RIBONUCLEOPROTEINS AND RIBONUCLEIC ACIDS

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PREPARATION AND COMPOSITION

by

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Preface

The investigators who are presently engaged in the areas of nucleic acid research and those who will enter this field in the next few years will be contributing their efforts during the closing years of the first century of research on these substances. The earliest investigators were able to accomplish sufficient progress to reveal a complex and difficult field. With a few exceptions the limited number of research workers in biochemistry during the first half of the twentieth century did not seem to find the rewards of research on the nucleic acids to be commensurate with the difficulties, hence progress was slow. Coincident with the entrance of large numbers of research workers into biochemistry in the last twenty years, research on nucleic acids has received widespread attention and progress has been rapid such that a survey of the published research of the last five years reveals that during this short period of time more publications that are concerned with the preparation and composition of ribonucleic acids have appeared than in all of the earlier years taken together.

The goal before the group who are interested in the isolation of ribonucleic acids is that of the possession of a representative ribonucleic acid in a state comparable to that in which it must have been in living tissues during the period in which it accomplished its biological function. In spite of the many publications whose authors seemingly believe themselves to have

achieved this goal a careful perusal indicates only that progress has been made toward the goal. It is evident that even the latest of refined techniques have weaknesses that must be studied and corrected before progress toward the goal is again resumed. The isolation of even a worthy degraded specimen of ribonucleic acids requires extreme attention to detail, in fact the worthiness of the product varies directly with such attention.

The aim of the present text is not to cover every publication in which a ribonucleic acid was produced but rather to provide the exact details and background of those methods and the modification thereof no matter how minor which represent some bit of progress toward the goal. It is hoped that the appearance of these details under one cover will stimulate the emergence of the ultimate method from out of the best points of past methodology.

San Francisco
March 1962

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CHAPTER I

The First Fifty Years of Research on Ribonucleic Acids. The Isolation of Crude Ribonucleic Acids and Study of Components

1. Introduction

The characterization of nucleic acids has remained one of the major problems of biology. This problem appears far less easy of solution today than it did to the investigator of the late nineteenth century. Soon after becoming aware of their existance in tissues early investigators very conveniently divided the nucleic acids into two kinds, one present in animal tissues and one present in plant tissues. With equal certainty the early investigators soon endowed each nucleic acid with four nitrogen-containing components so that their most difficult problem seemed to be to arrive at an agreement regarding the mode of linkage of these components to form the nucleic acid. The chemistry of the nucleic acids was believed to be so simple that it was felt by many at the time that to arrive at this information one had only to prepare pure specimens of each of the nucleic acids and the problem would be solved for all time.

The passage of time has taught much concerning the kinds of linkages between components and has also shown that with the attainment of such information the problems increase in complexity. That modern biochemistry recognizes that there are two main classes of nucleic acids each of which is present in all types of tissues shows the modifications which earlier and simpler concepts had to undergo. These classes of nucleic acids are now termed the deoxyribonucleic acids and the ribonucleic acids and it is recognized that there are a large number of representatives

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in each class. Thorough search by the use of techniques unavailable to early workers has uncovered many new nitrogen-containing components among the building blocks of the nucleic acids until it now appears that certain of the ribonucleic acids which were in the lead in this regard at the last count may contain no less than four times the number of nitrogen-containing constituents than were known in the first half of the century.

Regarded in the light of more recent research many of the early procedures for the isolation of nucleic acids contain at least one step sufficiently drastic such that actual rupture of linkages within the nucleic acid molecule might be possible. Such steps were regarded as necessary since the reasoning of the period demanded that the isolation procedure be capable of producing a completely protein-free, lipid-free and salt-free nucleic acid. To this was also added the criterion that a preparation of ribonucleic acids must be free of deoxyribonucleic acids and *vice-versa*. No one of these procedures attained their objective yet their study can not be neglected since many of these methods did exclude the possibility of enzymatic degradation during isolation procedures.

Following the realization that specific linkages in the nucleic acids were labile to certain treatment in the isolation procedures, the methods for the preparation of ribonucleic acids became characterized by the complete abandonment of earlier drastic procedures involving the use of extremes in pH and prolonged treatment with heat. The characteristics of these procedures are (*a*) extraction of nucleoproteins by the use of dilute solutions of salts, (*b*) precipitation of the nucleoproteins at pH values no lower than 4.2, and (*c*) removal of the proteins by either concentrated solutions of salts or surface active agents such as dodecyl sulfate or chloroform–octyl alcohol. In discarding drastic methods and accepting milder methods it soon became apparent that the way had been opened for enzymatic degradation as well as the relatively unstudied effects of concentrated salt solutions and surface active agents.

The methods which have been published during the past six

years have approached the problem of partial enzymatic degradation during isolation and have eliminated this as a factor. However it is not certain whether or not the methods chosen to eliminate enzymatic degradation are sufficiently mild to prevent the rupture of internucleotide linkages, also during this latter period the reproducible fractionation of certain ribonucleic acids has been achieved. This points up the fact that methods which would permit the isolation of all of the ribonucleic acids that may be present in a given tissue do not exist. The ribonucleic acids that are lost during isolation have been tacitly assumed to have the same properties as those which are isolated.

Regardless of the number and diversity of the published isolation procedures and of the claims made for the products obtained by the use of these procedures it is doubtful if the isolation of a protein-free, deoxyribonucleic acid-free specimen of completely undegraded ribonucleic acids has ever been achieved. It may well be that certain of the preparations of the last six years approach closer to this achievement than those of the previously published procedures but much improvement is still necessary. The problem of homogeneity is seemingly limitless in its complexity since each newly discovered component adds to the number of possible combinations of nucleotides which might possibly be found to occur in a nucleic acid. The concept of homogeneity in ribonucleic acids cannot be pursued with profit until such time as a given ribonucleic acid can be shown to have one definite role.

The methods of isolation which have been developed to date have provided specimens of ribonucleic acids from many sources but many important tissues remain to be investigated. As a result of such preparations the greater percentage of internucleotide linkages are probably known. The research of recent years has expanded the number of nitrogen-containing components of the ribonucleic acids from four to approximately sixteen known and several others of unknown composition reported in the literature. Further refinements in isolation techniques covering a wider range of tissues will undoubtedly uncover a few more. These

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findings are of the greatest possible significance in the comparison of one ribonucleic acid with another and certainly the characterization of a ribonucleic acid according to its composition in terms of only major constituents can no longer have meaning. For example, to report the percentage composition of uridylic acid as 25 per cent in one specimen and 26 per cent in another may permit the statement that within the limits of analyses and experimental error both results are the same. However to find that the percentage composition of 5-ribosyluracil is 1 per cent in one specimen and 2 per cent in another leaves no doubt that the difference in this case is 100 per cent.

Most of the methods to be described in the following pages attempt the isolation of ribonucleic acids directly from whole tissues. In a few instances, simple beginnings have demonstrated that nucleoproteins as well as the ribonucleic acids derived from them can be fractionated. Owing to the possibilities of the inclusion of enzymatic and chemical degradation products in samples of ribonucleic acids the validity of some fractionation procedures must be accepted with reserve yet there remains no doubt regarding the value of continued research in this area. The particulate components of cells such as membranes, nuclei, mitochondria, microsomes and supernatant fluid are in many cases simply and easily prepared. The isolation and fractionation of either the ribonucleoproteins or the ribonucleic acids from these particulate components and their characterization on the basis of both major and minor components has had an auspicious beginning and should provide a useful bank of information against the time when specific ribonucleic acids can be classified according to role. The achievements of the last several years indicate success in this area may be expected.

The details for the isolation of ribonucleic acids as explained and used by the early investigators appear in the original literature and in books by Jones[1] and by Levene and Bass[2]. Both the original literature and the books were published in editions which have proved far too limited to supply the many libraries to which the ever increasing numbers of research workers interested in

the nucleic acids must turn for their information. In recognition of this lack which occurs in all but the oldest and largest of libraries and in full awareness that the intelligent development of new methods is dependent upon the understanding of previous attempts and achievements the following pages attempt to give the details of those methods which are felt to have bearing on the development of present methods or may have a bearing on future developments. All terminology is modernized.

The first publicized preparations which can safely be said to contain a recognizable percentage of nucleic acids were the protein-rich fractions from cell nuclei which were isolated and described by Miescher[3] and a comparable fraction which was isolated from yeast and tersely described by Hoppe-Seyler[4]. Until a few years ago any investigator who read and evaluated Miescher's results unhesitatingly believed his descriptions to apply and be in accord with the properties of a deoxyribonucleoprotein while Hoppe-Seyler's lack of description was construed as applying to a ribonucleoprotein. Since it is generally agreed that nuclei contain small quantities of ribonucleoproteins in addition to deoxyribonucleoproteins Miescher's descriptions become more understandable especially the reference to a certain instability to alkali in nucleic acids when thought of in terms of a deoxyribonucleoprotein contaminated with ribonucleoproteins. Also, since deoxyribonucleic acids have now been isolated from yeast, Hoppe-Seyler's preparation is certainly a very crude ribonucleoprotein contaminated with deoxyribonucleoprotein. The first preparation to contain a major quantity of ribonucleic acids is described by Kossel[5] and isolated from yeast. The N content of this preparation was 15.3–15.9 per cent and the P content 6.1–6.3 per cent.

2. *Ribonucleic Acids from Yeast (Altmann)*

The first stepwise description for the isolation of ribonucleic acids in quantity from yeast was devised by Altmann[6]. In this

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preparation 2 liters of yeast fresh from the brewery is mixed with 6 liters of water. 200 g of sodium hydroxide are dissolved in 500 ml of water and added to the mixture while stirring vigorously for 5 minutes. The greater part of the hydroxide is immediately neutralized by the use of a concentrated solution of hydrochloric acid to a slightly alkaline reaction. The remainder of the alkali is finally neutralized by acetic acid and allowed to stand 24 hours.

The supernatant liquid is decanted and filtered and as much concentrated hydrochloric acid is added to the extract such that the first turbidity that is formed is redissolved and a final permanent precipitate is formed. The amount of hydrochloric acid which must be added must be sufficient to neutralize the sodium acetate and provide an excess equal to 3 to 5 parts per thousand. The mixture is added to an equal volume of alcohol which contains 3 to 5 parts hydrochloric acid per thousand and permitted to stand one to two days. The precipitate is collected by filtration. After it is collected the precipitate is rubbed to a fine powder with a solution which contains not less than 50 per cent alcohol and 3 parts of hydrochloric acid per thousand. The powder is finally washed with alcohol and ether and dried in warm air.

3. Nucleoprotein from Pancreas (Hammarsten)

Five years later Hammarsten[7] announced the finding of a protein which later work showed to be ribonucleic acid-containing and which at this time was termed β-nucleoprotein. The β-nucleoprotein was to be the subject of many investigations during subsequent years. The ribonucleic acid component by its appearance in animal tissues kept the thoughtful biochemist ill at ease concerning our lack of knowledge of the nucleic acids while many others succeeded in disregarding its seemingly irregular existance.

The β-nucleoprotein is easily prepared from cattle pancreas. The finely ground pancreas is mixed with water and finally brought to boiling. The mixture is cooled and filtered. Sufficient

hydrochloric acid to make the filtrate 1 to 2 per cent hydrochloric acid in concentration or sufficient acetic acid to make the filtrate 5–10 per cent acetic acid in concentration is added. A white flocculent precipitate forms. The precipitate is removed by filtration. It is suspended in water and brought into solution by the addition of a dilute solution of sodium hydroxide and reprecipitated from 5–10 per cent solution of acetic acid. This precipitate is collected and washed and dried.

4. *Ribonucleic Acids from Wheat Germ (Osborne and Harris)*

The ribonucleic acids from wheat germ represent the second tissue to be shown to contain these nucleic acids if the criterion of actual isolation of the nucleic acids is taken into consideration. From the later evidence wheat germ is actually the third tissue to be investigated and found to contain ribonucleic acids. These ribonucleic acids were to be the subject of several investigations in the hope of proving their identity with the ribonucleic acids from yeast.

Osborne and Harris[8] prepared the ribonucleic acids according to the following method. 9 kilogram of lipid-free meal from wheat embryos is shaken with 60 liters of water which has been saturated with thymol and permitted to stand in a cool place for 24 hours. The mixture is filtered through silk. The filtrate is saturated with sodium chloride and acidified with acetic acid until a precipitate forms. The precipitate is filtered off and washed with water in order to remove adhering salt and acid. The precipitate is suspended in water to which is added an equal volume of 4 per cent solution of hydrochloric acid containing 3 g of pepsin. The mixture is set aside at 40° for 24 hours for digestion. The undissolved and undigested portion is filtered off and resuspended in 6 liters of 2 per cent solution of hydrochloric acid containing 1.5 g of pepsin and set aside at 40° for 24 hours for digestion. This operation is repeated. The undissolved material which remains at the conclusion of the third digestion period

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