ON THE CHEMISTRY OF THE CHROMATIN SUBSTANCE OF THE NERVE-CELL.[1]

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The importance of the chromatin substance of a cell for its various functions and for the processes of growth and regeneration is being constantly realized and its part in heredity to-day is firmly established. It is natural that the chromatin substance of the nerve-cell should be the center of attention to the neurologist. It is true that a few years ago much more light was expected from the study of the morphological elements of the nerve-cell than has really been obtained, but for this disappointment the efforts to establish the nature of the morphological elements of the nerve-cell and the character of their changes in physiological and pathological conditions should not be abandoned.

With the view of gaining some knowledge of the nature of Nissl's granules, and of their relationship to the chromatin of the nucleus, I undertook the study of the nucleoproteids of the brain.

It was established, especially by work done in Kossel's laboratory by Lilienfeld and by Lilienfeld and Malfatti, that chromatins belong to the class of substances commonly designated as nucleo-compounds.

The nucleo-compounds, however, differ greatly in their composition. The point of similarity of all of them lies in the fact that they are all derivatives of a phosphorized organic acid named nucleic acid. The chromatin may be a compound of a nucleic acid with a very simple proteid substance, a protamin like that of the heads of the spermatozoa of fishes, or it may be a compound with a more complex proteid substance, a histon. Further, a nucleic acid may combine with more than one different proteid substance to form a chromatin, as, for instance, that of the

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thymus; or it may combine with both proteids and carbohydrates to form a chromatin, as, for instance, that of the pancreas.

It is apparent that the distinction between different individual chromatins may be due to the nature of the different nucleic acids or to the other components entering in combination with the nucleic acid and thus forming the chromatin.

It was assumed, a priori, that in the higher organism the chromatins of different tissues are distinct in their functions. It was also assumed that distinction in function was to a certain degree caused by difference in chemical nature, and there was a certain tendency to ascribe the individual function of a chromatin to the nucleic acid present in its molecule.

That nucleic acids may possess a different chemical composition was assumed by Kossel, who was one of the first to undertake a thorough study of nucleo-compounds.

The components of nucleic acid, as far as known at present, are phosphoric acid, purin bases, pyrimidin bases, and a carbohydrate. A great variety of purin bases is known, but in the molecule of nucleic acids only two were found with certainty, namely, adenin and guanin. Of the pyrimidin group many bases were obtained synthetically, but only three were demonstrated in the molecule of nucleic acids, namely, thymin, cytosin, and urocil. Of all possible sugars the hexose and the pentose are those that most frequently occur in nature.

It is evident that nucleic acids may differ by the character of the substances of each of the three groups of substances which combine with phosphoric acid in order to form a nucleic acid.

Theoretically, it is true that there are many different combinations possible, but in nature only few distinct acids are supposed to exist. Thus at one time Kossel thought to have isolated an acid with only one purin base, adenin, and he designated that acid in distinction from the rest as Adenilic Acid. He soon discovered, however, that guanin also

could be found among the decomposition products of his acid.

Recently Bang has described an acid containing in its molecule only one of the purin bases, guanin, and named his acid Guanilic Acid.

Nucleic acids may also differ in the nature of their pyrimidin bases, and also by the presence or absence of these bases in their molecule. Indeed, Bang claimed that the nucleic acid of the pancreas contained no pyrimidin bases at all, and Osborne and Harris could detect in the nucleic acid of the wheat embryo only one of the three possible bases, namely, uracil.

Nucleic acids may also differ by the nature of the carbohydrate entering into their molecule.

Thus the question as it presented itself when I first undertook the study of the chemical nature of the chromatins of the brain was very complex. The first aim was to establish the nature of this chromatin in a general way. The analysis of the substance demonstrated that it belonged to the so-called "true nucleoproteids," to which group most of the chromatins known at that time belonged. It was, therefore, impossible to form any opinion as to the distinction of the nerve-chromatin from the chromatin of any other cell. I realized then that a thorough study of different components of the nucleoproteid was urgent. I also realized that the chemical composition of a cell constituent can serve to explain its part in the function of the cell only then, when it can be compared with the composition of analogous components of cells with a distinctly different function. However, at the time when I undertook the work on the nucleoproteids of the brain, the knowledge of the chemical nature of nucleoproteids in general was limited. I saw that in order to establish the part of the different components of the nucleoproteids of the brain they would have to be compared with analogous components of nucleoproteids of different origin.

As already stated, to the nucleic acid was ascribed more

importance than to any other component of chromatin, and my attention was, therefore, directed to that substance.

The attempts to obtain a nucleic acid of the brain by the methods then existing were futile. It was necessary to devise another process for obtaining the substance. The result of the efforts was a very simple process, by means of which nucleic acids could be obtained from nearly every tissue. This method made it possible to compare the elementary composition of different nucleic acids, and it was found that from that standpoint nucleic acids varied only a little.

A study of the different components was then undertaken. Attention was first directed to the purin bases. It was found that all the acids analyzed contained the same bases of that group, namely, adenin and guanin. Osborne and Harris made the same observation on the nucleic acid of the wheat embryo.

A study of the pyrimidin bases then followed. At the time the work was begun, only two bases of that group were known to be present in the molecule of nucleic acids — thymin, discovered by Kossel, existing only in acids of animal origin, and uracil existing only in acids of plant origin. Uracil was first discovered in the nuclein of yeast by Ascoli, who worked under Kossel, and later by Osborne in the acid of the wheat embryo. The following acids were analyzed in this direction: That of the thymus, spleen, fish spermatozoa, and yeast by Kossel, that of wheat embryo by Osborne and Harris, and Wheeler, and that of the yeast, spleen, pancreas, liver, testis, and brain by me. This work led to my obtaining a new base of the pyrimidin group. Simultaneously the same base was discovered by Kossel.[1] This work also led to the discovery of uracil in nucleic acids of animal origin. Uracil was found first by myself, and then by Kossel and Steudel.

The quantities of nucleic acid of the testis and of the brain available were too small to enable me to demonstrate uracil among its decomposition products.

[1] Kossel's publication appeared in Germany only a few days before my communication was made in Washington.

Acids of animal origin were thus shown to contain all the three known bases of that group, and those of plant origin only uracil and cytosin.

Thus from this standpoint, also, nucleic acids differed but little.

Regarding the carbohydrate, only the furfurol-giving substance was shown to be present in all the acids analyzed.

EXPERIMENTAL.

Method of obtaining the nucleic acid of the brain. — Fresh brains of cattle were freed from their membranes, chopped and boiled one hour in a ten per cent solution of sodium chloride. Sodium acetate was then added to make a five per cent solution. On cooling, a solution of potassium hydrate was added to make the mixture contain five per cent of caustic potash. The mixture was allowed to stand over night. The proteids were removed by means of picric and acetic acids, and to the filtrate from the proteids, hydrochloric acid was added until the solution turned slightly turbid. The nucleic acid was precipitated by copper chloride. The precipitate was dissolved in a solution of potassic hydrate containing rochelle salt, and the acid was reprecipitated by means of hydrochloric acid. This operation was repeated twice and a proteid free nucleic acid was thus obtained. This substance was not absolutely free from inorganic material. It contained some copper, iron, and other mineral bases. The free acid, however, contained 8.00 per cent of phosphorus; 0.1524 gr. of the substance left on ignition 0.0533 gr. of Ash; 0.2900 gr. of the substance on fusion with sodium hydrate and potassium nitrate gave 0.0654 gr. Mge $P_2 O_7$. The acid reacted negatively to the biuret and Milon's tests. On heating with dilute mineral acids the substance did not reduce Fehling's solution. With orcin and anilin acetate, it gave positive tests for furfurol. In this respect the nucleic acid of the brain resembled the acids of other origin, and it is probable that, like the others, it contained in its molecule a sugar belonging to the pentose group.

Purin bases of the nucleoproteid of the brain. — For the purpose of obtaining the bases belonging to this group, the nucleic acid was heated two hours with one per cent solution of sulphuric acid in an autoclave at 125° C. The filtrate was then rendered alkaline by means of ammonia, and concentrated. A precipitate of Guanin was thus formed. It was redissolved in dilute acid, and reprecipitated by means of ammonia. The excess of ammonia was removed by heating on the water-bath, and the guanin was then washed with distilled water until the washings ceased to form a cloudiness with picric acid. The base was finally transformed into its silver nitrate salt and analyzed.

0.1245 gr. of the substance gave 0.0420 gr. Ag.

for $C_5 H_5 N_5 O$. $AgNo_3$.

Calculated.	Found.
Ag — 33.65 per cent	— 33.73 per cent

The filtrate from the guanin was treated with an ammoniacal silver solution, and a precipitate of the other purin bases was thus obtained. This silver precipitate was decomposed by means of dilute hydrochloric acid, and the filtrate treated with a solution of sodium picrate. The adenin picrate thus obtained was recrystallized out of hot water, and after drying in a toluol bath was analyzed.

0.1680 gr. of the substance gave over 50 per cent KOH solution. 44.00 cc. of nitrogen at p — 763 mm. and t° 21° C.

for $C_5 H_5 N_5$. $C_5 H_2$. $(NO_2)_3 OH$.

Calculated.	Found.
N — 30.71 per cent	— 30.63

No other basis of this group could be detected. Thus, in this respect also, the nucleic acid of the brain resembles those of other origin.

Pyrimidin bases of the nucleic acid of the brain. — For the purpose of obtaining the bases of this group, the following process was employed: The acid was taken up in a

twenty-five per cent solution of sulphuric acid and digested in an autoclave for three hours between 150 and 175° C. The sulphuric and phosphoric acids were then removed by means of baryta water, and the filtrate concentrated at very low pressure. On standing over night a precipitate was formed which consisted chiefly of thymin and also of cytosin. To remove the latter, the thymin was recrystallized from a ten per cent solution of sulphuric acid. It was then dried and analyzed.

0.1200 gr. of the substance gave over 50 per cent KOH solution. 23.5 cc. of nitrogen at p — 760 m t° — 24° C.

for $C_5 H_5 N_2 O_2$.

Calculated.	Found.
N — 22.22	— 22.52

The mother liquids of the thymin were then joined, the excess of barium removed by sulphuric acid, and the filtrate treated with a concentrated solution of picric acid. The solution was concentrated under low pressure to a small bulk and cytosin picrate was allowed to crystallize. The picrate was dissolved in hot water, filtered from the insoluble part and decomposed by means of sulphuric acid, toluol, and ether. When all the picric acid was removed, the solution was treated with a solution of barium hydrate until it ceased to react acid to congo, but reacted acid to litmus. The filtrate was concentrated under diminished pressure to a very small bulk and allowed to crystallize. Beautiful needles of the basic-cytosin sulphate formed immediately.

0.1450 gr. of the substance dried in Toluol bath gave 0.0588 gr. of $BaSO_4$.

for $^4 (C_4H_4N_3O)$. H_2SO_4. 2 H_2O.

Calculated.	Found.
S — 5.53 per cent.	— 5.57 per cent.

Part of this salt was then transformed to the chlorplatinate.

0.1364 gr. of substance dried in Toluol bath gave 0.0421 gr. pt.

for 2 $(C_4H_5N_3O)$ Pt Cl_4. 2 HCl.

Calculated.	Found.
Pt. — 30.84 per cent.	30.86 per cent.

The filtrate from the cytosin picrate was not sufficient to enable us to detect the presence of uracil in it.

Thus in the character of its pyrimidin bases the nucleic acid of the brain resembles those of other tissues, but only the nucleic acids of animal origin.

Whether or not all nucleic acids of animal origin are identical in their nature is, however, not yet known. It remains to establish the nature of the carbohydrate present in their molecule, and further to establish the proportions of the different components in the acids of different tissues. It seems probable from our present experience that the proportion of the purin bases to the pyrimidin bases, as well as the proportion of the different bases of each group, varies considerably in acids of different tissues. I hope to be in a position to establish this in the near future.

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