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THE NUCLEIC ACID OF THE LYMPH CORPUSCLES

By Rokuro Nakaseko

(From the A. C. James Research Laboratory, Kyoto)

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I. INTRODUCTION

It has been shown by biologists that protoplasm is the physical basis of life, and the cell is its ultimate visible structural unit. It was Dujardin who in 1835 first distinctly called attention to the importance of the "primary animal substance" or "sarcode," although he did not clearly recognize this substance as the seat of life. In 1850 Cohn definitely maintained not only that animal sarcode and vegetable protoplasm were essentially of the same nature, but also that this substance is the real seat of vitality, and hence to be regarded as the physical basis of life. To Max Schultze (1860) is generally assigned the credit of having finally placed this conclusion upon a secure basis; and by him the meaning of the word "protoplasm" was so extended as to include all living matter, whether animal or vegetable.[1]

The nucleus of a cell is a round body suspended in the cell substance; it is distinguishable from the latter by its higher refractive power, and by the intense color which it assumes when treated with staining fluids. It is surrounded by a very thin membrane, ond consists internally of a clear substance (achromatin), through which extends an irregular network of fibers (chromatin). It is especially these fibers that are stained by dyes. Many investigators now hold the view that inheritance has its seat in the nucleus and that chromatin is its

The greater part of this investigation was carried out in the years 1900–1901 at the Sheffield Laboratory of Physiological Chemistry of Yale University, and a brief preliminary report of it was published in the Journal of the Tokyo Chemical Society, Vol. xxiii, pp. 708–720, (1902).

(1) "General Biology" by Sedgwick and Wilson, New York, 1895.

physical basis. As the cell prepares for division the reticulum of chromatin becomes, in most cases, resolved into a thread coiled into a skein which finally breaks up into a number of bodies known as chromosomes. It has been proved in a considerable number of cases that in the fertilization of the ovum by the spermatozoa each germ cell contributes the same number of chromosomes, and the wonderful fact has been established, with a high degree of probability, that the paternal and the maternal chromatic substances are equally distributed to the cells formed at the first segmentation of the ovum.

From this brief introductory remark, it is evident how supremely interesting it is to study the chemistry of the cell nucleus, the seat of the great mystery of life—heredity.

One of the most essential constituents of the cell-nucleus is nucleic acid and it was Friedrich Miescher[2] who first discovered it in 1874, in the spermatozoa of the Rhine salmon. From this date until his death in 1895 he was constantly occupied in the investigation of this substance, as well as of other related substances, and carried the method of its preparation nearly to its perfection. Besides, Altmann.[3] Kossel,[4] Schmiedeberg,[5] and their pupils contributed the great bulk of our earlier knowledge of the nucleic acids.

Miescher's[6] Method of preparation of nucleic acid is the model of scientific exactness, thorough-going precaution, and technical ingenuity, and I cannot refrain from reproducing it here. He says: "Only after a great number of experiments with a constant control of the filtrates, I finally succeeded in preventing, in a certain measure, the splitting of xanthin bodies and in obtaining the preparations whose analysis gave

(2) Verhandl. d. Naturforsch. Gesells. in Basel. VI. Heft. 1. 138–208.
(3) Arch. f. An. u. Phys., Phys. Abth. 1889, s. 524.
(4) Arch. f. Anat. u. Phys., Phys. Abth. 1891. s. 184; 1894 s. 194–200; 1898 s. 157–164; Ber. d. Deuts. Ch. Ges. (1894) XXVII, 2217; XXVI, s. 2754.
(5) Arch. f. Exp. Path. u. Pharm. XLIII. 57 (1899).
(6) Arch. f. Exp. Path. u. Pharm. XXXVII, 1 (1896).

the true composition of the nucleic acid with a very great approximation. In this case, as in the preparation of haemoglobin, the cold plays a prominent role. The room must be so cool that the liquid cannot rise in temperature during the filtration and centrifugation beyond two or three Centigrades. The most rigorous cooling to zero during any longer contact with acid or alkaline liquids, so far as it is possible, is the necessary condition. These conditions are, unfortunately, not too often to be fulfilled in our climate.....The organs (salmon testicles) are crushed, stirred in water, the strained emulsion is precipitated in powdery form with a few drops of acetic acid, and the precipitate is filtered or separated by centrifugation. The white mass is then extracted several hours with strong alcohol at about sixty degrees and washed each time on a Bunsen's filter with alcohol and ether. This operation repeated four times is sufficient. If the further work is not to be undertaken at once, the fat-free material must be kept under alcohol. In a powdery form, even in closed vessels. it soon decomposes in the air, at least to such an extent as it no more meets the necessary requirement.....The mass of sperma, after being extracted with 0.5% hydrochloric acid, is buried in ice, and all the reagents to be used must be cooled before use to zero. As the mass of sperma swells up in the pure water, it is ground in 0.25—0.5% hydrochloric acid, and neutralized with phosphorus-free sodium hydroxide solution until the mixture tastes on the tongue distinctly caustic, and then immersed at once into the ice or snow water, where it has to remain about one hour. In the meantime fifteen small fluted filters are prepared; and the choice of the paper, which is a matter of experience, means a great deal. After one hour, the filtration is commenced; the filter easily stops up, and hence the need of having many of them. The filtrate, perfectly clear and wine yellow, is at once cooled, and, as soon as a sufficient quantity is collected, *is* neutralized by dropping in

half concentrated hydrochloric acid from a burette, so long as the turbidity formed disappears again, and is then again cooled down. Finally the precipitation is carried out under vigorous stirring with the same hydrochloric acid ; it is, however, only complete after the addition of two volumes of strong alcohol. In the meanwhile, there is prepared a cooling jar in the ice water, and each portion of the precipitate is at once poured into it. The precipitate must be a snow-white, coarse powder which easily settles to the bottom. The preparations which remain hanging on the glass rod as a sticky mass are to be thrown away as failures.....The separation of the precipitate must begin at once by means of the centrifuge, as the standing over night with the excess of acid is destructive even at zero. The precipitate is then stirred into a pretty large volume of 60% alcohol and centrifugalized. After repeating this twice, the substance can be considered as pure. It contains no more chlorine, or only a small trace of it, which can be determined simultaneously with phosphorus, and can thus be taken into the calculation as NaCl."

The following materials were already used by different investigators for the isolation of the nucleic acids :

(1) Spermatozoa of salmon by Miescher,[7] Altmann,[8] Schmiedeberg,[9] Herlant.[10]
(2) Spermatozoa of herring by Mathews.[11]
(3) „ and ova of codfish by Levene.[12]
(4) „ „ „ „ sturgeon by Noll.[13]
(5) „ „ „ „ sea-urchin by Mathews.[14]
(6) „ of bull by Kossel.[15]

(7) loc. cit.
(8) loc. cit.
(9) loc. cit.
(10) Arch. f. exp. Path. u. Pharm. XLIV, 148 (1900).
(11) Zeits. f. Phys. Ch. XXIII, 379 (1897).
(12) Journ. of Am. Ch. Soc. (1900) XXII.
(13) Zeits. f. Phys. Ch. XXV (1898) 430.
(14) loc. cit.
(15) Zeits. f. Phys. Ch. XVIII, 545.

(7) Thymus gland of calf by Altmann,[16] Lilienfeld u. Kossel,[17] Kossel u. Neumann,[18] Herlant.[19]

(8) Yeast by Altmann.[20] Kossel,[21] Herlant,[22] Miescher.[23]

(9) Pancreas-nucleo proteid by Bang.[24]

(10) Spleen by Kossel.[25]

(11) Wheat embryo by Osborne.[26]

(12) Bacillus tuberculosis by Levene.[27]

(13) Brain by Levene.[28]

(14) Liver by Levene and Mandel.[29]

(15) Sperm of burbot by Alsberg.[30]

(16) Mucous membrane of intestines by Araki.[30a]

The nucleic acids are characterized by the following general properties. In the dry state they are white, non-hygroscopic powder. Their solubility in cold water is very slight. In the free state they are decomposed by boiling water. They are very easily soluble in alkalies to form the neutral solutions, and are not precipitated from the solutions by the excess of acetic acid, but are precipitated by the mineral acids. Alcohol has no solvent action on them, but fromth e aqueous solutions of their salts, an equal volume of 90% alcohol containing HCl precipitates them in a white gelatinous form.

About the molecular weight and structure[31] of the nucleic

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(16) loc. cit.
(17) Arch. f. Anat. u. Physiol, Phys. Abth. (1893), 157–164.
(18) loc. cit. (19) loc. cit.
(20) loc. cit. (21) loc. cit.
(22) loc. cit.
(23) Arch. f. exp. Path. u. Pharm. XXXVII, 1 (1896).
(24) Zeits. f. Phys. Ch. XXVI, 133 (1898).
(25) Ber. d. d. ch. Gesels. XXVII, 2217 (1894).
(26) "The Conn. Agr. Exp. Station." 1899. 307–310.
(27) loc. cit.
(28) Zeits. Phys. Chem. XXXIX, 481 (1903); XLIII, 201 (1904).
(29) Bioch. Zeits., X.
(30) Arch. Exp. Path. u. Pharm. LI.
(30a) Araki, Z. f. Phys. Chem. XXXVIII, 98 (1903); Inouye, Z. Phys. Chem. XLVIII, 181 (1906).
(31) Levene and Jacobs, J. Biol. Ch., XII, 411 (1912); Steudel, Z. f. Phys. Chem. LXXVII, 497; Jones, J. Bio. Chem. XXXI, 338 (1917).

acids we are yet somewhat uncertain. The study of the decomposition products points to very complex molecular structure. The most important of the decomposition products are:

1. The purine bases: xanthine, guanine.
2. The pyrimidine bases: uracil, cytosine, thymine.
3. Phosphoric acid.
4. Carbohydrates.

THE ANATOMICAL STRUCTURE OF THE LYMPHATIC GLANDS

The glands are oval or bean-shaped bodies, situated in the course of the lymphatic and lacteal vessels, so that the lymph and chyle pass through them on their way to the blood. Lymphatic glands consist of (1) a fibrous envelope, or capsule, from which a framework of processes (trabeculae) procede inward, dividing the gland into open spaces (alveoli) freely communicating with each other; (2) a quantity of gland pulp, occupying these spaces without completely filling them; (3) a free supply of blood vessels, which are supported on the trabeculae; and (4) the afferent and efferent vessels.

The gland pulp, the lymphoid tissue, does not completely fill the alveolar spaces, but leaves between its outer margin and the trabeculae forming the alveoli a channel or space of uniform width throughout. This is termed the lymph-path, or lymph-sinus. Running across it are a number of trabeculae of retiform connective tissue, the fibers of which are, for the most part, covered by ramified cells. The gland pulp is made up of a delicate reticulum of retiform tissue, which is continuous with that of the lymph paths, but marked off from it by a closer reticulation; in its meshes are closely packed lymph corpuscles, travereed by a dense plexus of capillary blood vessels.[1]

(1) Grey's Anatomy, edited by American authors. Lea Brothers & Co., 1897.

METHOD OF PREPARATION OF THE LYMPHO-NUCLEIC ACID

The lymphatic glands preserved in ice are cleansed as much as possible from the adhering foreign tissues and fats. Two to five kilograms of the cleansed glands are passed three times through the hash-machine and the pulp is stirred into about double its weight of chloroform water, and allowed to stand for twenty-four hours, with frequent stirring, and then strained through cheese cloth. By repeating this process two or three times most of the lymph corpucles disengage themselves from the reticulum of the adenoid tissue and pass through the meshes of the cheese cloth. The milky liquid thus obtained is now treated with a hot saturated solution of $Ba(OH)_2$, so long as the precipitate continues to appear by each addition of fresh portions of $Ba(OH)_2$ and is then set aside for twenty four hours. The clear liquid is thrown off and the sediment is filtered through several large fluted filter papers, and washed with a cold saturated $Ba(OH)_2$ solution.

The precipitate is now carefully removed from the filter to a large evaporating dish, carefully neutralized with acetic acid, diluted with about three times its bulk of water, boiled one hour on wire gauze with the free flame, and then filtered through the fluted filter. The residue on the filter is again boiled with water and filtered, and this operation repeated four times. All the filtrates are now combined and after standing over night they are poured with vigorous stirring into an equal volume of 95% alcohol, containing 2% of fuming hydrochloric acid. The precipitation of the nucleic acid in alcoholic hydrochloric acid is always carried on in a bath of melting ice. The nucleic acid now separates in pure white flakes which soon settle on the bottom, and adhere to each other, forming a soft dough. The alcohol is poured off, the soft dough is stirred in 60% alcohol containing HCl, decanted and treated with a little

stronger alcohol containing HCl, and so until ninety-five per-cent alcohol is reached. By this time the mass, which was at first rather sticky, becomes very brittle, and is ground in a mortar with a small quantity of 95% alcohol, and is repeatedly washed with pure 95% alcohol, then with absolute alcohol and finally with ether. It is now spread in a shallow evaporating dish, and dried in a vacuum over sulphuric acid.

The product thus obtained weighs about one per cent of the original weight of the glands taken, and still contains 10–15% of barium, which comes out, on its ignition, as barium phosphate. For purification, the method of Kossel is followed with the following modification. Ten grams of the crude nucleic acid is dissolved in 100 c.c. of water, containing 10 c.c. of 10% ammonia solution, is warmed to about 60° in a water bath, poured into the previously warmed tubes of a centrifuge, and centrifugalized for several hours in a warm room. (In the cold the nucleic acid becomes gelatinized). The clear solution is poured off from the sediment and stirred into an equal volume of 95% alcohol containing 10 c.c. of fuming hydrochloric acid. The nucleic acid precipitated is again dissolved in ammoniacal water and reprecipitated with alcoholic hydrochloric acid. (By each precipitation it is very easy to lose from ten to twenty per cent of the original material). The substance is now gathered on filter paper, repeatedly washed with alcohols of different strengths containing HCl, and finally with absolute alcohol and ether.

By this method of purification I could get a specimen yielding only 1.2% of ash, about half of which consisted, presumably, of phosphoric acid. The substances thus prepared possess all the characteristic properties of the nucleic acid mentioned in the introduction and for the sake of brevity the name "lympho-nucleic acid" will be applied to it.

ANALYSIS OF LYMPHO-NUCLEIC ACID.

Preparation I. A.

18 grams of the crude nucleic acid were obtained from 1,600 grams of well cleansed lymphatic glands of cattle. It was once purified by the method above described, dried in a steam bath and reduced to a fine powder. A certain amount was taken into a weighing tube and dried in an air bath successively for periods of varying duration, and at varying temperatures. The change of weight was noticed as follows:

2	hours	at	110°	..... 6.6775	grams.
3	,,	,,	,,	..... 6.6545	,,
1	,,	,,	,,	..... 6.6353	,,
4	,,	,,	103°	..... 6.6059	,,
5	,,	,,	,,	..... 6.5917	,,
2	1/2	,,	,,	..... 6.5883	,,

The experiment shows that even at the temperature of 103° the lympho-nucleic acid undergoes a gradual loss of weight and becomes more and more brown in color. The loss of weight does not cease even at the end of 17½ hours, when it reaches the amount of 2.79%. The wide variation of the analytical data of different samples of lympho-nucleic acid is thus partly due to the variable amount of moisture contained in them and partly to the variable degree of partial decomposition.

0.6495 gr. of this dry substance was taken into a platinum crucible and ignited. The ash found was 0.2090 gr.=32.18%.

5.9392 gr. of the same substance were subjected to a further purification, and the purified product dried at 102° for 7 hours, when it weighed 3.1456 gr.=52.96%.

(1) 0.2670 gr. of this newly purified product gave on ignition 0.0078 gr. of the ash=2.92%.

(2) 0.3750 gr. gave 0.05738 gr. of nitrogen by Kjeldahl =15.30%.

(3) 0.1495 gr. gave 0.02295 gr. of nitrogen=15.35%.

(4) 0.3658 gr. gave 0.0554 gr. of nitrogen=15.15%.

The determination of phosphorus made by Liebig's method gave the following results:

(5) 0.4018 gr. of the substance gave 0.1490 gr. $Mg_2P_2O_7$ ||10.33% of P.

(6) 1.0481 gr. of the substance gave 0.3853 gr. $Mg_2P_2O_7$ =10.24% of P.

From these data it is found that the proportion of phosphorus to nitrogen in this particular specimen is, on the average, 10.29% to 15.27%. In the atomic ratio it is therefore $P_3 : N_{10}$.

Preparation I. B.

10.38 gr. of the crude lympho-nucleic acid of Preparation I. A. were subjected to three repeated purification processes, and the purified product was found to weigh after three hours' drying at 94°—95°, 4.4522 gr.=42.89%.

(1) 0.2765 gr. of the substance gave on ignition 0.0033 gr. ash=1.2%. Assuming the ash to consist entirely of $BaHPO_4$, the barium contained in the substance was=0.71%.

4.1757 gr. of the substance kept 2 hours at 94°—100° lost 0.0100 gr. in weight; kept 3 hours more at 94°—96° it lost 0.0010 gr. further.

The substance thus dried altogether 8 hours at 94°—100°, was subjected to the following analyses:

(2) The substance taken=0.5185 gr. CO_2 found was 0.7285 gr.=38.32% of C. H_2O found=0.2173 gr.=4.63% of H.

(3) The substance taken=0.3789 gr. CO_2 found was 0.5311 gr.=38.23% of C. H_2O found was 0.1162 gr.=3.47% H.

(4) The substance taken=0.1635 gr. CO_2 found was 0.2310 gr.=38.53% C. H_2O found was 0.0602gr.=4.09% H.

(5) The substance taken=0.3855 gr. N found was 0.05843 gr.=15.16%.

(6) The substance taken=0.2887 gr. N found was 0.04294 gr.=14.88%.

(7) The substance taken=0.1719 gr. $Mg_2 P_2 O_7$ found was 0.0593 gr.=9.61% of P.

(8) The substance taken=0.3714 gr. $Mg_2 P_2 O_7$ found was 0.1328 gr.=9.94% of P.

The result of this series of analyses is summarized in the following table:

	Found.	Average.	Calculated for $C_{41} H_{55} N_{14} P_4 O_{25}$.
C	38.23 38.32 38.53	38.36	38.53
H	3.47 4.63 4.09	4.06	4.31
N	15.16 14.88	15.02	15.39
P	9.61 9.94	9.77	9.72
Ba*	0.71	0.71	0.71
O		31.78	31.34
		100.00	100.00

* The ash content was also taken into consideration in calculating up the figures in the last column.

Preparation II.

4450 gr. of well cleansed cattle lymphatic glands were treated in the usual manner, but the first aqueous extract of the barium hydroxide precipitate was washed separately from the succeeding extracts.

The first extract constitutes Preparation II. A.

The succeeding extracts constitute Preparation II. B.

Preparation II. A.

The crude lympho-nucleic acid was dissolved in 1100 c.c. of dilute ammonia and filtered with a suction pump and acidified with acetic acid. On standing a short while the whole content became gelatinized. It was then liquified by warming to 53° in a water bath, and poured into 1500 c.c. of 87% alcohol without addition of hydrochloric acid. The moist nucleic acid, after pressing between filter folds weighed now 88 grams. It was dissolved again in dilute ammonia and filtered with the suction filter and poured into alcoholic hydrochloric acid and the product thus obtained gave, after drying at 50°—70°, the ash content of 11.2%. It is therefore further purified by dissolving in dilute ammonia, acidifying with acetic acid, filtering through the suction filter, pouring into alcoholic hydrochloric acid, and repeatedly washing with alcohol of different strengths. This process was repeated twice more, and the substance dried in a vacuum desiccator over sulphuric acid. The following analyses were made of the substance:

(1) The substance taken=0.3475 gr. The ash found=0.0070 gr.=2.01% of ash=1.18% of Ba.

(2) The substance taken=0.3201 gr. CO_2 found=0.4194 gr.=35.73% of C. H_2O found=0.1160 gr.=4.02% H.

(3) The substance taken=0.2621 gr. CO_2 found=0.3362 gr.=34.98% of C. H_2O found=0.0974 gr.=4.13% H.

(4) The substance taken=0.2657 gr. CO_2 found=0.3526 gr.=36.2% of C. H_2O found=0.0797 gr.=4.71% H.

(5) The substance taken=0.2977 gr. CO_2 found=0.3812 gr.=34.94% C. H_2O found=0.1270 gr.=4.74% H.

(6) The substance taken=0.4518 gr. N found=0.0561 gr. =12.41%.

(7) The substance taken=0.3893 gr. N found=0.0489 gr. =12.56%.

(8) The substance taken=0.5367 gr. N found=0.0649 gr. =12.09%.

(9) The substance taken=0.3207 gr. $Mg_2P_2O_7$, found= 0.1096 gr.=9.52% of P.

(10) The substance taken=0.9188 gr. $BaSO_4$ found= 0.0200 gr.=0.3% of S.

The result of this series of analyses is summarized in the following table:

	Found.	Average after correcting for ash and sulphur.	Calculated for $C_{39}H_{61}N_{12}P_4O_{29}$ or $C_{39}H_{53}N_{12}P_4O_{25}+4H_2O$.
C	34.94 34.98 35.73 36.20	36.51	36.40
H	4.02 4.13 4.71 4.74	4.80	4.78
N	12.09 12.41 12.56	12.70	13.10
P	9.52	9.67	9.65
O		36 30	36.07
Ba	1.18		
S	.30		
		100.00	100.00

For the sake of comparison I may better represent this nucleic acid by the formula

$$C_{39}H_{58}N_{12}P_4O_{25}+4H_2O$$

It will at once appear to the eye by a comparison of this formula with that of Preparation I. B. that the nucleic acid in this case has lost two atoms each of C, H, and N, in the proportion to form adenine. This is presumably due to the partial splitting of the base by an unusual treatment to which it was exposed. It is also very interesting to note that the formula above given resembles very closely that given by Miescher for the salmon nucleic acid, namely :

$$C_{39}H_{57}N_{12}P_4O_{25}+4H_2O^{(32)}$$

which he found later to be the product of a partial splitting of the xanthine bases from the pure nucleic acid $C_{40}H_{54}N_{14}P_4O_{27}$.

Preparation II. B.

The crude lympho-nucleic acid weighing 57 grams in the moist state, was purified three times by the ordinary method, using the filtration with a suction pump instead of centrifugation. The product thus obtained was found to weigh, after drying over H_2SO_4 in vacuum, about 9 grams.

0.2238 gr. of the substance gave 0.0218 gr. ash=9.74%.

5 grams of the substance was further purified twice by the usual method, and after a thorough washing, dried in vacuum over sulphuric acid. The analytical data are as follows :

(1) The substance taken=0.1328 gr. The ash found= 0.0070 gr.=5.27%=3.10% of Ba (calculated by assuming the ash to consist of $BaHPO_4$).

(2) The substance taken=0.2000 gr. The ash found= 0.0130 gr.=6.50%. This ash was further analysed for P_2O_5. $Mg_2P_2O_7$ found=0.0120 gr.=0.0077 gr. of P_2O_5. The difference=0.0053 is assumed to be BaO=2.37% of Ba.

(32) This formula was obtained by multiplying Miescher's original formula by 4/3. Verhandlungen D. Naturforsch. Cesell. in Basel VI. Heft. 1. 138—208. 1874.

(3) The substance taken=0.0883 gr. CO_2 found was 0.1266 gr.=39.15% of C. H_2O found=0.0441 gr.=5.56% H.

(4) The substance taken=0.1511 gr. CO_2 found 0.2100 gr. =37.904% C. H_2O found =0.0673=4.94% of H.

(5) The substance taken=0.1120 gr. CO_2 found =0.1570 gr.=38.23% C. H_2O found=0.0505 gr.=5.01 H.

(6) The substance taken=0.2667 gr. N found=0.0378 gr.=14.17%.

(7) The substance taken=0.2152 gr. N found=0.0308 gr.=14.31%.

(8) The substance taken=0.1280 gr. N found=0.01876 gr.=14.65%.

(9) The substance taken=0.1275 gr. $Mg_2P_2O_7$ found= 0.0436 gr.=9.52% of P.

(10) The substance taken=0.2748 gr. $BaSO_4$ found= 0.0050 gr. =0.25% of S.

This series of results can be summed up in the following table:

	Found.	Average after correcting for the Ash and Sulphur.	Calculated for $C_{41}H_{54}N_{14}P_4O_{25}$.
C	37.904 38.23	39.09	38.53
H	4.91 5.01 5.56	5.11	5.05
N	14.17 14.31 14.65	14.77	15.39
P	9.52	9.77	9.72
O		31.26	31.31
Ba	2.37		
S	0.25		
		100.00	100.00

A comparison of this result with those of Preparation I. B. and II. A. shows that the present sample of the lympho-nucleic acid is richer in hydrogen than the others. In the other respects the composition is identical in the first and last samples. To show this relationship more clearly to the eye, and to compare my results with those of other investigators on nucleic acids from the different sources, I shall put together these results in the following table :

Lympho-nucleic acid, Preparation II. B.	$C_{41}H_{64}N_{14}P_4O_{25}$
Lympho-nucleic acid, Preparation I. B.	$C_{41}H_{55}N_{14}P_4O_{25}$
Lympho-nucleic acid, Preparation II. A.	$C_{39}H_{53}N_{12}P_4O_{25}+4H_2O$
Salmon nucleic acid, Schmiedeberg and Herlant	$C_{40}H_{56}N_{14}P_4O_{26}$
Salmon nucleic acid, Miescher and Schmiedeberg	$C_{40}H_{54}N_{14}P_4O_{27}$
Salmon nucleic acid, Miescher	$C_{39}H_{57}N_{12}P_4O_{25}+4H_2O$
Lympho-nucleic acid, Nakaseko, Prep. II. A.	$C_{39}H_{53}N_{12}P_4O_{25}+4H_2O$
Thymus nucleic acid, Kossel	$C_{40}H_{69}N_{12}P_4O_{23}$
Thymus nucleic acid, Schmiedeberg and Herlant	$C_{40}H_{53}N_{14}P_4O_{26}$
Thymus nucleic acid, Steudel	$C_{43}H_{61}N_{15}P_4O_{34}+9H_2O$
Yeast nucleic acid, Altmann, Miescher and Schmiedeberg	$C_{40}H_{54}N_{14}P_4O_{27}(OH)_5$
Yeast nucleic acid, Herlant	$C_{36}H_{60}N_{14}P_4O_{33}$
Yeast nucleic acid, Kossel	$C_{34}H_{52}N_{12}P_4O_{28}$
Yeast nucleic acid, Levene	$C_{38}H_{55}N_{15}P_4O_{32}$
Wheat embryo nucleic acid, Osborne	$C_{41}H_{61}N_{16}P_4O_{31}$

A comparison of the above formulas shows a close relation between the nucleic acids of salmon spermatozoa, of thymus glands of calf, and of the lymph corpuscles of cattle. One is almost tempted to conjecture that lympho-nucleic acid is a homologue of salmon nucleic acid, only richer than the latter by CH_2. On the other hand one can easily see the wide variations of the composition of the nucleic acids of the different sources, and of the same source according to the methods of preparation. So far, there is not yet a method known which absolutely guarantees the purity of the product prepared by its use, and it must be confessed that there still remains a certain

degree of uncertainty over the ultimate composition of any nucleic acid.

THE PARTIAL DECOMPOSITION OF THE NUCLEIC ACIDS.

The ease with which the nucleic acids are decomposed was dwelt upon in the foregoing chapters, and it was pointed to as the chief cause of the difficulty of obtaining pure preparations. Indeed, the wide variation in composition of the nucleic acids of the different authors must be chiefly attributed to different degrees of the decomposition undergone by the respective preparations.

A. Neumann[33] claims that the nucleic acids obtained by the method of Kossel and Neumann is a mixture of three acids. The fact that any two preparations made by the same method did not give the same composition made him suspect that there was no homogeneous compound in hand. He claims to have worked out a new method by which he can get any of the three acids at will. He does not, however, give, so far as I am aware, any analytical data to enable one to judge whether each of the three acids is a chemical individual or again a mixture of other compounds.[33]

THE COMPLETE DECOMPOSITION OF THE NUCLEIC ACIDS.

I. The Purine-Bases.

Kossel[34] expressed the opinion that there may be four different nucleic acids each containing one of the four purine bases: xanthine, guanine, hypoxanthine, and adenine. Ivar

(33) Du Bois Reymond's Arch. f. Anat. u. Physiol. 1899. Suppl. s. 555; 1898, 374; Chem. Centralbl. 1898, [2] 1211.
(34) Zeits. f. Physiol. Chem. 22, 74 [1896].

Bang[35] thought he actually found one of these hypothetical acids in the form of his guanylic acid, which contains about 36% of guanine and no other purine-base according to him. P. A. Levene[36] obtained, on the contrary a nucleic acid from the pancreas of cod-fish, and found that "it contains in its molecule adenine in addition to guanine and that this acid does not much differ in its composition from the acids obtained from different sources within the last year by Schmiedeberg, Herlant, etc." Schmiedeberg[37] also concluded that one molecule of the salmon-nucleic acid contained both adenine and guanine. The fact must be noted here that Kossel and Inoko found all the four purine-bases in the testicles of bull, bear, salmon, and carp.

When Kossel and Neumann[37a] first obtained the thymus nucleic acid they thought it contained only adenine and called it "adenylic acid." Their later study convinced them, however, that it contained also guanine[38] and another base which they called "cytosine." Still later Neumann[39] found all the four purine bases in his nucleic acids "A" and "B" of the thymus glands and only adenine and hypoxanthine in his nucleo-thyminic acid.

From these considerations it was quite reasonable that all the four purine bases were also expected from my lympho-nucleic acid. This expectation was fulfilled as the following experiments show, which were made quantitatively and qualitatively on the purine-bases of the lympho-nucleic acid.

A few gramms of the substance were treated with dilute hydrochloric acid (sometimes with dilute sulphuric acid) several

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(35) Bang, Zeits. Phys. Chem. XXVI, 133 [1898]; XXXI (1901), 411–427. Inoko, Ibid. 18, 540 [1898].
(36) Am. Journ. Physiol. (1901) V, No. 2.
(37) Arch. f. Exp. Path. u. Pharm. XLIII, 57 [1899].
(37a) Arch. f. Anat. u. Physiol., Physiol. Abth. [1894], 19; Ber. d. deut. chem. Gesells. [1894], XXVII, 2217.
(38) Zeits. f. Physiol. Chem. XXII, 74 [1896].
(39) Du-Bois Reymond's Archive f. Anat. u. Physiol., 1899, Sup. pl. 552.

hours over the water-bath, filtered and the filtrate was made ammoniacal, whereupon the solution became turbid and gradually deposited a voluminous precipitate. This ammoniacal liquid was then treated with ammoniacal silver nitrate solution. The silver precipitate of the purine bases was well washed with a weak ammonia solution and finally with water. Xanthine in this precipitate was identified by two methods:

[1]. The silver precipitate was decomposed by boiling with hydrochloric acid and the filtrate was evaporated to dryness and then moistened with water and again evaporated to dryness. This operation was repeated several times and finally the xanthine which was now in the free insoluble state, was separated from the soluble hydrochlorides of the other bases.

[2]. The silver precipitate was boiled with the nitric acid of the specific gravity 1.1, previously treated with urea, and this solution was filtered boiling hot. The xanthine silver nitrate which remains in solution after cooling was filtered off and precipitated with ammonia; the precipitate was again dissolved in a small quantity of boiling nitric acid of the sp. gr. of 1.1 containing urea and after cooling filtered off from the small amount of the precipitate formed. The purified xanthine silver was now precipitated as such by an addition of ammonia. The insoluble silver nitrate combinations of the other bases were decomposed by suspending the mass in hot water and passing H_2S gas.

The solution containing the hydrochlorides, or the nitrates of the remaining three bases was now treated with ammonia to precipitate guanine, and the filtrate from the guanine was evaporated to expell the excess of ammonia and slightly acidified with nitric acid and then treated with picric acid. The adenine picrate was immediately filtered off and the filtrate was treated with an ammoniacal silver nitrate solution, whereupon a voluminous precipitate of hypoxanthine silver, strongly coloured yellow, was thrown down. The guanine was purified

by digesting with a warm solution of strong ammonia, and the adenine picrate was purified by dissolving in a few drops of an alkali solution and reprecipitating by acidification.

Experiment A.

The alcoholic hydrochloric acid which was used to precipitate the lympho-nucleic acid was distilled and the residue filtered off and the filtrate treated with the above method for the determination of the proportion of the purine-bases.

Xanthine silver found...	=0,0360 gr.	=0,0143 gr. xanthine.
Guanine found	=0,0018 gr.	=0,0018 gr. guanine.
Adenine picrate found...	=0,0789 gr.	=0,0293 gr. adenine.
Hypoxanthine silver ...	=0,2742 gr.	=0,1040 gr. hypoxanthine.

Experiment B.

Another sample of the alcoholic hydrochloric acid used for the purification of the lympho-nucleic acid was distilled, the residue filtered off, and the filtrate was treated by the same method as above for the separation of the purine-bases. The amounts of xanthine and guanine were determined by the Kjeldahl estimation of nitrogen in the xanthine silver and guanine respectively. Adenine was weighed as adenine picrate, hypoxanthine as hypoxanthine silver picrate.

Nitrogen found in the xanthine silver was	=0,0261 gr.	=0,0706 gr. xanthine.
Nitrogene found in the guanine	=0,0316 gr.	=0,0681 gr. guanine.
Adenine picrate found	=0,0568 gr.	=0,0211 gr. adenine.
Hypoxanthine silver picrate found	=0,3900 gr.	=0,1125 gr. hypoxanthine.

The molecular ratio of the four bases is therefore approximately:

$$3C_5H_4N_4O_2 : 3C_5H_5N_5O : C_5H_5N_5 : 5C_5H_4N_4O$$

xanthine guanine adenine hypoxanthine.

EXPERIMENT C.

0,8035 gr. of a sample of the lympho-nucleic acid was taken into a 100cc. flask and heated with 50cc. of 2% HCl in the water bath for two hours and the purine-bases were determined quantitatively by the above method.

Xanthine silver found	=0,0240 gr.	=0,0095 gr. xanthine. [1,18%].
Guanine found	=0,0022 gr.	=0,0022 gr. guanine. [0,27%].
Adenine picrate found	=0,0065 gr.	=0,0024 gr. adenine. [0,30%].
Hypoxanthine silver found...	=0,0370 gr.	=0,0140 gr. hypoxanthine. [1,75%].
The total purine-bases		3,42%.

The filtrate from the silver combinations of the purine-bases was treated with the magnesia mixture.

$Mg_2P_2O_7$ found =0,0805 gr. =0,0224 gr. phosphorus =2,79% P.

EXPERIMENT D.

2,1535 gr. of a sample of the lympho-nucleic acid were boiled for one hour with 50cc. of 40% [by weight] sulphuric acid under an inverted condenser over a free flame. The reaction product was, after cooling, shaken with ether for extracting the levulinic acid formed and then treated with the phosphotungstic acid and filtered. The precipitate was boiled with barium hydroxide solution and filtered and the excess of the barium in the filtrate was precipated with H_2SO_4 and filtered. The filtrate was now treated with the ammoniacal silver nitrate, etc. as usual. Xanthine, guanine and hypoxanthine were calculated from the Kjeldahl determinations of nitrogen in xanthine silver, guanine, and hypoxanthine silver respectively.

Xanthine silver found	=0,0843 gr.	
Nitrogen found in it	=0,0148 gr.	=0,0402 gr. xanthine. [1,87%].

Nitrogen found in guanine...	=0,0161 gr.	=0,0347 gr. guanine. [1,61%].
Adenine picrate found	=0,0978 gr.	=0,0363 gr. adenine. [1,69%].
Hypoxanthine silver found.	=0,3832 gr.	
Nitrogen found in it	=0,0713 gr.	=0,1728 gr. hypoxanthine. [8,02%].
The total of the purine bases found.....		13,19%
The total of the purine-nitrogen found..		5,62%

Now, the three concordant determinations of the nitrogen in the same sample of the lympho-nucleic acid gave the values: 14,23%, 14,29%, and 14,27%. The average is therefore 14,26% for the total nitrogen contained in this particular specimen of the lympho-nucleic acid. Subtracting from this figure the total purine-nitrogen above mentioned there are left 8,64% of nitrogen in the nucleic acid still to be accounted for.

$$\begin{array}{r} 14{,}26\% \\ 5{,}62\% \\ \hline 8{,}64\% \end{array}$$

It must not be imagined, however, that the above ratio of the purine-bases represents the actual proportion of the respective atom-groups in a molecule of the lympho-nucleic acid. Neither should it be imagined that the figure 13,19% above given represents the total percentage of the purine bases separable from the substance. There are three possibilities still to be taken into consideration:—[1] the action of 40% [by weight] sulphuric acid at the boiling temperature for one hour may not be sufficient to separate all the purine bases from the lympho-nucleic acid, or [2] its action under the above circumstances may be destructive on the purine bases already separated, or [3] these two conditions may exist simultaneously. Indeed Ivar Bang[40] has already shown that guanine liberates ammonia by two hours' boiling with 2% hydrochloric acid while the three hours' boiling with 5%

(40) Zeits. f. Physiol. Chem. XXVI., 140 [1898]; Ibid. XXXI, 420 (1901).

H_2SO_4 has no such effect. It therefore remains to be shown what conditions are the optimum for obtaining the greatest possible quantity of the purine-bases from the lympho-nucleic acid.

I could also identify the thymine[41] by its characteristic crystalline forms in the filtrate from the phospho-tungstic acid precipitate of the experiment D. The crude product that remained after a few chemical examinations weighed 0,0332 gr. and was subjected to the Kjeldahl determination of nitrogen without any previous purification. The nitrogen found was 0,068 gr. =20,5%, while theory requires 22,22%.

The lack of material prevented me from the search after the other pyrimidine bases, cytosine and uracil. Neither was it, so far, possible for me to make a study of the nucleotides and nucleosides expected to be formed by the hydrolysis of the lympho-nucleic acid by heating it with ammonia in an autoclave, or by its neutral hydrolysis at 175°.[42]

II. CARBOHYDRATES.

A. Kossel[43] found that boiling dilute acids produce some reducing substances from the nucleic acid of yeast. These reducing substances do not undergo fermentation by yeast, but give osazones with phenyl hydrazine. By the fractional crystallization he succeeded in separating them into two osazones. One of them melted at 204°—205° and yielded, by the elementary analyses, figures corresponding to the formula of the phenylglucosazone. The second osazone melted at about 150°

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(41) Sits. d. Gesells. z. Beförd. d. ges. Naturw. z. Marburg No. 2, Jan. 1901; Zeits. f. Physiol. Chem. XXXII., Heft. I. u. II.; Gulewitch; Zeits. f. Physiol. Chem. XXVII, 292 [1899].

(42) Jones, W., and co-workers: J. Biol. Chem XX, 25 (1915); XXIX, 111 (1917); XXXI, 48 (1917); Levene and Jacobs: Ber. d. deuts. Chem. Ges. XLI, 2703 (1908); XLII, 335, 1198, 2102, 2469, 2474, 2703, 3247 (1909).

(43) Arch. f. Anat. u. Physiol., Phys. Abth. 1893, 157–164.

and showed a higher percentage of carbon, suggesting the existence of a pentose. He then distilled the original mixture of the reducing substances with sulpuric acid and obtained in the distillate an oil floating on the water and showing very distinctly the properties of furfurol. This distillate was then rectified and treated with ammonia, whereupon there separated out the crystalline needless of furfuramide. It becomes clear from this statement that there are a hexose-derivative and a pentose-derivative among the decomposition-products of the yeast nucleic acid. On the other hand Kossel showed that these carbohydrates cannot be obtained from the nucleics acids of salmon-spermatozoa and carp-spermatozoa. Neither could he get it from the leuco-nucleic acid of thymus.

Bang[44] boiled his guanylic acid with 5% H_2SO_4 for three hours over a water bath and then neutralized it with alkali and filtered after a lapse of some time. The filtrate being titrated with Fehling's solution gave the figures corresponding to 30-36% of dextrose. This reducing substance was not fermentable but gave the pentose-reaction of Tollens and yielded an osazone which crystallized easily in fine needless aggregated in rosettes and melted between 151°–154°.

Kossel and Neumann[45] obtained from the thymus nucleic acid, by the action of 20% (by volume) sulphuric acid at 150°, levulinic acid in a not inconsiderable quantity. A. Noll[46] obtained levulinic acid from the nucleic acid of sturgeon's sperma by the action of 30% H_2SO_4 for two hours at 150°. Araki and Inouye obtained it from the nucleic acid of the intestial mucous membrane.[46a]

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(44) Zeits. f. Physiol. Chem. XXXI, S. 411–427, (1901).

(45) Ber. d. deuts. Chem. Gesells., 1894.

(46) Zeits. f. Physiol. Chem. XXV., [1898] 430–434.

(46a) Zeits. Phys. Chem. XXXVIII, 98 (1903); XLII, 117, (1904).

Experiment E.

About two gr. of the lympho-nucleic acid were boiled for one hour with 50cc. of 40% [by weight] H_2SO_4, with an inverted condenser on a wire-gauze. The reaction-product was, on cooling, shaken with ether and the ether extract evaporated on the warm water. There remained a brownish oily material which solidified into radiating plates in coming in contact with a drop of water. It had a slight smell of the oil of bitter-almond. The aqueous solution of this substance caused, on the addition of a little iodine and a drop of KOH solution, a strong evolution of iodoform in the cold. The quantity of this material was very small and I did not succeed in obtaining either the phenylhydrazone or the silver salts.

Experiment F.

About half a gr. of the lympho-nucleic acid was taken into a small flask with an inverted condenser and heated with 50cc. of 5% [by weight] H_2SO_4 in a boiling water bath. After the lapse of 12 hours, the flame was withdrawn. The following morning, 10cc. of the solution were taken out, neutralized with KOH and heated with the Fehling's solution. The solution became strongly greenish and on standing a greenish flocky mass separated out, but there was no trace of Cu_2O noticeable. The contents of the flask were further heated for $3\frac{1}{2}$ hours more. A portion was taken out and treated with the Fehling's solution which gave the flocky greenish precipitate but no Cu_2O. Another portion treated with iodine and KOH gave a strong smell of iodoform which separated out in the form of yellow powder.

These two preliminary experiments rather tend to show that the carbohydrates as such do not exist among the products of the partial or complete decomposition of the lympho-nucleic acid. There is, however, among them a compound, probably

levulinic acid, which gives the pronounced iodoform reaction and must, therefore, contain either one of the two groups[47]..... CH_3COC..... and CH_3CH (OH) C..... If it is actually the levulinic acid, as to be expected from the results of Kossel, Araki, Inouye, and Noll, then it is an evidence of the presence of a hexose-atomgroup in the constitution of the lympho-nucleic acid. It is also possible that this iodoform yielding substance is a derivative of glycerine which Ivar Bang[48] claims to have identified among the decomposition products of guanylic acid.

This investigation was carried on chiefly in the Sheffield Laboratory of Physiological Chemistry of Yale University, and the author's thanks are due to Prof. Lafayette B. Mendel for his kindly suggestions and encouragement. The author also wishes to acknowledge his obligation to Prof. William J. Gies of Columbia University who was kind enough to obtain the material for him from time to time in New York City. It is the author's desire to continue this investigation further in a near future.

SUMMARY OF THE RESULTS.

I. The lympho-nucleic acid was isolated from the lymph-corpuscles of the cattle.

II. It has an elementary composition, $C_{41}H_{55-64}N_{14}P_4O_{25}$, closely resembling that of the salmon nucleic acid and thymus-nucleic acid of Miescher, Schmiedeberg, and Herlant.

III. The nucleic acids are very unstable compounds and the widely different compositions given by the different authors are due to the different degrees of the partial decomposition.

IV. The ratio of phosphorus to nitrogen in the original lympho-nucleic acid is $P_4 : N_{14}$, or $P_2 : N_7$.

V. The purity of a nucleic acid can not be, by any means, judged by the determinations of the phosphorus alone.

(47) Lieben, Liebig's Ann. d. Chem. Suppl. 7, 218, 377.
(48) Zeits. f. physiol. Chem. XXXI, (1901), 424.

VI. All the four purine-bases, namely xanthine, guanine, adenine, and hypoxanthine, are found among the decomposition products of the lympho-nucleic acid.

VII. The existence of the carbohydrates as such among the decomposition products is uncertain. A compound containing either of the two groups, CH_3COC.....and CH_3CH (OH) C....., exists among the decomposition products of the lympho-nucleic acid.

VIII. The thymine exists among the decomposition products of lympho-nucleic acid.

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END.