SPECIAL ARTICLES

THE MICROCHEMISTRY OF NUCLEAR INCLUSIONS IN VIRUS DISEASES

THE cellular response in diseases caused by filterable viruses is very pronounced and to the best of our knowledge quite characteristic.[1] Curious "inclusion bodies" are produced in the nucleus, in the cytoplasm or in both. For the cytologist the nuclear ones are particularly interesting because it is distinctly unusual for nuclei to react so profoundly and apparently so specifically in diseased conditions.

The classical studies of Lipschütz and Luger and Lauda published in 1921 relative to the nuclear inclusions in herpes constitute the point of departure for most of the recent work. They gave rise to a controversy which has never been settled. Lipschütz and his followers regard the inclusions as combinations of living virus and nuclear material in which the causative micro-organisms themselves are microscopically visible in the form of extremely minute particles of uniform size; whereas Luger and Lauda look upon them as stages in a non-specific type of degeneration which they style "oxychromatic" because the inclusions are "oxyphile" in the sense that they have a strong affinity for "acid" dyes, such as eosin. Inclusions something like those in herpes had previously been reported in several diseases, of which the following may be mentioned: smallpox, chicken-pox, Borna disease of horses, and the salivary gland disease of guinea-pigs. Since 1921 other inclusions have been found in Virus III disease of rabbits and it is probable that still others await discovery.

By most investigators these nuclear inclusions are considered to be pathognomonic of the action of filterable viruses. But knowledge of their wide occurrence in man and animals has developed so rapidly and has been so directly of practical value that the difficult question of their chemical composition has been rather set aside. Because they are colored in the same way by non-specific stains means but little and is not a good reason to assume that they are alike in the different diseased conditions. It is the purpose of this paper to briefly report their reactions to the Bensley-Macallum test for masked iron and the Feulgen reaction, as well as to determine a few of their solubilities.[2]

[1] T. M. Rivers, "Filterable Viruses," Williams & Wilkins Co., Baltimore, 1928.

[2] Thanks are due to Dr. T. M. Rivers for tissues from cases of chicken-pox and for Virus III and to Dr. P. K. Olitsky for the virus of herpes and for tissues from a case of Borna disease.

The test for iron gave consistently negative results with the intranuclear inclusions of chicken-pox herpes, Virus III disease of rabbits and the salivary gland disease of guinea-pigs, so we may conclude that they are all alike in so far that they do not contain detectable amounts of iron in organic combination.

In applying the Feulgen, or the nucleal reaction,[3] as it is sometimes called, paraffin sections of material fixed in equal parts of saturated aqueous corrosive sublimate and absolute alcohol were passed through xylol and alcohol to water, then

(1) Placed in a staining jar containing normal HCl (82.5 cc HCl, sp. gr. 1.17–1.185 per liter of water) at room temperature for one minute.

(2) Transferred to normal HCl, at 60° C. and there hydrolyzed for four minutes.

(3) Treated with the fuchsin sulphurous acid reagent in a staining jar for one and one half hours. (This reagent was made up as follows: One gram of basic fuchsin is dissolved in 100 cc of distilled water with the aid of a little heat. The solution is filtered while still warm and 20 cc of normal HCl is added to the filtrate. The resulting fluid is then cooled and one gram of dry sodium bisulfite ($NaHSO_3$) is added. Then, after standing for about twenty-four hours, the reagent is ready for use and should have a pale straw color.)

(4) Passed through a series of three jars, each containing a solution made by adding 10 cc of a molecular solution of sodium bisulfite (*i.e.*, 104 grams per liter), to 200 cc of tap-water, allowing one and one half minutes in each and agitating frequently.

(5) Washed in tap-water for five minutes, dehydrated, cleared and mounted in balsam.

This reaction was likewise wholly negative for the same inclusions, with the exception of those in the submaxillary glands of adult guinea-pigs, which were tinged, but so very lightly as to leave some doubt of the presence of reacting material. The inclusions in Borna disease were also tested and were found to be negative. The available evidence seems to indicate that a positive result is only given by substances containing thymonucleic acid. However this may be, the reaction is specific and elective for a distinctive nuclear component of some kind and has the advantage of being easily applied. It is far more specific than any basic stain which will color in addition to nuclear chromatin the Nissl bodies and the chromidial material of gland cells, substances left entirely untouched by the Feulgen reaction.

All the inclusions studied were found to be remarkably resistant to solvents. They were well preserved in mixtures containing large amounts (80 per cent.) of acetic acid and chloroform and were not noticeably dissolved by 95 per cent. ethyl alcohol.

[3] *Enzy. f. Mikr. tech.*, 1927, Bd. III, 1729.

Attempts to secure a clearly positive Millon's reaction were unavailing. Other microchemical studies are in progress and will be reported elsewhere. Among the reactions which should be applied are Macallum's tests for potassium and for chlorides, because these may furnish clues to the nature and source of substances added when the nuclei undergo hypertrophy, as they often do.

From the results obtained thus far, it seems clear that, although the viruses are obviously distinct, the material forming the inclusion bodies appears to be in some respects alike throughout the series of diseases examined. But this similarity should not be taken at all as a measure of a hypothetical and corresponding similarity in the mode of action of the viruses; for the reactivity of the nucleus is so restricted that it might well respond to different stimuli in the same way. The available material for building up the inclusions is limited both in amount and in variety as compared with that existing in the cytoplasm, and the nucleus by its position is sheltered from environmental influences acting upon the periphery of the cell. Such influences may be different in kind, and there is reason to believe that they actually do differ within certain limits, while the transmitted effects to the nucleus may be identical. Consequently, in striving to know the viruses by their deeds, which is about all we can do at present, we must proceed cautiously. It is this that makes the problem largely a cytological one.

The results are, moreover, at variance but not absolutely incompatible with the theory that the inclusions are composed in large part of the causative microorganisms themselves—if indeed the inciting agents are actually living, which has certainly not been proved in the case of any of those mentioned. In the first place, iron, which is known to be of widespread occurrence in detectable amounts in living things, was observed in the nuclear inclusions to be conspicuous by its absence.

Secondly, the material responsible for the Feulgen reaction, which is probably thymonucleic acid, while not quite so ubiquitous is nevertheless an essential constituent of animal cells including the pathogenic protozoa, yet, like iron, it was absent in all the inclusions, with the possible exception of some in the submaxillary glands of old guinea-pigs. The questionable reaction which these gave should not be weighed in the balance one way or the other. They constitute a special case, for, as will be shown in a later communication, the cells tested, though persisting in the living animal, had been dead for days, perhaps for weeks, before the examinations were made.

Further evidence pointing in the same direction has been available for some years, but has not been stressed. It is known that some of the inclusions that we are considering are occasionally feebly basophilic but that the majority throughout their whole history are strongly acidophilic (or oxyphilic). A parallel is not easily found of any existing microorganism which is so consistently acidophilic in its reaction. Even the Rickettsia of Rocky Mountain spotted fever, which approximate most closely to the inclusions, being in a stage of their life history parasitic in masses within nuclei, are distinctly basophilic.

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THE EFFECTS OF X-RAYS IN PRODUCING MUTATIONS IN THE SOMATIC CELLS OF DROSOPHILA MELANOGASTER

THE signal success of my colleague, Dr. H. J. Muller, in producing mutations and rearrangements of genes in the germ cells of *Drosophila* by X-radiations, suggested the possibility of securing similar results by this method in somatic cells. It was thought that if the genes in the somatic cells could be changed by the means of X-rays, the facts thus obtained would be of importance, especially in studying the mutation rate and in determining the behavior of specific genes during development.

With this in view, a series of experiments, involving the use of several different genes, has been planned and is now being carried out in this laboratory. For the first set of experiments, the sex-linked genes for eye color were selected as suitable characters for study. The compound eye of insects is especially well adapted to this kind of work. It is composed of a series of definite units, the ommatidia, in which any change of color of one or more of the units can be detected. In this paper we shall report the results obtained from raying the F_1 eggs and larvae of crosses between the normal red-eyed fly and the white-eyed mutants.

The relatively low frequency with which mutations occur, in the individual genes, even after X-ray treatment, makes remote the chance that two identical dominant genes in the same somatic cell would be changed at the same time. The object of making the cross is, therefore, to obain females heterozygous for the sex-linked genes. Since the male has but a single X-chromosome, his somatic cells will have either the dominant or recessive gene, but not both.

The cultures to be rayed were made up in a manner such that the difference in age between the youngest and oldest eggs or larvae in any given culture would not exceed twelve hours. These cultures were