin would then be identical with Behrend's methyl uracyl, which it is not. It must, therefore, have the position 5 and thymin is consequently 2.6 dioxy 5 methyl pyrimidin and is related to the ureides, uric acid and the purin bodies. Its formula is:



Continuing his work with nucleic acid, P. A. Levene[46] gave the results of analyses of preparations of nucleic acids of different origin.

In order to remove a little glycogen, which he found in some

[45] Zeit. f. physiol. Chem. 32, 241, 1901.
[46] Zeit. f. physiol. Chem. 32, 541, 1901.

of his preparations, he converted the nucleic acid, obtained by his method, published the year before, into its copper salt, by means of copper chloride. The copper nucleate was then washed with water, until copper chloride was removed, then with alcohol and dried for analysis. Analyses of these preparations gave the following results, calculated copper-free:

	C.	H.	N.	P.
Pancreas I.....	....	....	17.10	8.66
" II.....	36.50	4.69	16.70	8.73
" IV.....	....	....	15.85	9.00
" V.....	36.67	5.10	17.18	8.65
Spleen.....	36.40	5.24	17.30	9.03
Cod fish milt.....	34.76	5.16	16.77	9.15
Yeast	36.65	4.57	17.89	8.93

Levene points out that the nucleic acid from the pancreas is a wholly different substance from Bang's guanylic acid. It contained guanin and adenin, but no xanthin or hypoxanthin could be obtained from it. The presence of thymin was probable, but not established with certainty.

The preparations of nucleic acid obtained from the tubercle bacilli showed no definite composition since they contained from 9.04-13.22 per cent. of phosphorus and 9.42-14.19 per cent. of nitrogen. Guanin and adenin were found among its decomposition products.

E. Fischer and Hagenback[47] have recently prepared both thymin and uracyl synthetically and have confirmed Steudel's view that Ascoli's substance from yeast neuclein is in fact uracyl, identical with that artificially produced.

From the results of all these numerous investigations it appears to be established:

1. That the nuclei of cells contain a relatively large amount of phosphorus which is a constituent of a complicated organic acid.

2. That the nucleins and nucleoproteids obtained from tissues rich in nucleated cells are compounds of this acid with protein, the proportion of these constituents being variable, those containing a large proportion of nucleic acid forming the so called nucleins, those with a smaller proportion, the nucleoproteids.

[47] Ber. 34, 3751, 1901.

3. That there are phosphorus- and protein-containing substances of similar solubilities which, however, do not contain the true nucleic acid, but other acids from which the characteristic decomposition products of nucleic acid cannot be obtained. These latter acids are now known as para- or pseudo-nucleic acids.

4. That the true nucleic acids are strong polybasic acids, containing the purin, pyrimidin and carbohydrate groups and yield on hydrolysis orthophosphoric acid.

5. That there is at least one other acid which contains the purin and carbohydrate group and also yields orthophosphoric acid, but is a substance of a different order since it contains glycerine and lacks the pyrimidin group.

6. That there are at least two true nucleic acids—one containing thymin, the other uracyl.

7. That the ultimate composition of these acids is not yet settled, though the more carefully purified preparations have a similar composition.

The results of the analyses of different nucleic acids have led to the following formulas:

Salmon Milt.

$C_{44}H_{64}N_{12}P_4O_{32}$ Miescher.*
$C_{40}H_{54}N_{14}P_4O_{27}$ Miescher and Schmiedeberg.
$C_{40}H_{56}N_{14}P_4O_{26}$ Schmiedeberg.
$C_{40}H_{56}N_{14}P_4O_{26}$ Herlant.

Thymus of Calf.

$C_{40}H_{69}N_{12}P_4O_{25}$ Kossel.*
$C_{40}H_{56}N_{14}P_4O_{26}$ Herlant.

Arbacia.

$C_{40}H_{54}N_{14}P_4O_{27}$ Mathews.

Pancreas.
$C_{42}H_{64}N_{16}P_4$
$C_{42}H_{73}N_{17}P_4$

Spleen.
$C_{42}H_{74}N_{17}P_4$

Codfish Milt.
$C_{40}H_{70}N_{17}P_4$

} Levene.†

* Calculated to a basis of four P atoms.
† Ratio calculated by the writer from Levene's analyses.

YEAST.

$C_{40}H_{59}N_{16}P_4O_{32}$ Schmiedeberg.
$C_{36}H_{60}N_{14}P_4O_{38}$ Herlant.
$C_{36}H_{52}N_{14}P_4O_{30}$ Herlant.
$C_{42}H_{63}N_{18}P_4$ — Levene.†

WHEAT.

$C_{42}H_{62}N_{16}P_4O_{30}$ Osborne.*

Although these analyses indicate that nucleic acids of different origin have a similar composition, nevertheless the differences between many analyses of preparations obtained from the same source are so considerable that it is impossible to determine what may be the true composition of the acid or the relations of the different preparations to one another. These differences in composition are probably largely due to the fact that doubtless most, if not all, the preparations of the free acid which have been analyzed were acid salts, in which the base has been overlooked. Since the nucleic acids are probably all highly polybasic acids, a number of different salts may be formed, so that on attempting to precipitate the free acids from their alkaline solutions the product obtained consists largely of a mixture of acid salts. If these acid salts are wholly inorganic, only the ratio of the oxygen atoms will be affected, but if organic bases are present the results of analysis are entirely misleading.

III. PREPARATION OF TRITICO-NUCLEIC ACID.

The embryo of wheat, and doubtless of other seeds, is very rich in nucleated cells. Sections stained with iodine green show that the tissues of the embryonic plant consist of a mass of nucleated cells of which the nuclei are so large that in many places the stained tissue looks as if it contained no other material. On extracting the embryo with water a large part of the nucleic acid, which it contains, passes into solution, in combination with protein matter and can be separated from the latter in the way described in the following pages.

The material used for this investigation was a commercial product of the modern milling process, consisting almost entirely

* Calculated to a basis of four P atoms.
† Ratio calculated by the writer from Levene's analyses.

of the embryo of the wheat kernel, flattened into thin scales by passing between smooth steel rolls and being practically free from bran or endosperm was an excellent material from which to obtain constituents of the embryo. This meal when fresh yielded about 1.25 per cent. of tritico-nucleic acid. It was necessary, however, to use very fresh meal, as we found that after a few weeks the meal from which we at first obtained 1.25 per cent. of nucleic acid yielded a very much smaller amount.

The following experiment indicates that only about one-third of the tritico-nucleic acid present in the meal was extracted by water.

Ten grams of the meal were boiled with 4 per cent. hydrochloric acid for four hours, the insoluble matter filtered out and washed, the filtrate made alkaline with ammonia and precipitated with a solution of silver oxide dissolved in ammonia. The silver compound of the purin bases was washed free from the greater part of the ammonia, suspended in water and distilled with magnesia, until the ammonia was completely expelled. The solution was then mixed with 20 cc. of sulphuric acid concentrated by evaporation, and the nitrogen, which was contained in the purin bases, determined by Kjeldahl's method.

We thus found 0.0360 gram of purin nitrogen in the 10 grams of meal, which corresponds to 0.0730 gram of equal parts of guanin and adenin, equivalent to 0.356 gram of tritico-nucleic acid or 3.56 per cent. if all these bases were originally contained in nucleic acid. The quantitative determination of the purin bases contained in tritico-nucleic acid is described in the following pages.

It was not practicable to use an alkaline solution in extracting the meal, whereby a larger yield of acid would, doubtless, have been obtained, because the extracts made with such solutions could not be filtered.

Tritico-nucleic acid was prepared from the wheat embryo meal in the following manner.

a. *First Extraction.*

Nine kilograms of finely ground, oil-free wheat embryo-meal was agitated with about 60 liters of water, the extract strained through bolting cloth and allowed to settle during 24 hours in a cool place, protected by thymol. The somewhat turbid extract

was then siphoned from the deposit, saturated with sodium chloride and strongly acidified with acetic acid. The large precipitate, thus produced, was filtered out, washed with water until most of the salt and acid was removed, suspended in water, an equal volume of 0.4 per cent. hydrochloric acid containing 3 grams of active pepsin was added and the mixture digested for 24 hours at 40°. The insoluble matters were filtered from the solution, suspended in 6 liters of 0.2 per cent. hydrochloric acid, 1.5 gram of pepsin added and the digestion continued for 24 hours longer at 40°. The insoluble matter was filtered out and again digested as before. The filtrate from this last digestion contained but little proteose, showing that all the proteid matter soluble by peptic digestion had been removed from the insoluble crude nuclein. This nuclein was washed, suspended in 3 liters of water, strained through fine cloth, to ensure complete subdivision, and an aliquot portion was neutralized to phenolphthalein with a measured quantity of potassium hydroxide solution. The whole of the mixture was then neutralized by the calculated quantity of the alkali and the nearly clear solution which resulted was filtered clear and divided into two equal parts, A and B.

Part A was treated with so much decinormal hydrochloric acid as just sufficed to produce a flocculent precipitate which separated readily from solution and was easily filtered out. The slightly brownish, strongly acid filtrate that was obtained was treated with an excess of hydrochloric acid which produced a characteristic precipitate that settled rapidly, forming a dense deposit that contracted to a solid mass which could be ground to a course powder under water. This latter was washed with water, dissolved in dilute potassium hydrate solution and reprecipitated with hydrochloric acid. The precipitate, thus produced, was dissolved with potassium hydrate and the solution of the potassium salt was poured into ten times its volume of strong alcohol. In order to cause the precipitate to separate, a little ammonium acetate was added and the precipitate washed extensively with absolute alcohol and dried over sulphuric acid. This preparation, 1, weighed 12.8 grams, was a white amorphous powder, readily soluble in water, its concentrated solution having a yellowish brown color. This and the following preparations were dried at 110° to constant weight and analyzed accordingly to the following methods:

Methods of analysis.

Carbon and hydrogen. The substance was mixed with a large proportion of freshly ignited calcium phosphate and burned with a current of oxygen in a tube filled with copper oxide and a roll of metallic copper gauze. The calcium phosphate absorbed the fusible metaphosphate, resulting from burning the substance, and rendered a complete combustion possible.

Nitrogen was determined by the Kjeldahl method.

Phosphorus. The substance was fused with sodium hydroxide and oxidized with sodium peroxide in a nickel crucible. The fusion was dissolved with nitric acid and the phosphoric acid precipitated with ammonium molybdate and weighed as magnesium pyrophosphate in the usual way.

Potassium and *sodium.* The substance was burned in a porcelain crucible, and the residual, fused metaphosphate, weighed. This was then dissolved by long boiling with hydrochloric acid and the amount of phosphorus determined and calculated as PO_3. This quantity was deducted from the weight of the metaphosphate and the difference taken as representing the quantity of potassium or sodium according as the preparation was obtained from potassium or sodium nucleate. While the results thus obtained are obviously not entirely accurate, they are more satisfactory than those obtained by a direct determination of the bases and are sufficiently near the truth to answer all the purposes for which they have been used.

ACID POTASSIUM NUCLEATE, *Preparation* I.

	Per cent.	Atomic Ratio.
C	33.06	42.12
H	4.22	64.52
N	14.96	16.32
P	8.11	4.00
K	8.72	3.40
O	30.93	29.56
	100.00	
Ash	28.60	
PO_3	19.88	
K	8.72	

Free acid $=C_{42}H_{68}N_{16}P_4O_{30}$

The other half, B, of the alkaline solution of the nuclein, referred to on page 390, was treated, according to Levene's method,[48] with a strong solution containing 18 grams of picric acid, the molecular equivalent of the hydrochloric acid added to the other half of the solution. This appeared to be very nearly the necessary quantity because complete separation of the protein did not take place until nearly all of the picric acid solution had been added.

The filtered solution thus freed from protein, when treated with an excess of hydrochloric acid gave a flocculent precipitate, which soon settled to a coherent layer, having the characteristic properties of this nucleic acid. After extensively washing this precipitate with water, it was dissolved with potassium hydroxide, reprecipitated by an excess of hydrochloric acid, washed with water, again converted into the potassium salt and reprecipitated by pouring into 10-12 volumes of strong alcohol to which were added a few drops of ammonium acetate solution in order to promote its separation. The nearly colorless preparation 2 thus obtained, when dehydrated with absolute alcohol and dried over sulphuric acid, weighed 10 grams and, dried at 110°, had the following composition:

ACID POTASSIUM NUCLEATE, *Preparation* 2.

	Per cent.	Atomic Ratio.
C	33.00	41.52
H	4.07	61.44
N	15.99	17.24
P	8.20	4.00
K	6.11	2.20
O	32.63	30.84
	100.00	
Ash	25.40	
PO_3	19.29	
K	6.11	

Free acid = $C_{42}H_{63}N_{17}P_4O_{31}$.

The formula of this preparation shows one atom more of nitrogen and one atom less of potassium than that of preparation 1.

Since ammonium acetate was used to cause the potassium salt to separate from the alcoholic solution, it is quite possible that

[48] Jour. Am. Chem. Soc. 22, 329, 1900.

a little ammonium has combined with the nucleic acid. This supposition was confirmed by the strong reaction for ammonia which this preparation showed when treated with lime, but unfortunately at the time this preparation was analyzed, not enough of it was left for a quantitative determination of the ammonia.

If we assume that one atom of the nitrogen of the above formula belongs to ammonia and deduct this with the corresponding three atoms of hydrogen, the resulting formula of the free acid will be in close agreement with those obtained for the other purer preparations, namely, $C_{41}H_{60}N_{16}P_4O_{31}$.

The potassium salts forming these two preparations were acid salts, since their aqueous solutions reacted strongly acid towards litmus. As both, when dissolved in water, yielded pale yellow solutions, what remained of them was united, dissolved in water, the solution made alkaline to litmus, and, in order to separate any combined basic coloring matter or other basic organic substance, the solution was precipitated by pouring into an excess of alcohol. The voluminous precipitate thereby produced was filtered out, washed with alcohol, redissolved in water, the solution made alkaline to phenolphthalein with potassium hydroxide and again precipitated with alcohol. The precipitate was filtered out, dissolved in water and the free acid precipitated by adding decinormal hydrochloric acid. The flocculent precipitate that separated soon settled to a coherent layer, which was ground to a powder under water and thoroughly washed with water, dilute and absolute alcohol and, dried over sulphuric acid, gave 10.15 grams of preparation **3**. This had the following composition when dried at 110°.

NUCLEIC ACID, *Preparation* **3**.

	Per cent.	Atomic Ratio.
C	35.41	41.84
H	4.37	62.00
N	16.07	16.28
P	8.75	4.00
K	0.32	
O	35.08	31.00
	100.00	
Ash	3.52	
PO_3	3.20	
	0.32	

Free acid $= C_{42}H_{62}N_{16}P_4O_{31}$.

b. *Second Extraction.*

Four portions of oil-free embryo meal, each weighing 12 kilograms, were separately agitated with 60 liters of water, the extract strained through coarse cloth and the suspended matters allowed to deposit during the night. The somewhat turbid liquid was then siphoned off, saturated with sodium chloride and made distinctly acid with hydrochloric acid. The large precipitate that separated was filtered out, removed from the paper, suspended in water and again thrown on a filter. After the water had run out it was suspended in 0.2 per cent. hydrochloric acid, pepsin added and the digestion continued for three days at the room temperature. The undissolved residue was filtered out, washed twice, by suspending in water and draining on filters, and then treated with an excess of potassium hydroxide solution and a large quantity of picric acid. After about an hour, the mixture was acidified with acetic acid, the insoluble protein picrate filtered out and the clear filtrate treated with strong hydrochloric acid, as long as a precipitate was formed. The very large flocculent precipitate soon settled to a coherent deposit that rapidly contracted to a dense cake, which retained the form of the vessel in which it had settled. By transferring the precipitate to a smaller vessel, before it had become too dense, it was obtained in four round cakes about 6.5 centimeters in diameter and 2.5 centimeters thick. These four cakes, which contained but little combined water, weighed about 100 grams each and were so dense that they closely resembled vulcanite.

The crude nucleic acid from the two portions first extracted were separately dissolved in a small excess of potassium hydroxide solution, an operation which required much agitation and time, owing to the density of the substance, and the two solutions which resulted were then poured into a large quantity of strong alcohol containing an excess of hydrochloric acid. This process was then repeated, the alcoholic filtrate from the second precipitation being much less colored than was that from the first. The two flocculent precipitates were dehydrated with absolute alcohol and dried over sulphuric acid, giving preparations **4** and **5**, which weighed, respectively, 36 and 18 grams. The crude nucleic acid of the third and fourth portions was together treated in the same way, giving 28 grams of preparation **6**, which was more colored than **4** or **5** and appeared less pure.

The acid alcohol, from which the preceding preparations **4**, **5** and **6** had been separated, was treated with twice its volume of strong alcohol and 10 grams of preparation **7** were obtained, which was nearly free from color.

It is evident that much loss of nucleic acid was incurred in thus reprecipitating the crude acid, since from the dense precipitates, which contained but little water and weighed nearly 400 grams, only 92 grams were finally obtained in the four preparations above described.

When dried at 110° and analyzed, these four preparations gave the following results:

NUCLEIC ACID.

	Preparation **4.**		*Preparation* **5.**		*Preparation* **6.**		*Preparation* **7.**	
	Per cent.	Atomic Ratio.	Per cent.	Atomic Ratio.	Per cent.	Atomic Ratio.	Per cent.	Atomic Ratio.
C	34.37	40.92	32.92	42.72	32.73	44.68	34.25	44.76
H	4.33	61.84	4.32	66.80	4.32	70.76	4.15	65.12
N	16.12	16.44	15.10	16.68	15.55	18.16	16.33	18.28
P	8.69	4.00	8.02	4.00	7.57	4.00	7.90	4.00
K	1.62	0.59	4.41	1.74	2.86	1.20	3.25	1.30
O	34.87	31.12	35.23	34.04	36.97	37.84	34.12	33.44
	100.00		100.00		100.00		100.00	
Ash	10.73		18.98		12.95		13.35	
PO_3	9.11		14.57		10.09		10.10	
K	1.62		4.41		2.86		3.25	

Free acid=

$C_{41}H_{62}N_{16}P_4O_{31}$—$C_{43}H_{69}N_{17}P_4O_{34}$—$C_{45}H_{72}N_{18}P_4O_{38}$—$C_{45}H_{66}N_{18}P_4O_{34}$.

c. *Third Extraction.*

The nuclein, obtained from another lot of 12 kilograms of the embryo meal in the same manner as in the preceding extraction, was dissolved by a slight excess of potassium hydroxide and a solution of potassium bichromate and acetic acid added, as long as a precipitate was produced.

The latter was filtered out and washed once by suspending in water and returning to the filter. The filtrate and washings were then strongly acidified with hydrochloric acid and the very large precipitate, with the peculiar properties characteristic of this acid, was thoroughly washed with water and with alcohol

and dried over sulphuric acid. In this way 120 grams of preparation 8 were obtained which, when dried at 110°, had the following composition:

NUCLEIC ACID, *Preparation* 8.

	Per cent.	Atomic Ratio.
C	36.53	44.60
H	4.51	66.08
N	15.87	16.44
P	8.45	4.00
K	0.52	0.20
O	34.12	31.36
	100.00	
Ash	6.40	
PO_3	5.88	
K	0.52	

Free acid $= C_{44}H_{66}N_{16}P_4O_{21}$.

Since this preparation represents the whole of the acid that can be obtained from this meal, and has the same properties and nearly the same composition as the purified preparations of the acid, it seems almost certain that we have but one nucleic acid to deal with in all these preparations.

d. *Fourth Extraction.*

Another preparation of nucleic acid was made as in the preceding experiments and then treated with a strong solution of sodium acetate, whereby a part passed into solution as sodium nucleate. From this solution the nucleic acid was precipitated by hydrochloric acid and found to contain much less coloring matter than did any of the previously obtained crude products. This precipitate was dissolved in a slight excess of sodium hydroxide and the resulting solution poured into alcohol. The sodium nucleate thus precipitated was dissolved in water and its solution poured into alcohol containing an excess of hydrochloric acid. The voluminous precipitate of the free acid was then washed with alcohol and dehydrated with absolute alcohol.

The 200 grams of nucleic acid thus prepared formed a pure white, dusty powder, which had the following composition when dried at 110°:

NUCLEIC ACID, *Preparation* 9.

	Per cent.	Atomic Ratio.
C	35.15	44.88
H	3.93	63.60
N	15.59	18.08
P	7.63	4.00
Na	1.42	1.00
O	36.28	36.84
	100.00	
Ash	17.22	
PO_3	15.80	
	1.42	

Free acid = $C_{45}H_{65}N_{18}P_4O_{37}$.

In order to obtain as pure a preparation as possible, about 120 grams of the crude nucleic acid was dissolved in an excess of potassium hydroxide solution, three grams of picric acid added and the solution acidified with acetic acid. A small precipitate of protein picrate was filtered out and the perfectly clear filtrate precipitated with an excess of hydrochloric acid. The nucleic acid thus precipitated was washed with water, redissolved with potassium hydroxide and the clear solution poured into five volumes of strong alcohol.

The precipitate thus produced was dissolved in water and the solution again poured into five volumes of alcohol, which threw down the potassium nucleate, leaving the alcohol only slightly colored. After filtering out and washing with alcohol, the potassium nucleate was again dissolved in water and reprecipitated as the free acid by pouring its solution into several volumes of strong alcohol containing an excess of hydrochloric acid, whereby a very voluminous, white precipitate resulted, which was washed with alcohol, dehydrated with absolute alcohol and dried in the air, yielding a white powder, which weighed 53 grams. This was then dried at 110° and analyzed with the following results:

NUCLEIC ACID, *Preparation* 10.

	Per cent.	Atomic Ratio.
C	34.65	40.76
H	4.30	60.72
N	15.88	16.00
P	8.70	4.00
K	1.42	
O	35.05	30.92
	100.00	
Ash	13.71	
PO_3	12.29	
Na	1.42	

Free acid = $C_{41}H_{61}N_{16}P_4O_{31}$.

This preparation was free from chlorine, iron and all other mineral bases which with phosphoric acid form compounds insoluble in alkaline solutions.

The analyses of these several preparations of nucleic acid show wide differences, similar to those presented by the analyses of nucleic acids heretofore published by others.

This is chiefly due to the fact that all these preparations contained a variable proportion of mineral base. As this nucleic acid is polybasic, it forms acid salts with potassium or sodium, which are insoluble in water and whenever an attempt is made to precipitate the free acid from a solution of its alkaline salt, a part is thrown down as an acid salt, from which it has been impossible to remove all the base.

If the atomic ratio is calculated from the analyses, the differences caused by this combined base largely disappear and formulas are obtained which are not unlike those previously given for the nucleic acids obtained by other investigators.

In the more carefully purified preparations the ratio of phosphorus to nitrogen is approximately 4 to 16, but in the less pure it is greater, reaching even 4 to 18. It would appear that it is almost as difficult to remove organic bases as inorganic and that consequently an excess of nitrogen is usually present.

Preparations **3** and **10** were more extensively purified than the others, and their analyses doubtless most closely represent the true composition of this nucleic acid. It is to be noted that the ratio of phosphorus to carbon in **3** is 4 to 42, whereas in **10** it is 4 to 41. In the latter the ratio of phosphorus to nitrogen is 4 to 16, but in the former 4 to 16¼. Since in the other preparations the ratio of carbon to phosphorus increases with that of nitrogen, it is probable that preparation **10** is somewhat purer than **3** and that there are 41 and not 42 atoms of carbon for every 4 of phosphorus in the molecule of the pure acid.

We may, therefore, take the formula found for **10** as most closely representing the molecule of the tritico-nucleic acid, namely, $C_{41}H_{61}N_{16}P_4O_{31}$.

IV. Properties of Tritico-nucleic Acid.

a. *Solubility in Water.*

Compared with nucleic acids of animal origin, that from the wheat embryo is very insoluble in water. Definite statements concerning the solubility of the former are not to be found in the literature, but the practice of precipitating these acids by the addition of one or more volumes of alcohol, containing hydrochloric acid, indicates that they are soluble in water to a notable extent.

Owing to the difficulty encountered in preparing tritico-nucleic acid free from base, it is not possible to make precise statements in regard to its solubility in water. None of the preparations just described were soluble in cold water to a noticeable degree. In boiling water, most of them formed a pasty mass, of which very little was soluble.

One preparation, not previously described, which was very finely divided, and contained about 3 per cent. of sodium, when boiled with water for a few minutes dissolved almost completely, its solution being acid to litmus. After boiling for some time, its solution gave only a slight precipitate with hydrochloric acid, which, however, did not have the peculiar character of the unchanged acid. From this it would appear that, by boiling with water, tritico-nucleic acid is altered to such an extent that it is no longer precipitated by hydrochloric acid. It is probable that the free tritico-nucleic acid is very slightly, if at all, soluble in boiling water, except in so far as it is altered thereby with the formation of soluble products.

b. *Inorganic Salts of Tritico-nucleic Acid.*

With potassium, sodium or ammonium, tritico-nucleic acid forms salts that are soluble in water. Potassium, sodium or ammonium form readily soluble acid salts whose aqueous solutions are strongly acid to litmus. Tritico-nucleic acid, like the nucleic acids described by others, is soluble in solutions of alkali acetates. Strong solutions of potassium nucleate are precipitated abundantly by barium or calcium chloride, the precipitate being moderately soluble in water, but are only incompletely precipitated by magnesium sulphate solutions.

With zinc sulphate a voluminous gelatinous, white precipitate forms; with ferric acetate a light reddish, bulky precipitate; with mercuric nitrate a more dense, colorless precipitate, and with silver nitrate a gelatinous, white one. With copper salts a pale greenish precipitate results in which only a part of the copper is present in the usual form of basic combination.

Thus a quantity of the acid was dissolved with just enough potassium hydroxide to give a neutral reaction with phenolphthalein, and was then treated with a dilute solution of copper sulphate until precipitation was complete. The copper nucleate was filtered out, washed, suspended in water and the substance, thus very finely divided, was poured into a large volume of alcohol containing an abundant excess of hydrochloric acid. The free nucleic acid was filtered out, thoroughly washed with dilute alcohol and dried over sulphuric acid. When moist, this substance had a very pale green color; when dry it was white, with a just perceptible greenish tint. Dissolved in an excess of ammonia it gave a clear yellow solution, with no trace of the blue color caused by the copper ammonium ions usually formed under such conditions. On ignition this preparation left an ash of copper phosphate, which formed 17.43 per cent. of the dry substance. The remainder of this preparation was dissolved in ammonia, the solution acidified with acetic acid, ammonium chloride added and hydrogen sulphide passed through the solution. After filtering out the copper sulphide, the nucleic acid was precipitated by hydrochloric acid and recovered with unchanged properties and composition.

Schmiedeberg[49] observed a similar behavior of salmo-nucleic acid, for he says that in no case could the nucleic acid be freed from copper by precipitating with hydrochloric acid or even by dissolving in concentrated hydrochloric acid. If the copper nucleate was dissolved in ammonia and the solution treated with barium chloride, the precipitated barium nucleate still contained copper which could not be removed by washing with ammonia.

c. *Attempts to Crystallize Potassium Tritico-nucleate.*

A quantity of tritico-nucleic acid was dissolved with enough potassium hydroxide to give a slightly alkaline reaction with phenolphthalein, enough alcohol added to give a faint turbidity

[49] Archiv. f. Exper. Path. u. Pharm. 43, 57.

and the mixture allowed to evaporate over calcium oxide. The amorphous deposit that formed was redissolved and treated as before, but no crystals could be obtained, even after the several fractional precipitations had been again treated in this way. No crystals resulted from the addition of more alcohol to the solutions decanted from these deposits and further evaporation over lime.

d. *The Basicity of Tritico-nucleic Acid.*

A silver salt of this acid was prepared by adding silver nitrate to the aqueous solution of the acid potassium salt. Dried to constant weight over sulphuric acid, this was found to contain

	Found.	Calc. for $C_{41}H_{55}N_{16}P_4Ag_6O_{31}$.
Ag	31.62	31.76 per cent.
P	5.97	6.08

In harmony with this result, it was found that 4.4 cc. of decinormal potassium hydroxide solution were made neutral to phenolphthalein by 1.4 grams of preparation **3**, and that on neutralizing the added alkali with decinormal nitric acid the solution became turbid when 3 cc. of the added alkali were still unneutralized by the nitric acid. When the nucleic acid was precipitated by completely neutralizing the whole of the added alkali, the precipitated acid was redissolved and a clear solution obtained with 3.1 cc. of decinormal potassium hydroxide solution. Since the calculated molecular weight of this acid is 1397, the above quantity of nucleic acid corresponds in its capacity for reaction to 1 cc. of decinormal alkali. We see, therefore, that with three atoms of potassium a soluble acid salt is formed, and with a little more than four atoms, one that is dissociated, under the conditions of the experiments, to such an extent as to give an alkaline reaction with phenolphthalein.

The salt with three atoms of potassium reacts acid with litmus, that with four atoms, alkaline.

V. Hydrolytic Decomposition Products of Tritico-nucleic Acid.

a. *Purin Bases.*

Most of the preparations of this acid, as already stated, when heated with boiling water form a plastic mass, of which little

is dissolved. One preparation of an acid sodium salt, which was insoluble in cold water, dissolved almost completely in boiling water and, after an hour and a half, its solution gave no trace of a precipitate with silver oxide-ammonia, although a considerable quantity of orthophosphoric acid was found in the solution.

Hot 1 per cent. hydrochloric acid rapidly dissolves tritico-nucleic acid, and if the solution is at once tested with ammoniacal silver solution no precipitate of purin bases is obtained. If, however, the 1 per cent. hydrochloric acid solution is boiled for 30 minutes, these bases are completely separated and the solution yields no more by further treatment with boiling acid. These facts show that in tritico-nucleic acid these bases are in ester combination, as Kossel found them to be in thymo-nucleic acid, and not in salt-like combination, as Schmiedeberg supposed them to be in salmo-nucleic acid. Boiling for a longer time and with stronger acids does not increase the yield of purin bases. Thus one gram of preparation **8**, when boiled for 30 minutes with 1 per cent. hydrochloric acid, gave 11.4 per cent. of guanin, when boiled for 90 minutes with 1.5 per cent. hydrochloric acid, 11.6 per cent., and when boiled with 5 per cent. sulphuric acid for 7 hours, 11.2 per cent.

A considerable quantity of adenin is also obtained by hydrolysis and indeed in nearly equi-molecular proportion with the guanin. The identity of these two bodies was established by their reactions and the crystalline form of their salts, which were throughout those characteristic of these substances.

The nitrogen of the two bases was determined with the following results:

	Guanin.		Adenin.	
	Found.	Calc.	Found.	Calc.
N.....	46.04	46.36 per cent.	51.49	51.85 per cent.

A quantity of the adenin was also converted into the picrate, which had the following composition:

	Found.	Calculated for $C_{11}H_8N_8O_7$.
C.....	36.07	36.27
H.....	2.51	2.19
N.....	30.28	30.77

The proportion of these purin bases was found by boiling two one-gram portions of preparation 8, air dried, with 25 cc. of 2 per cent. sulphuric acid for 30 minutes, making the solution alkaline with ammonia and precipitating the guanin and adenin with a solution of silver oxide in ammonia. After washing out the greater part of the free ammonia and its salts, the precipitate was freed from the last traces of these by continued boiling with an excess of magnesia. The remaining nitrogen was then determined by Kjeldahl's method and 0.0942 and 0.0950 gram of nitrogen was found. The one air-dry gram of substance taken contained 0.1531 gram of nitrogen, one-sixteenth of which is 0.00957 gram, from which it appears that the nitrogen of the purin bases is ten-sixteenths of the whole, a proportion required by the presence of one molecule of guanin and one of adenin.

The amount of each of these bases was then determined in a number of different preparations of the acid by hydrolyzing, as above, and precipitating the guanin by adding an excess of ammonia to the solution, which had a volume of 100 cc. After filtering out the guanin on asbestos in a Gooch crucible, the adenin was precipitated by ammoniacal silver oxide solution. The precipitates were washed until every trace of ammonia was removed and nitrogen determined in them with the following results:

WEIGHT IN GRAMS OF PURIN NITROGEN IN 1 GRAM OF TRITICO-NUCLEIC ACID.

Preparation	4	5	7	8		10		Calculated for 1 mol. of each.
				I	II	I	II	
Guanin N....	0.0579	0.0572	0.0548	0.0474	0.0463	0.0573	0.0532	0.0500
Adenin N....	0.0494	0.0448	0.0433	0.0441	0.0459	0.0488	0.0459	0.0500
	0.1073	0.1020	0.0981	0.0915	0.0922	0.1061	0.0991	0.1000

These figures correspond to the following quantities of guanin and adenin:

WEIGHT OF GUANIN AND ADENIN IN 1 GRAM OF NUCLEIC ACID.

Preparation	4	5	7	8		10		
				I	II	I	II	Calculated
Guanin ...	0.1249	0.1235	0.1182	0.1022	0.0999	0.1235	0.1148	0.1080
Adenin ...	0.0953	0.0865	0.0835	0.0850	0.0885	0.0941	0.0885	0.0964
	0.2202	0.2100	0.2017	0.1872	0.1884	0.2176	0.2033	0.2044

The total purin nitrogen, thus found, in the purest of these preparations, namely, **4, 5** and **10,** is very nearly equal to the amount calculated for one molecule of each of these bases for every four atoms of phosphorus, whereas that found in **7** and **8,** which are not quite so pure, was a little less.

Although in these determinations the amount of guanin nitrogen was found to be higher than the adenin nitrogen, the amounts are so nearly the same that there can be no doubt that these bases are present in equal molecular proportions and that the differences observed are due to the difficulties presented in completely separating the two bases, a little adenin being doubtless precipitated with the guanin.

It would appear from these results that all of the purin bases are easily precipitated from the solutions containing the other hydrolytic products of decomposition of tritico-nucleic acid and that no such difficulties are met with as those described by Schmiedeberg,[50] who states that he was unable to completely precipitate the silver salt of these bases in the presence of the other decomposition products of salmo-nucleic acid. He confirmed, therefore, Kossel's[51] assertion that thyminic acid, from thymo-nucleic acid, prevents the precipitation of guanin and adenin as silver salts. Schmiedeberg also found that a very considerable amount of some other substance was precipitated with the silver purin compounds, which on evaporation with hydrochloric acid yielded melanin-like decomposition products.

In our determinations of guanin and adenin, we have largely avoided errors due to such products by determining the nitrogen in the precipitates, instead of weighing them, for we found that the results obtained by weighing were always somewhat higher than those calculated from the nitrogen content of the precipitate.

Kossel[52] obtained a compound of guanin with ammonia, which was stable at 110°, and it might therefore be thought that such a compound was formed under the conditions of these experiments. A distillation with sodium hydroxide of the guanin obtained in the determinations made in preparation **10,** as above given, showed no trace of ammonia, so that it is

[50] Archiv. f. Exper. Path. u. Pharm. **43,** 57.
[51] Zeit. f. physiol. Chem. **22,** 74, 1896.
[52] Zeit. f. physiol. Chem. **7,** 17.

probable that in these analyses an ammonium compound of guanin was formed in very small quantity, if at all.

These determinations make it almost certain that an equal number of molecules of the two bases are contained in these preparations of the nucleic acid, and since **8** represents the whole of the nucleic acid obtained from the wheat embryo, while **4**, **5** and **10** are purified fractions of the acid, it is certain that both of these bases are present in each molecule of nucleic acid and that the preparations are *not* mixtures of two acids, one containing guanin, the other adenin, for it is hardly possible that two acids should have so nearly the same solubility as to escape separation, to a recognizable extent, by the fractional precipitations occurring during the process of purification.

By treating tritico-nucleic acid with alkalies, it has been found that the purin bases are removed very slowly and incompletely. The rate at which these bases are split off was determined by boiling one gram portions of the acid with 100 cc. of normal sodium hydroxide solution for various times, neutralizing with hydrochloric acid, adding an excess of ammoniacal silver oxide solution, filtering out the silver purin precipitate, washing it free from ammonia and determining the amount of nitrogen in it. In this way the following percentages of the purin nitrogen were split off during the times indicated.

PERCENTAGE OF THE PURIN NITROGEN SPLIT OFF FROM TRITICO-NUCLEIC ACID BY BOILING WITH ALKALI.

After boiling	1¼	hours	2.1 per cent.
"	4	"	17.9 "
"	6	"	20.0 "

These figures show that the bases are separated much more slowly by alkalies than by acids, a result in harmony with those obtained by Stokes,[53] who found that in amido-phenylphosphoric acids the amido binding, while very unstable in acid solutions, was very stable in alkaline, whereas the reverse was true for the hydroxyl binding. We may, therefore, conclude that the purin bases are joined to the phosphorus by a nitrogen and not by a carbon atom.

[53] Amer. Chem. Jour. 16, 123, 1894.

b. *Ammonia.*

Kossel and Neumann[54] found ammonia among the decomposition products of thymo-nucleic acid. We, also, have found it among those of tritico-nucleic acid, but only after boiling the nucleic acid with a strong mineral acid for some time.

Thus, when one gram of the acid was boiled for 30 minutes with 2 per cent. sulphuric acid and the resulting solution distilled with an excess of magnesia, no trace of ammonia was found.

When one gram was boiled for 6½ hours with 12 per cent. hydrochloric acid, the solution diluted with twice its volume of water and precipitated with phosphotungstic acid, the precipitate washed, dissolved in sodium hydroxide solution and distilled, 0.0110 gram of nitrogen was evolved as ammonia.

Another gram, hydrolyzed under the same conditions, gave a solution, which, when freed from the greater part of its hydrochloric acid by evaporation and the residue distilled with an excess of magnesia, gave 0.0101 gram. Two portions of nucleic acid weighing one gram each, when boiled with 12 per cent. hydrochloric acid for ten hours and the solution distilled, after standing over night, yielded respectively 0.0170 and 0.0180 gram of nitrogen. This ammonia was unquestionably, for the most part, yielded by guanin, adenin and uracyl, as we found that, when 0.1 gram of guanin, 0.1 gram of adenin and 0.16 gram of uracyl were similarly boiled for ten hours with 12 per cent. hydrochloric acid and the ammonia determined by distilling with magnesia, the following quantities of nitrogen, as ammonia, were produced from each:

Guanin	0.0020 gram.
Adenin	0.0103 "
Uracyl	0.0010 "
Total	0.0134 "

As Kossel[55] has stated that adenin may be boiled for hours with hydrochloric acid without being changed, we repeated this experiment by boiling 0.1 gram of adenin with 20 per cent. hydrochloric acid for seven hours and found 0.0100 gram of nitrogen as ammonia.

[54] Ber. 27, 2215, 1894.
[55] Zeit. f. physiol. Chem. 12, 248.

One gram of tritico-nucleic acid was distilled with normal sodium hydroxide solution and the water lost by distillation continuously replaced. The first distillate, 220 cc., contained .0180 gram of nitrogen as ammonia, which is very nearly two-sixteenths of the total nitrogen; the second .0040, the third .0043, and after this the amount fell to .0010 or .0020 gram of nitrogen in each distillate, until after four days only insignificant quantities were found. During this time about eight liters of water had distilled over. The total nitrogen found in the distillates was 0.0742 grams or 46.4 per cent. of the total, equivalent to more than seven of the sixteen atoms of the nitrogen in the nucleic acid molecule.

Since guanin and adenin yielded not more than minute traces of ammonia when we boiled one gram of each under the same conditions, it seems almost certain that uracyl, two molecules of which, as we shall next show, are present in the nucleic acid, also would not. It would seem, then, as if the atoms in the radicals yielding guanin, adenin or uracyl exist under different conditions than in these bodies when isolated, for at least five of the seven atoms thus passing over as ammonia must have belonged to one or another of these complexes.

c. *Pyrimidin Compounds.*

Twenty grams of air-dry tritico-nucleic acid, which contained phosphorus equal to 16.22 grams of the pure acid, was digested in an autoclave for two hours at 150-160°, with 45 cc. of 20 per cent. sulphuric acid. A large black mass of undissolved matter remained, which was treated with hot water and thoroughly washed.

The filtrate and washings were then made to contain 5 per cent. of sulphuric acid, and phosphotungstic acid added as long as a precipitate formed. This was filtered out, washed with water, dissolved with sodium hydroxide solution and reprecipitated with an excess of sulphuric acid, equivalent to 5 per cent. of the solution, and a little more phosphotungstic acid added. The resulting precipitate was filtered out and washed, and the filtrate and washings added to those first obtained. This solution was freed from phosphotungstic acid by barium hydroxide and the filtrate from the precipitate, so produced, was

freed from barium by a slight excess of sulphuric acid. An excess of silver nitrate was then added to the solution, filtered from the barium sulphate, and ammonia enough to neutralize the free acid. The voluminous white precipitate that formed was filtered out, washed with water, decomposed with hydrogen sulphide, the silver sulphide filtered out and washed and the solution evaporated to dryness. The residue, which was left, weighed 1.78 grams, equal to 11 per cent. of the original acid. This residue was dissolved in a little hot water and allowed to cool slowly, whereupon a large quantity of colorless crystals separated in microscopic balls and bunches of needles. The mother liquor from these crystals, on concentration, yielded a second crop of similar crystals and the small quantity of substance in the second mother liquor, on further concentration, did the same, showing that practically the whole of the original 1.78 grams of residue consisted of this substance. To these crystalline products was added about one-half as much more substance obtained in the same way in a preceding experiment, and the whole, after decolorizing with animal charcoal, was repeatedly recrystallized. The pure product, thus prepared, melted, with decomposition, on rapid heating at 337° (uncor.), which agrees well with the melting point of uracyl determined by E. Fischer and Hagenbach[56] to be 336°.

This substance when dried at 110° and analyzed was found to have the composition of uracyl:

	Found.		Calculated for $C_4H_4N_2O_2$.
Carbon.....	43.08	43.21	42.83
Hydrogen	....	3.65	3.59
Nitrogen	....	25.09	25.05

From these results there can be no doubt that this substance is uracyl and that the tritico-nucleic acid, in this respect, resembles the yeast nucleic acid, in which Ascoli[57] found this substance. One molecule of uracyl in the molecule of nucleic acid is equal to 8 per cent. of the latter. In the above described experiment 11 per cent. of uracyl was found, from which we must conclude that there are at least two molecules of this

[56] Ber. 34, 3751, 1901.
[57] Zeit. f. physiol. Chem. 31, 161, 1900.

body in the nucleic acid molecule. That the quantity found should fall so far short of the 16 per cent. required for two molecules, is not surprising in view of the long process involved in its separation and the great bulk of the phosphotungstic acid and barium hydroxide precipitates.

That the guanin and adenin, which contain the pyrimidin ring, are not the source of the uracyl is shown by the fact that when the purin bases were removed by a brief hydrolysis and the residual portion of the nucleic acid subjected to further hydrolysis, uracyl was found among the decomposition products. The fact that thymo-nucleic acid, which also contains these bases, yields no uracyl, but thymin in its stead, is further evidence in this direction.

A 1 per cent. aqueous solution of the uracyl thus obtained is not precipitated by phosphotungstic acid nor by barium hydroxide. With lead acetate and ammonia, silver nitrate or mercuric nitrate, it gives voluminous white precipitates; with copper acetate no precipitate, even after adding an excess of alcohol.

If a solution of copper acetate is added to an aqueous solution of uracyl and then dilute sodium hydroxide solution, drop by drop, the precipitate of copper hydroxide first formed redissolves and a very considerable amount passes into solution before any remains undissolved.

Uracyl is much more firmly bound within the molecule of nucleic acid than are the purin bases and has been obtained in sufficient quantity for identification only after the profound decomposition of the acid caused by strong sulphuric acid at high temperature or by very long continued action of strong hydrochloric acid. Thus after boiling 10 grams of the acid with 100 cc. of 10 per cent. sulphuric acid for four hours no uracyl could be found in the solution, but by prolonged treatment with hot concentrated hydrochloric acid it was found in quantity among the decomposition products.

d. *The Carbohydrate Group.*

Like the other nucleic acids previously described, triticonucleic acid gives no copper oxide reduction even after boiling for some time with hydrochloric acid.

Kossel and Neumann, *l. c.*, found both formic and laevulinic acids among the decomposition products of thymo-nucleic acid, from which they concluded that this nucleic acid contains a hexose group.

No formic acid was found in the distillate from 10 grams of tritico-nucleic acid when boiled for seven hours with 5 per cent. sulphuric acid, nor was any laevulinic acid found by shaking the residual acid solution with ether. We therefore conclude that there is no hexose group in this nucleic acid.

The distillate produced by boiling with acids contains, however, large quantities of furfurol, in which respect the tritico-nucleic acid resembles the yeast nucleic acid, from which Kossel[58] likewise obtained this substance.

The furfurol is evolved slowly, and is found in the distillate in appreciable quantities even after boiling for many hours. In this fact we have, perhaps, an explanation of the failure to obtain a copper oxide reduction, as it is possible that the sugar is decomposed by the acid as rapidly as it is separated from the nucleic acid.

The following figures show the amount of furfurol-phloroglucide obtained from one air-dry gram of preparation 8 in each successive 500 cc. of distillate:

WEIGHT OF FURFUROL-PHLOROGLUCIN.

	I.	II.
1st distillate.....	0.2165	0.2325
2d "	0.0205	0.0255
3d "	0.0180	0.0260
4th "	0.0083	0.0100
Total.....	0.2633	0.2940

The amount of furfurol was calculated from the weight of the phloroglucide by dividing the quantities greater than 0.2 gram by 1.895 and those less than 0.2 gram by 1.82. Subtracting 0.0104 gram from the furfurol found in each of the above determinations and multiplying by 1.91, we find 27.5 and 30.6 per cent. respectively of xylose in the nucleic acid dried at 110°; or, multiplying by 2.35 we find 33.8 and 37.6 per cent. of arabinose. Three molecules of pentose are equal to 32.2 per cent. of the nucleic acid, with which the above determinations

[58] Verhandl. der Physiol. Gesellsch. zu Berlin, 14 Oct., 1892.

agree more closely than with the quantity calculated for four molecules. There can be little doubt, therefore, that the nucleic acid molecule contains three pentose groups.

In this connection it is interesting to note that very recently Neuberg[59] has found that the pentose contained in the pancreas and derived, presumably, from guanylic acid, is xylose.

To confirm the preceding figures, two portions of 1 gram each of preparation **4**, air-dry, were distilled for seven hours with 12 per cent. hydrochloric acid and 0.2520 and 0.2495 gram of furfurol-phloroglucide were obtained. These quantities are equal to 25.4 and 25.1 per cent. of xylose in the nucleic acid dried at 110° or to 31.2 and 30.9 per cent. of arabinose.

e. *Phosphoric Acid.*

Kossel and Neumann[60] found that the aqueous solution of thymo-nucleic acid, when heated on the water bath for about ten minutes, underwent hydrolysis to such an extent that the purin bases were wholly removed and that, when this operation was carefully conducted, none of the phosphorus appeared as orthophosphoric acid.

This process cannot be applied to tritico-nucleic acid, because it is not soluble in water and when heated therewith forms a dense and gummy mass that permits only a superficial action of the water. This acid is, however, much more resistant to hydrolysis than thymo-nucleic, as it must be boiled with 1½ per cent. hydrochloric acid for at least 30 minutes before all the purin bases are set free.

When heated for 30 minutes with 1½ per cent. hydrochloric, or with 2 per cent. sulphuric acid, all the purin bases are removed, but the solution contains also some orthophosphoric acid. When 1 gram of preparation **8** was boiled for 30 minutes with 2 per cent. sulphuric acid, and the orthophosphoric acid at once precipitated with baryta and its amount determined in the usual way, it was found that 20.76 per cent. of the total phosphorus of the nucleic acid had been converted into orthophosphoric acid by the boiling acid. In another experiment the phosphoric acid was precipitated directly with ammonium

[59] Ber. 35, 1467, 1902.
[60] Zeit. f. physiol. Chem. 22, 74, 1896.

molybdate, the precipitate at once filtered out, to avoid further hydrolytic action of the acids and 22.8 per cent. of orthophosphoric acid was found. The remaining phosphorus appears to be mostly contained in a complicated organic acid, which is difficult to separate and purify.

This acid might be thought to correspond to the thyminic acid which Kossel and Neumann (l. c.) obtained from thymo-nucleic acid, and to the nucleotinic acid which Schmiedeberg assumed to be formed when the purin bases were removed from salmo-nucleic acid. The results of analysis of the barium salts of the acid derived from the tritico-nucleic acid, however, indicate that it is formed by a furthergoing decomposition of the nucleic acid by which a part of the carbohydrate and phosphorus are split off as well as the purin bases.

This acid was separated by the same process that Kossel and Neumann used in preparing their thyminic acid.

The nucleic acid was boiled for about 45 minutes with 2 per cent. sulphuric acid and a sufficient excess of baryta water added to give a distinct alkaline reaction with phenolphthalein. After standing over night the precipitate, which contained guanin, together with barium phosphate and sulphate, was filtered out and one and one-half volumes of alcohol were added to the filtrate. A very voluminous precipitate was thus produced, which settled slowly. This was filtered off, after standing over night, dissolved again in water, of which a considerable quantity was required, the solution filtered from a little insoluble matter, chiefly barium carbonate, and again precipitated by mixing with one and a half volumes of alcohol.

When dried to constant weight over sulphuric acid, the substance thus prepared gained in weight when dried in air at 110°. Three different preparations so made were dried and analyzed with the following results:

	11	12	13
Carbon	....	20.80	20.72
Hydrogen	....	3.37	3.19
Nitrogen	6.52	6.52	6.68
Phosphorus	5.60	6.08	6.22
Barium	30.65	31.16	30.99

On distilling with acid, preparation **12** yielded furfurol equal to 22.7 per cent., that from **13** to 21.3 per cent. of pentose, calculated for the mean between xylose and arabinose.

Preparation **13**, when dried in a current of hydrogen at 100°, lost 4.28 per cent. of moisture. Calculating the formula for the free acid, after deducting the moisture and barium, we have $C_{26}H_{48}N_7P_3O_{26}$.

It has already been shown that, by the brief hydrolysis employed in preparing this acid, all the guanin and adenin are split off and, at the same time, very nearly one-fourth of the phosphorus appears as orthophosphoric acid.

The amount of pentose found in these barium salts corresponds very closely with the quantity calculated for two molecules of this sugar for every three atoms of phosphorus, which shows that one of the three pentose groups of the original acid is also split off.

The sum of the atoms contained in the radicals which represent the groups split off from the original nucleic acid, is $C_{15}H_{17}N_{10}PO_6$.

If this is subtracted from the formula of the nucleic acid, we have as a remainder $C_{26}H_{45}N_6P_3O_{25}$, which agrees closely with that of the acid contained in the barium salts, $C_{26}H_{48}N_7P_3O_{26}$.

The excess of one atom of nitrogen is probably due to a little adenin, in the presence of which the barium salt was precipitated, for an examination of preparation **13** showed that it contained a little of this base. The excess of three atoms of hydrogen and one of oxygen may well be due to a molecule of water, which was not driven off at 100°. If these assumptions are correct, it would seem probable that one of the four molecules of phosphoric acid, present in the tritico-nucleic acid, is united to a guanin, an adenin and a pentose group, and that this is easily split off from the rest of the nucleic acid molecule and at the same time decomposed into its constituent groups.

In alkaline solutions the hydrolysis proceeds very differently from that in acid solutions, as already indicated in connection with the purin bases, which are separated with difficulty by alkalies, though easily by acids.

On boiling with alkalies, orthophosphoric acid is the most abundant decomposition product detected, the only other ones found being the two purin bases, though the remaining constituents of the nucleic acid can easily escape detection, owing to the difficulty of isolating them.

Gram portions of the nucleic acid were boiled with 100 cc. of normal sodium hydroxide solution for different definite lengths of time and then treated, while boiling hot, with baryta solution, the precipitates were filtered out, washed with hot water and the amount of phosphorus determined in them. Other gram portions were similarly boiled for the same definite lengths of time, the free purin bases precipitated by silver oxide, dissolved in ammonia, the purin silver compounds washed free from ammonia and the nitrogen determined in them. The following figures show the percentage of the total phosphorus and of the total purin bases which were thus found to have been split off by boiling for the times indicated:

	Phosphorus.	Purin bases.
1¼ hours.	21.5 per cent.	2.1 per cent.
4 "	47.0 "	17.9 "
8 "	58.3 "	20.0 "

From these figures it would seem as if the part of the nucleic acid molecule to which the purin bases are attached is less easily broken up by alkalies than is the other part.

It would appear that nearly 25 per cent. of the phosphorus is readily split off, since 21.5 per cent. was obtained after boiling for only one and one-quarter hours, which suggests that one of the four atoms of phosphorus is present in the nucleic acid molecule under different conditions from the others.

By boiling for two hours, an aqueous solution of an acid sodium nucleate, which dissolved in water with an acid reaction to litmus, 7.35 per cent. of the phosphorus appeared as orthophosphoric acid, while no trace of any free purin bases could be detected with ammoniacal silver solution.

It is, however, to be remembered that, by long boiling with alkali, we found that nearly one-half the nitrogen of the nucleic acid was evolved as ammonia, so that it is possible that a notable proportion of the purin bases, that were liberated by the alkali, may have been destroyed and also that the presence of the other decomposition products of the nucleic acid may have hindered the precipitation of the purin silver compounds, as Kossel stated was the case with thymo-nucleic acid when boiled in acid solution.

f. *An unidentified decomposition product of Tritico-nucleic Acid.*

Kossel and Neumann[61] described, as cytosin, a decomposition product of thymo-nucleic acid, which was precipitated by phosphotungstic acid or by silver nitrate in neutral solution, but its character was not established, owing to the difficulty encountered in isolating a sufficient quantity of it.

We, also, have found a substance which, under the above conditions, is precipitated from solutions containing the hydrolytic decomposition products of tritico-nucleic acid, but, as yet, we have been unable to separate it in sufficient quantity for identification. Although this substance is precipitated from its solution by phosphotungstic acid, it cannot be afterwards separated from the precipitate by decomposing this with baryta, because its barium compound is insoluble and remains undissolved with the barium phosphotungstate.

By suspending the phosphotungstic acid precipitate in acidified water and shaking out with ether, according to Winterstein's method,[62] it has been possible to obtain a little of the body in question in an impure condition. The decomposition of the phosphotungstic acid precipitate could not be made complete in any way that we tried, and, as its bulk was so great, it was almost impossible to wash it free from phosphoric acid, which subsequently went into the aqueous solution and contaminated the basic body that we were attempting to separate. The product thus obtained yielded a gummy precipitate with picric acid, which dissolved on adding more water, and by silver nitrate, in neutral solution, was precipitated, but the product contained so much silver phosphate that it was not suitable for analysis.

g. *Melanin-like decomposition products of Tritico-nucleic Acid.*

Schmiedeberg[63] states that the ground substance of nucleic acid, that is, the part of the acid other than the purin bases, is characterized by a great tendency to form melanin. He collected the black melanin formed by repeatedly evaporating nucleic acid with hydrochloric acid, washed the precipitate

[61] Ber. 27, 2215, 1894.
[62] Zeit. f. physiol. Chem. 34, 153, 1901.
[63] Archiv. f. Exper. Path. u. Pharm. 43, 57, 1899.

with dilute acid, dissolved it in ammonia and treated the resulting solution with magnesia mixture. After filtering out the ammonium magnesium phosphate he precipitated the melanin with hydrochloric acid and found that it contained much phosphorus, from which he concluded that it was a phosphorized melanin.

We have observed the formation of similar black products when tritico-nucleic acid is boiled with sulphuric or hydrochloric acid, it being especially abundant when the solution is repeatedly evaporated with the latter acid.

On decomposing the nucleic acid by heating with 20 per cent. sulphuric acid at 150° for two hours, a large amount of this melanin-like matter was obtained, which was filtered out, washed with water and absolute alcohol, and dried at 110°. In this condition it was a bulky, brownish black powder, which in one case, *a*, formed 24.4 and in another, *b*, 23.3 per cent. of the nucleic acid. Another preparation, *c*, was obtained by boiling the nucleic acid with 12 per cent. hydrochloric acid for ten hours and then evaporating the solution to a syrup on the water bath. The insoluble residue was washed thoroughly with water and alcohol and dried at 110°. This product formed 16.2 per cent. of the original acid and appeared similar in all respects to the substance produced by sulphuric acid. These preparations were dried at 110° and analyzed with the following results:

	a.	*b.*	*c.*
Carbon	62.03	60.57	63.14
Hydrogen	4.09	3.70	4.36
Nitrogen	9.63	10.67	5.07
Oxygen	24.25	25.06	27.43
	100.00	100.00	100.00

All these preparations were phosphorus-free and are evidently not the same as Schmiedeberg's *phosphorized* melanin. The amount of them was so great it is improbable that they can have originated from any *one* of the constituents of the nucleic acid.

Kossel[64] states that adenin, heated with zinc and hydrochloric acid, is decomposed and that from the solution, so obtained, he separated about 40 per cent. of the adenin as a black mass, which

[64] Zeit. f. physiol. Chem. 12, 249. 1888.

contained from 28.8 to 33 per cent. of carbon and 47 per cent. of nitrogen. This product he considered to be a mixture, and suggests that it may have some relation to the so-called alzuminic acids. It may well be that our melanins were mixtures of somewhat similar decomposition products of the purin bases, with the humus-like decomposition products of the carbohydrate.

h. *Glycerin.*

Bang[65] found a considerable quantity of glycerin among the decomposition products of guanylic acid. Although we have several times examined relatively large amounts of tritico-nucleic acid for glycerin, we have not been able to detect it among the decomposition products nor have we obtained any reactions which would indicate its presence. We are, therefore, satisfied that tritico-nucleic acid contains no glycerin.

VI. The Constitution of the Molecule of Tritico-nucleic Acid.

It has been shown that for every four atoms of phosphorus there are sixteen of nitrogen in the molecule of tritico-nucleic acid, and that of the recognized decomposition products containing nitrogen we have found one molecule of guanin, one of adenin and two of uracyl, for every four of phosphorus. These products contain fourteen of the sixteen atoms of nitrogen, leaving two unaccounted for. We have also shown that a basic decomposition product occurs, which it was not possible to isolate in sufficient quantity for identification, and it is probable, though not proved, that this contains the two remaining atoms of nitrogen.

Tritico-nucleic acid also yields furfurol, equivalent to three molecules of pentose for every four atoms of phosphorus, and a silver salt with six atoms of silver for every four of phosphorus, from which we conclude that there are six hydroxyl groups in the molecule.

Assuming that the four atoms of phosphorus are united by three of oxygen, we have the following constituents in the molecule of tritico-nucleic acid:

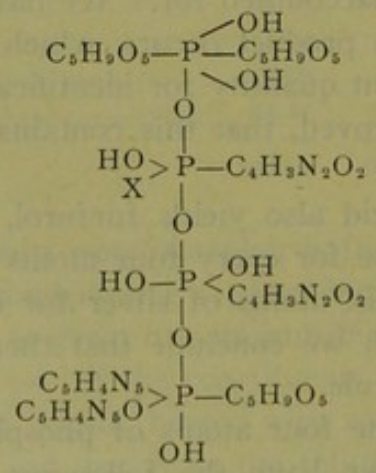
[65] Zeit. f. physiol. Chem. 31, 416.

		Mol. Wt.
Guanin,	1 molecule, $C_5H_5N_5O$	151
Adenin,	1 " $C_5H_5N_5$	135
Uracyl,	2 " $2(C_4H_4N_2O_2)$	224
Pentose,	3 " $3(C_5H_{10}O_5)$	450
Hydroxyl,	6 groups, $6(HO)$	102
Phosphorus,	4 atoms, P_4	124
Linking Oxygen,	3 atoms, O_3	48
		1234

Deducting seven atoms of hydrogen, eliminated when the four first mentioned substances unite with the phosphoric acid, we have 1227 parts of the 1397, which constitute the molecular weight of the tritico-nucleic acid, or 87.8 per cent. There remains, therefore, 12.2 per cent. unidentified, which probably belongs to the basic substance already mentioned.

The sum of the atoms in the radicals of the substances contained in the above table is $C_{33}H_{47}N_{14}P_4O_{29}$, which is less than the formula of tritico-nucleic acid by $C_8H_{14}N_2O_2$. We consider that it is probable that this difference belongs to the unidentified decomposition product, but of this we have as yet no evidence which has much weight.

Assuming, however, that this unknown substance belongs to a single group, and designating it by X, the possible structure of tritico-nucleic acid may be represented by the following formula, in which four atoms of phosphorus are united in much the same way as Bang assumed them to be in guanylic acid:



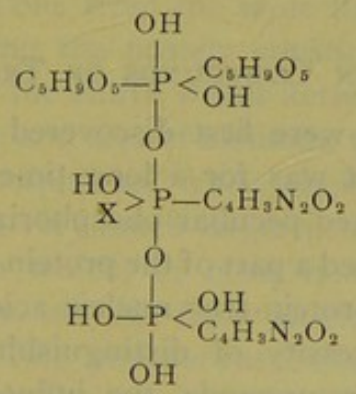
This formula presupposes the union of four $P(OH)_5$ groups, although $P(OH)_5$ is unknown in the free state.

Stokes[66] has shown, however, that the esters of pentahydroxyl phosphoric acid are stable bodies and there can, therefore, be no objection to the above formula on this ground. The position of the various groups in the formula is arbitrarily assigned, with the exception of guanin and adenin and one pentose group which are united with a terminal phosphorus, as there is strong evidence that this phosphorus, with the groups attached to it, is readily split off from the rest of the molecule.

It is assumed that the guanin and adenin are united to the phosphorus by a nitrogen atom, because this union was found to be far more stable in alkaline than in acid solutions.

Uracyl is represented as bound in this way also, though this is not in harmony with the difficulty encountered in separating it from the nucleic acid. If the uracyl were united by a hydroxyl group two atoms of oxygen would be required and there would then be no oxygen remaining for group X, which, however, may in fact contain none.

We have shown that after a brief hydrolysis guanin, adenin, one pentose and one phosphorus atom are split off and that a barium salt of a complicated acid can be obtained from the solution, which has a composition corresponding pretty closely with an acid of the following structure:



VII. The Relation of Tritico-nucleic Acid to the Nucleic Acids previously described.

The nucleic acids of animal and vegetable origin have similar properties and their composition as determined by analysis are much alike. All the later investigators agree in assigning four atoms of phosphorus to their empirical formulas. In salmo-

[66] Amer. Chem. Jour. 16, 123, 1894.

and thymo-nucleic acids there appears to be fourteen atoms of nitrogen for four of phosphorus. In the other animal nucleic acids this ratio is not definitely ascertained.

In yeast nucleic acid the proportion of nitrogen to phosphorus is variously stated by different investigators, but is mostly given higher than in the animal nucleic acids.

The animal nucleic acids as well as the vegetable contain both guanin and adenin. The pyrmidin group is represented in the animal nucleic acids, so far as they have been investigated, by thymin, which is a methyl uracyl; in the yeast and wheat nucleic acids by uracyl. In the thymo-nucleic acid the carbohydrate group appears to be a hexose, in the yeast and wheat nucleic acids to be a pentose. We thus have an apparent distinction between animal and vegetable nucleic acids, but further investigations are required to show in how far this holds good.

Whether all the animal nucleic acids are the same cannot be determined from the information now obtainable, but an examination of the literature indicates that this may prove to be the case. It is also possible that yeast nucleic acid may be identical with tritico-nucleic acid, but this, too, cannot be determined without further investigation.

VIII. The Protein Compounds of Tritico-nucleic Acid.

The nucleic acids were first discovered in combination with protein bodies and it was for a long time supposed that these compounds represented peculiar phosphorized proteids in which the phosphorus formed a part of the protein molecule. Although Miescher obtained protein-free nucleic acid and Altmann later pointed out the necessity of distinguishing the nucleic acids from their protein compounds, the influence of the old view respecting phosphorized proteids persisted and even now seems to have force, as shown by the almost universal practice of writers, who treat the nucleic acids in intimate connection with the proteins as though the two classes of bodies were chemically related.

Since the nucleic acids undoubtedly form a special class of phosphoric acid esters which can readily unite with protein bodies to form artificial compounds, similar to, if not identical

with, those found in the tissues or separated from them, it would seem important to abandon the designation phosphorized proteids as applied to these compounds with nucleic acid, and consider more carefully their true character. Since the protein bodies are now recognized as basic substances, there can be no question but that they can form true salts with nucleic acid. Miescher showed that the salmo-nucleic acid, and we, that tritico-nucleic acid is hexabasic, so that at least six different salts may be formed by each of these acids with any one of the protein bodies. Further, since the proteins are probably all polyacid bases, the number of possible salts that may be formed is still further increased, and consequently when nucleic acid and protein occur in the same solution a large number of different salts may be formed, according to the proportion in which the two substances exist and the nature and proportion of the other bases and acids present. In view of these facts it is not surprising that different investigators have obtained nucleins and nucleo-proteids with very different phosphorus content even when working under apparently similar conditions, nor is the present confusion which exists in regard to these substances to be wondered at.

That, in fact, a great variety of such nucleates can be obtained under different conditions from the same tissue, was shown by the writer[67] in studying the protein constituents of the wheat embryo. Extracts of the entire wheat kernel contained a small proportion of globulin, and also of albumin, which were obtained wholly free from phosphorus, while similar extracts of the embryo meal yielded a very much larger proportion of the same globulin and albumin which, in most cases, contained phosphorus, the proportion varying from an insignificant quantity to over 3 per cent. This phosphorus belonged to the tritico-nucleic acid, since when the amount of nucleic acid corresponding to the phosphorus content of the different preparations was subtracted from their analyses, the remainders all showed the composition of the globulin or albumin previously obtained, phosphorus-free, from the whole wheat kernel. By this investigation it is made evident that a considerable quantity of nucleic

[67] Report Conn. Agri. Expt. Station for 1899, also Jour. Am. Chem. Soc. 22, 379, 1900.

acid can combine with a protein substance without altering its behavior as a globulin or an albumin.

That existing conditions determine the proportion in which the nucleic acid combines with the protein is shown by the behavior of the extracts described in this paper.

The freshly prepared aqueous extract of the wheat embryo is at first neutral to litmus and contains a large amount of protein, chiefly albumin and some proteose, together with much nucleic acid, held in solution by the basic protein matter present in large proportion. If this neutral solution is saturated with sodium chloride, very little precipitate results, but if a small quantity of acid is added, a large precipitate forms at once which contains practically all of the nucleic acid. This precipitate is formed by the increase in the proportion of acid, since the soluble nucleates, containing a large proportion of protein base, can then no longer exist. The new compounds which are formed contain a much smaller proportion of the basic protein and are no longer soluble in the saturated salt solution nor in water. By digesting the resulting precipitate with pepsin, the proportion of protein is still further reduced and insoluble compounds are formed, which are incapable of further alteration by the pepsin. These products, which have the properties characteristic of "nucleins," are still merely protein salts of nucleic acid; no hydrolytic action is required to separate their two constituents; the nucleic acid can be largely removed as sodium nucleate by simply treating them with a solution of sodium acetate; and furthermore, the protein can be almost completely converted into protein picrate by adding picric acid to their alkaline solutions and acidifying with acetic acid, the nucleic acid remaining in solution as an alkaline nucleate.

It is evident, therefore, that there is no reason to assume that the union between the nucleic acid and the protein is different from that which exists between base and acid in other salts.

We have already stated that protein nucleates exist, which resemble globulins or albumins, the character of the nucleate being determined by the nature of the combined protein, the globulin yielding nucleates with the characteristics of globulin, the albumin those with the characteristics of albumin. In this respect nucleic acid behaves like other acids which, in small proportion, unite with protein substances to form salts, which,

as the writer has shown in the case of edestin, have the properties of globulin and are obtained crystalline by dialysis or by cooling their warm, concentrated solutions.[68] There is, therefore, no reason for considering these protein compounds of nucleic acid as in any way different in character from those of the other acids.

Whether all of the many "nucleo-proteids" which have been obtained from various cells and tissues are protein nucleates cannot, of course, be determined without a special examination of each, but it is not at all improbable that they are such and it would seem highly probable that the nucleic acid is contained within the cell in salt-like combination. The lack of uniformity in the composition of the many preparations of these substances, obtained by various investigators, especially the wide variations in their content in phosphorus, points strongly to this view of their nature. If this be so, the products isolated from the cells cannot be regarded as necessarily existing, as such, in them, for the proportion in which the protein and the nucleic acid unite would depend upon the conditions prevailing at any given moment. As these conditions during life must be constantly changing, the protein nucleates must be changing also and their great physiological importance is doubtless due, in large measure, to this fact.

The high basicity of nucleic acid enables it to enter into the formation of a multitude of chemically different products, forming many different salts not only with one and the same protein, but with all other basic bodies, as well as mixed salts with the several different bases which may be present.

As to the occurrence of the nucleins in nature, much the same may be said.

The compounds of protamine with nucleic acid have been regarded as salts since Miescher first discovered them and these doubtless exist as such in the cell. As to the insoluble protein compounds, which contain a large proportion of nucleic acid, compared with the nucleo-proteids, the facts are not so clear. Certainly the "nuclein," which we obtained in this investigation from the wheat embryo, did not exist as such in the tissues of

[68] Zeit. f. physiol. Chem. 33, 240; also Report Conn. Agri. Expt. Station for 1900.

the seed, but was formed during the process of preparing the nucleic acid. Whether the nuclein obtained by Miescher, by digesting pus cells with pepsin until all the other constituents were removed and only the nuclein remained apparently but little, if at all, changed, so far as microscopic examination showed, represented the unaltered substance of the cell nuclei, or not, is not entirely certain, but it is quite possible that it did. Cohnheim[69] states that the "nucleins" do not occur in nature, as such, but are always splitting products of nucleo-proteids. He adopts Kossel's and Lillienfeld's view that the nucleo-proteids on splitting yield protein and nuclein, which latter when further decomposed yields protein and nucleic acid. In what way these groups are united is unknown, but such facts as are known incline him to the idea that the protein and nuclein are combined as salts. Cohnheim considers, however, that this is not proved and that the formation of nuclein is here left out of consideration.

It seems to us that the formation of the nuclein may be easily explained by considering the relation of the proteins to acids. These bodies unite with small proportions of acids to form salts, in which the protein molecule retains its original properties unchanged. Thus edestin forms definite crystalline salts with one and with two molecules of hydrochloric acid, and to these primary salts many of the nucleo-proteids probably correspond. Under the hydrolytic action of acids, the acid capacity of the protein increases, the first product, standing very near to the unchanged edestin, combining with at least three molecules of hydrochloric acid,[70] while the total acid capacity of edestin, as tested by tropaeolin, corresponds to twenty molecules. The behavior of the other native proteins is similar. With the formation of the proteoses, the acid-combining power increases greatly, as has long been known.

As the nucleins are always obtained from solutions containing relatively much acid, it is highly probable that the acid capacity of the protein is thereby greatly increased, and consequently each molecule combines with several of nucleic acid, thus forming the compounds now known as nucleins.

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[69] Die Eiwesskorper. Braunschweig, 1901, p. 198.

[70] Osborne, Zeit. f. physiol. Chem. 33, 225; also Report Conn. Agri. Expt. Station for 1900, p. 388.

Nothing in this view precludes the possibility that the nucleic acid may also exist in the cell in some other form of combination than as a salt. It is quite possible that esters may also occur, but the evidence in respect to this has not yet been presented.

The former statements that nucleic acid occurs free in the cell are now no longer considered to be correct, and in view of the large proportion of protein associated with nucleic acid it would seem to be quite impossible that any should remain uncombined therewith.

IX. Relation of Nucleic Acids to Guanylic Acid.

The conception of the nucleic acids as esters of a complex phosphoric acid, formed by the union of four $P(OH)_5$ groups, brings them into relation with guanylic acid, to which Bang has assigned a somewhat similar constitution. Both may be considered to be esters of a similar phosphoric acid, but differ materially from one another in many essential points.

Guanylic acid, unlike all the true nucleic acids that have been sufficiently well studied, contains no adenin; the proportion of guanin is decidedly greater and the pentose is probably not united directly to the phosphoric acid, but to glycerin.

Guanylic acid may be called a purin ester of a glycero-phosphoric acid, tritico-nucleic acid a mixed ester of a glyco-phosphoric acid.

Such decided differences in constitution must correspond to differences in their physiological relations. It would seem, therefore, to be doubtful that guanylic acid is a constituent of the cell nuclei and probable that its physiological relations are quite different from those of the nucleic acids proper.

In this connection it is important to recall that Levene[71] has obtained an acid from the pancreas which had the properties of a true nucleic acid, contained both guanin and adenin in abundance, probably contained thymin, and gave on analysis atomic ratios for carbon, hydrogen, nitrogen and phosphorus, which were very similar to those found for tritico-nucleic acid.

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[71] Zeit. f. physiol. Chem. **32**, 541, 1901.

X. Relation of the Nucleic Acids to the Paranucleic Acids.

Some time ago one of us pointed out the possibility that those physiological phosphoric acids, which were not orthophosphoric acid, might prove to be pentahydroxyl phosphoric acid H_5PO_5, or its first anhydride $H_8P_2O_9$.[72] If this be so, the nucleic acids would be related to the paranucleic acids, as both would then be esters of a pentahydroxyl phosphoric acid.

Their genetic relations would lead one to suspect that a chemical relation exists between them, for the unincubated egg, which contains an abundance of paranucleic acid, but no true nucleic acid, contains much of the latter, after the embryo develops.

Levene and Alsberg,[73] who obtained from egg yolk vitellin preparations containing 10 per cent. of phosphorus, were unable, in any way, to further separate the protein which the substance still contained in abundance, although they used methods by which this was readily accomplished when applied to the protein compounds of true nucleic acids.

They conclude from their experiments, that it is probable that the phosphorus is in ester combination with the protein.

Osborne and Campbell (*l. c.*) found that by calculating the phosphorus of egg yolk vitellin as PO_4, the elementary composition of the remaining part was nearly the same as that of the similar part of the paranuclein derived from it, even when the phosphorus content of the latter was over five times greater than that of the original vitellin, as the following figures show:

			Calculated PO_4—free.	
	Vitellin.	Paranuclein.	Vitellin.	Paranuclein.
C	51.31	45.30	52.59	52.12
H	7.24	6.64	7.42	7.64
N	16.30	14.60	16.70	16.80
S	1.00	0.83	1.03	0.96
P	0.79	4.26	....	....
O	23.36	28.37	22.26	22.48
	100.00	100.00	100.00	100.00

[72] Osborne and Campbell, Report of the Connecticut Agri. Expt. Station for 1899, p. 346; also Jour. Am. Chem. Soc. **22**, 413, 1900.

[73] Zeit. f. physiol. Chem. **31**, 543, 1901.

If four $P(OH)_5$ groups were united in the paranucleic acid in the same way as they are represented to be in the triticonucleic acid, the ratio of phosphorus to oxygen would be as 1 to $4\frac{1}{4}$, which is so nearly equal to PO_4 that the result of the above calculation would not be materially changed if the analyses were calculated free from P_4O_{17} instead of from PO_4.

The fact that the paranucleic acid of the egg yolk, which serves as food for the growing embryo, gives place during development to the true nucleic acids, is strong evidence that the latter owe their origin to the presence of the former.

The experiments of Burian and Schur[74] show that, although only traces of the nuclein bases are to be found in milk, nevertheless, there is a great increase in the amount of these bases and also of nuclein phosphorus in the young animals which are fed exclusively therewith.

The fact that the embryo which develops in the egg is supplied with a large amount of paranucleic acid, among the substances which serve as its food, and that the growing mammal is also furnished with an abundant supply of the same, is a strong indication that this substance is essential for the rapidly growing organism and, as this is furnished in abundance at a time when the development of new cell nuclei is at a maximum, it seems most probable that the paranucleic acid is converted into the nucleic acid which forms a large part of the cell nuclei.

Whether paranucleic acids occur in plants is not yet demonstrated. Wiman,[75] according to the abstract by Hammarsten,[76] obtained a substance by extracting peas with dilute ammonia, which on digestion with pepsin, yielded variable amounts of an insoluble product, which contained phosphorus and which he regarded as a paranuclein. The product yielded "traces" of nuclein bases which were attributed to a contamination. He concludes that legumin is a nucleo-albumin and states that the legumin prepared according to the methods of Osborne and Campbell and of Ritthausen also yields this paranuclein. Whether the pea contains a nucleo-albumin, or not, cannot be determined from the rather indefinite statements contained in this abstract and we regret that we have been unable to refer to the original

[74] Zeit. f. physiol. Chem. 23, 55, 1897.
[75] Upsala Läkareförenings förhandlingar N. F. Bd. 2, 1897.
[76] Jahresberich für Thier-Chemie f., 1897.

paper. The statement that legumin prepared according to the method of Osborne and Campbell is a nucleo-albumin, is certainly incorrect, for in the purified preparations which we made from several different seeds we were not able to find any phosphorus whatever.

It is quite possible that nucleo-albumins may also exist in the pea, which, together with the legumin, are extracted from the seed by dilute ammonia. It is, however, certain that legumin, when properly prepared, is a true globulin and not a nucleo-albumin, as Wiman supposes.

It is indeed probable that true nucleic acid compounds exist in leguminous seeds, possibly together with paranucleic acid compounds, for these seeds, which contain no true endosperm, consist chiefly of an enlarged embryo in which the functions of the embyro and endosperm, of such seeds as wheat, are united.

That nucleo-albumins occur abundantly in seeds is certainly not true, and the repeated assertion that such is the case, founded on the uncertain experiments of Wiman, has led to grave errors and much confusion, respecting the true nature of their protein constituents.

XI. Conclusions.

1. The embryo of wheat contains a relatively considerable quantity of nucleic acid, for which the name tritico-nucleic acid is proposed. About 3.5 per cent. of the commercial embryo meal used in this investigation probably consisted of tritico-nucleic acid.

2. On keeping, the meal undergoes a change so that the unaltered nucleic acid is obtained from it in diminished quantity or not at all.

3. Tritico-nucleic acid has the properties of the true nucleic acids of animal origin, but is less soluble in water.

4. Its composition corresponds to the formula $C_{41}H_{61}N_{16}P_4O_{31}$. It forms acid salts with potassium, sodium or ammonium, which are readily soluble in water with a strongly acid reaction to litmus. In consequence of this, it is impossible to make preparations of the acid wholly free from base. The lack of agreement between the analyses of nucleic acids, heretofore published, is largely due to this fact.

5. On hydrolysis with acids, tritico-nucleic acid yields one molecule of guanin, one of adenin, two of uracyl and three of pentose, for every four atoms of phosphorus, and also an unidentified basic body.

6. Silver tritico-nucleotinate contains six atoms of silver for every four of phosphorus, from which the free acid is supposed to contain six hydroxyl groups.

7. The constitution of tritico-nucleic acid may be represented by the union of four $P(OH)_5$ groups in which the four atoms of phosphorus are united by three of oxygen, and all but six of the remaining fourteen hydroxyls are substituted by the groups named, thus forming a complicated ester of penta-hydroxyl phosphoric acid, an acid unknown in the free state, but which Stokes has shown forms stable esters.

8. By a brief hydrolysis with dilute acids, all the guanin and adenin are split off and, at the same time, about one-fourth of the phosphorus appears as orthophosphoric acid. By dilute alkalies, the purin bases are not easily split off, but orthophosphoric acid is rapidly and abundantly formed.

9. After a brief hydrolysis, in acid solution, a complicated phosphoric acid remains which contains no guanin or adenin and only two pentose groups for every three atoms of phosphorus. The composition of its barium salt indicates that it may be formed from the nucleic acid by splitting off one of the phosphorus atoms, to which are attached the guanin, adenin and one pentose.

10. Tritico-nucleic acid resembles the nucleic acids of animal origin, in that it contains the purin, pyrimidin and carbohydrate groups, together with phosphorus. The purin groups are the same in the animal and vegetable acids, but in the former the pyrimidin and carbohydrate groups are represented by thymin and hexose, in the latter by uracyl and pentose.

11. Tritico-nucleic acid closely resembles, and may be identical with, the nucleic acid of yeast, since both contain uracyl and a pentose and appear to have the same ultimate composition.

12. Tritico-nucleic acid resembles guanylic acid, in that both may be represented as complicated esters of a phosphoric acid formed by the union of four $P(OH)_5$ groups, but otherwise they present marked differences, which indicate different physiological relations.

13. The conception of tritico-nucleic acid as an ester of penta-hydroxyl phosphoric acid suggests a chemical relation that may possibly exist between paranucleic acid and the true nucleic acids, for the organic part of the paranuclein of egg yolk, as one of us has previously shown, has nearly the same composition as that of the organic part of the paranucleo-proteid from which it originated, as is seen when the analyses are calculated PO_4 free. (Four $P(OH)_5$ groups united by three oxygen atoms contain P : O :: 1 : 4¼).

14. The protein compounds of nucleic acid may be regarded as protein nucleates, those containing but little nucleic acid united with much protein forming the nucleo-proteids, those with much nucleic acid and little protein forming the nucleins. The proportion in which the protein and nucleic acid unite is determined by the relative proportion of bases and acids present in the solution at any given time.