MITOGENETIC ANALYSIS OF THE PROTEIN SUBSTRATE OF PROTOPLASM

PART II. DIVIDING CELLS AND CELLS WHICH HAVE EMERGED FROM THE "MERISTEMATOUS" STATE

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The methods at present available for the study of molecular changes proceeding in the protoplasm do not permit us to follow the course of rapidly succeeding changes in its chemical composition, and, in particular, of changes in the relative locations of molecules and their complexes. Such methods can inform us only of certain transiently existing states of the protoplasm – "structures".

TABLE 1

Spectra of the Phenolic Groups of Tyrosine, R–C_6H_4OH, and of the Carbonyl Groups R–C=O, from the Radiation of 18-hour and 3-day Yeast Cultures

Functional group	Wave length, A	Mean effect, %		Number of experiments (1) / (2)
		18-hour culture (1)	3-day culture (2)	
C=O (I)	1960—1975	58,2	69,2	5/5
C=O (II)	2100—2110	59	64,2	5/5
C_6H_4OH	2770—2790	70,5	62,8	21/19
Adjacent to C=O	1955—1960	2,2	61,4	5/5
	1975—1980	—6,6	76,4	5/5
	2090—2100	2,1	0.2*	5/5
	2110—2120	4,8	59,4	5/5
Adjacent to C_6H_4OH	2760—2770	—1	69,6	9/10
	2790—2880	2,7	112,2	10/10

* The band did not broaden towards the short wave side.

There is, however, experimental evidence that, in many of the basic processes taking place in the protoplasm, great importance is attached to weak, readily broken bonds between peptide chains. The number of such bonds, and their significance, appear to vary widely, depending on the functional state of the system, and may in many cases lead to establishment of conditions approaching localized reversible gelation of the protoplasm.

The fact that within the short period of mitotic division all the cell elements undergo reconstruction leads naturally to the concept that the microscopically observable processes of breakdown and reformation of microstructures have their analogous submicroscopic molecular counterparts. It was natural to postulate that an important part is played in mitosis by processes leading to disaggregation, i.e. to achievement of maximum mobility of protein and nucleoprotein elements of the cytoplasm and nucleus. One would, on the contrary, anticipate a lower mobility of the substrate, i.e. higher degree of association of molecular complexes, in the cells of tissues in which cell division is not proceeding.

TABLE 2

R−C=O and R−C_6H_4OH Bands in Newt Fin Radiation Spectra.

Functional group	Wave length, A	Average effect, %		Number of experiments	
		larvae (I)	adult newts (II)	on larvae	on adult newts
CO (I)	1960—1975	65	40	9	5
CO (II)	2100—2110	59	49	7	5
C_6H_4OH (I)	2720—2730	57	70	7	7
C_6H_4OH (II)	2770—2780	47	65	7	8
C_6H_4OH (III)	2780—2790	60	60	8	8
Adjacent to $CO_{(I)}$	1955—1960	1	43	6	5
The same	1975—1980	2	47	6	5
Adjacent to CO (II)	2090—2100	2,5	46	6	5
Adjacent to $C_6H_4OH_{(I)}$	2710—2720	0,3	61	7	7
The same	2730—2740	3	60	7	7
Adjacent to $C_6H_4OH_{(II)}$	2760—2770	0,6	57	7	8
Adjacent to $C_6H_4OH_{(III)}$	2790—2800	2	50	8	8

Our experiments were performed on material in two physiological states — the state of intensive multiplication of cells, and the state corresponding to physiological emergence from the "meristematous" state.

The mitogenetic method permits of the investigation of the extent of disaggregation and breakdown of large protein complexes held together by weak intermolecular bonds (bond energy 4-8 kcal / mole), in experiments performed on living, undamaged organisms.

Conclusions as to the presence and the extent of intermolecular bonding were based on analysis of mitogenetic spectra of selective scattering. Substrates not possessing intermolecular quasichemical bonds give spectra with narrow bands, corresponding closely with those of the standards; the bands become broader when quasichemical bond formation takes place in the substrate (see Part I).

As for the data obtained from our investigation of gelatin, we chose, for our in vivo investigations, one of the functional groups of the peptide chain, viz. the phenolic group of tyrosine and the carbonyl group of the peptide chain itself. As found for the standard prepared in our laboratory, the functional group R−C=O is represented in the selective scattering spectrum by bands at 1960-1975 and 2100 -2110 A. The spectrum of the phenolic group of tyrosine may be considered, with sufficient accuracy for the purposes of this research, to include bands at 2720-2730 and 2770-2790 A.

1. Experiments on Cultures of the Yeast Saccharomycetes Cerevisiae

We set up two identical cultures of the organisms in beer wort. One of the cultures was taken for examination after 18 hours, at the stage of intensive multiplication of the cells, and the other culture was examined after 3-4 days. By that time, not only had cell division ceased, but the fermentation process was also practically completed, so that the cells were all located at the bottom of the vessel, as a dense sediment. Both cultures were maintained at 27-28°.

The cells were washed with tap water before being taken for spectral analysis, in the form of slightly opalescent aqueous suspensions, using water of the same temperature.

TABLE 3

R−C=O Bands of Radiation of the Labial Mucous Membrane of Guinea Pigs

Functional group	Wave length, A	Effect, %	
		in newborn guinea pigs	in adult guinea pigs
CO (I)	1960—1975	39, 43, 52	31, 54, 45
CO (II)	2100—2110	39, 50, 48	55, 50, 41
Adjacent to CO (I)	1955—1960	2, —2, 3	36, 54, 48
The same	1975—1980	0, 2, 3	39, 35, 43
Adjacent to CO (II)	2090—2100	2, 1, —2	43, 40, 54

We examined only the 2770-2790 A band due to the phenolic group of tyrosine, since the 2720-2730 A band is subject to background interference in 18-hour cultures. As is evident from the data of Table 1, the characteristic spectra of the functional groups R−C=O and R−C_6H_4OH are represented by narrower bands in 18-hour than in 3-day cultures. The conclusion may hence be drawn, on the basis of the data found for model systems (Part I), that the process of ageing of the cultures — their physiological emergence from the stage of multiplication — coincides with the formation of weak, quasichemical bonds which interlink peptide chains, to form two- and three-dimensional structures.

2. Experiments on Newts

We examined the outer epithelium of the tail fin, which is a uniform stratified formation not containing glandular elements. Both larval and adult forms were used for the experiments.

In the larval stage, the tail fin epithelium emits so-called spontaneous mitogenetic radiation. The experiments on adult newts were conducted during the autumn and winter, on the same individuals which had undergone metamorphosis in the laboratory. The same functional groups were taken for spectral examination, viz. the R−C=O carbonyl groups, and the R−C_6H_4 phenolic group.

Apart from the analysis of selective scattering spectra, we examined the spontaneous radiation spectra of larval tissue. A certain lengthening of exposure was required for the detection of spontaneous radiation bands.

The newt fin was screened with a sheet of thick paper having a small aperture (0.3 × 0.3 cm) centered in front of the collimator slit of the spectrograph. The intensity of the ultraviolet radiation (from a physical source) incident on the tissue was lowered by interposing a number of mat quartz filters between the tissue and the radiation source. This is an essential condition of the application of the method of selective spectrum analysis to the study of aggregates present in living tissues, such as protein substrates in the crystalline or gel forms, since the general background of scattered radiation unavoidable in such experiments greatly weakens the specific effects, making them almost subthreshold even for the sensitive biological method of detection of mitogenetic radiation.

An active region is to be found in the emission spectrum of the fin of newt larvae, contiguous with the longer wave length part of one of the characteristic carbonyl group bands (2100-2110 A). In comparing this region of the spectrum for both larval and adult newts we could only make use of the short wave end of the band. There is no overlapping of either of the bands due to the phenolic group of tyrosine, so that both of them are available for comparative studies.

It is evident from the data of Table 2 that the selective scattering spectrum of the fin of adult newts is distinguished by the breadth of its bands, as compared with those of larval tissue. In other words, much of the same results were obtained with two such disparate materials as are yeast cultures and newt fins — the peptide chains of the cells of actively growing tissues are relatively little interlinked; in contrast, in tissues in which the cells have, even if only temporarily, ceased to multiply the peptide substrates are more highly aggregated, due to formation of weak intermolecular bonds.

3. Experiments on Guinea Pigs

We examined the surface epithelium of the mucous membrane of the lips of guinea pigs, which spontaneously

TABLE 4

R–C=O Bands of Radiation of the Corneal Epithelium of Rabbits

Functional group	Wave length, A	Effect, %
$CO_{(I)}$	1960—1975	43, 27, 58, 64
$CO_{(II)}$	2100—2110	45, 78, 80, 62
Adjacent to $CO_{(I)}$	1955—1960	17, 15, 10, 10
The same	1975—1980	7, 4, —9, —3
Adjacent to $CO_{(II)}$	2090—2100	0, 7, 3, 2
The same	2110—2120	—1, —5, 2, 3

emits mitogenic radiation. For spectrum analysis we everted the lower lip, covered it with a celloidin screen, and immobolized it. An aperture in the screen, exposing a small area of the lip surface (0.3 × 0.3 cm) was centered against the collimator slit of the spectrograph.

In this series of experiments we examined the carbonyl group bands in the spontaneous radiation spectrum of newborn guinea pigs (ages from 1 to 3 days) and of adult guinea pigs (weighing from 500 to 1000 g). The width of the first carbonyl group band of the radiation from the lips of newborn guinea pigs was very close to that of the corresponding bands found for actively dividing yeast cultures and for larval newt tail fins; the second band overlapped an active region of the spectrum (2110-2120 A), toward the side of increasing wave lengths. For this reason we confined our analysis of the second band to the short wave region of the spectrum only, without entering into a consideration of its origin.

We found that both bands were broadened in the emission spectrum of the mucous membrane of lips of adult guinea pigs (Table 3). In other words, cessation of division of mammalian epithelial cells is associated with the same effect as was seen in the other cases – the peptide substrate undergoes aggregation, i.e. weak bonds are formed between peptide chains.

4. Experiments on Rabbits

In this series of experiments we studied the spontaneous emission spectrum of rabbit cornea, which is known to be "meristem" with a high mitotic index, in which cell division persists in the adult animal. Animals of medium size and weight were taken for spectrum analysis, which was confined to the carbonyl group.

As can be seen from Table 4, both of the R–C=O bands are narrow, i.e. they resemble those found in the previous experiments on biological material in the "meristematous" state. This finding shows that constant physiological "meristem" is associated in the adult organism with absence of intermolecular bonds between peptide chains, as was found in other "meristems" of different kinds.

The application of mitogenetic spectrum analysis has thus enabled us to establish that in all the organisms examined the "meristematous" state is associated with presence of narrow bands due to the functional groups R–C:O and R–C_6H_4OH, while in a state of maturity these bands become more diffuse.

Comparison with the corresponding bands of the radiation spectrum of gelatin gel and sol shows that narrowness of the bands may be interpreted as indicating mutual independence of the peptide chains (in sols), whereas their broadening is associated with formation of weak (quasichemical) intermolecular bonds (in gels). It is thus very probable that a characteristic feature of cells during mitosis (and probably also interkinesis) is the smaller degree of aggregation of the peptide and protein substrates, i.e. there is much less interlinking of peptide chains. On the other hand, tissues which have emerged from the "meristematous" state are characterized by formation of considerable numbers of weak intermolecular bonds, leading to the formation of two- and even three-dimensional peptide and protein chain structures.

It is evident that mitosis is a process involving considerable and co-ordinated reconstruction of all the elements of the cell substrate, and should be associated with a preponderance of free, i.e. mobile, peptide chains.

SUMMARY

It has been shown, by means of mitogenetic spectrum analysis that during the proliferative stage of cells (yeast, newt larvae, newborn guinea pigs), and in proliferating tissues of adult animals (guinea pig labial epithelium, rabbit cornea) there is relatively little interlinking of cytoplasmic peptide chains. This is shown by the relative narrowness of the spectral bands due to the functional groups $R-C=O$ and $R-C_6H_4OH$. Where active proliferation has ceased, the peptide chains are interlinked by weak molecular bonds, giving rise to two- and three-dimensional structures.

The mitotic process thus involves profound and co-ordinated reconstruction of all cell components.

LITERATURE CITED

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