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CHANGES IN THE STAINING CAPACITY OF NUCLEAR COMPONENTS DURING CELL DEGENERATION

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Cell degeneration is often accompanied by distinct nuclear alterations which have been classified into several types (*cf.* Ries-Gersch, 1953) on the basis of microscopically observable structural changes. One of these is nuclear pycnosis: the chromatin condenses into one or several homogeneous masses, which, in microscopic preparations, stand out by virtue of their intense staining capacity. A previous cytochemical comparison of fresh and pycnotic tumor nuclei by Leuchtenberger (1950) has dealt with the quantitative behavior of desoxyribonucleic acid (DNA) and proteins during the degenerative process. More recently, a direct staining method for basic proteins has been developed, based on their ability to combine with acid dye at an elevated pH (Alfert and Geschwind, 1953). Routine surveys of various animal tissues by this procedure disclosed the fact that pycnotic nuclei always stain with great intensity, indicating that basic proteins are not lost, but greatly concentrated during this type of nuclear degeneration.

The present paper is concerned with a detailed cytochemical analysis of this degenerative process in a mammalian ovary where pycnotic nuclei are invariably found in old and atretic follicles. This investigation has revealed a pattern of cytochemical changes different from that previously described for tumor nuclei. The data also lend further support to ideas previously expressed by the author (Alfert, 1952), dealing with the mechanism of changes in methyl green stainability of DNA.

Material and Methods

A comparison between normal nuclei from the cumulus oophorus of young follicles and pycnotic masses of chromatin lining the antrum of old follicles was made on the ovaries of an adult guinea pig. Small pieces of the ovaries were fixed for four hours in Carnoy's acetic alcohol or in Baker's formol-calcium and subsequently washed overnight in 95% alcohol or in running water, respectively; the material was then embedded in paraffin and sectioned at various thicknesses. Four staining reactions were subsequently applied: methyl green and Feulgen staining for DNA, fast green staining for histones, and the Millon reaction for tyrosine. The first three of these reactions were quantitated by microspectrophotometric procedures (for details see Pollister, Himes and Ornstein, 1951), comparing the relative dye-binding capacities of normal and pycnotic chromatin in terms of their Feulgen/methyl green and Feulgen/fast green ratios. Several modifications of

the routine photometric technique were used in this case and are herewith described in some detail:

1) Rather than measuring a separate series of nuclei for each of the staining reactions used, these reactions can be applied in sequence to one slide, and the same nuclei can be used over again, for successive series of measurements. This procedure greatly increases the precision of the results since sampling errors among independent series of measurements are excluded. Thus the dye-binding ratios of individual nuclei can be computed and averaged in terms of optical densities, and variations in the amounts of stained substrate need not be taken into account. A multiple staining schedule similar to that proposed by Bloch and Godman (1955a) was employed:

The methyl green reaction according to Pollister and Leuchtenberger (1949) was applied to formalin-fixed sections, previously digested with ribonuclease (0.025% for two hours at 40° C. and at pH 6.8) to remove cytoplasmic methyl green staining induced by the fixation (*cf.* Alfert, 1952); series of normal and pycnotic nuclei were mapped out by means of a camera lucida and drawn at a magnification of 3000 ×; nuclear diameters were measured on the drawings and used for computations of nuclear volumes; microphotometric measurements were then made on nuclear cores comprising 50% of the total nuclear volume (core diameter = 0.6 of average nuclear diameter). Subsequently the coverslip was removed and the slide was hydrolyzed for the Feulgen reaction in 1 N trichloracetic acid (TCA) at 60° C. for 12 minutes (the methyl green is extracted during this hydrolysis). The slide was then treated with Schiff reagent and bleaches prepared by substituting TCA for the HCl ordinarily used, and the same nuclei were remeasured in the manner previously indicated. The coverslip was removed again, and the slide was subjected to treatment with 5% TCA at 90° C. for 12 minutes (leading to complete extraction of DNA and Feulgen dye); finally the slide was stained for histones with fast green at pH 8 according to Alfert and Geschwind (1953) and the same nuclei were measured again.

The TCA modification of the Feulgen reaction was designed by Bloch and Godman (1955a) to prevent loss of histones from the nuclei. Formalin fixation of the tissue is required for the same reason but may affect the methyl green stainability of DNA (Alfert, 1952); an independent series of measurements was therefore made, using methyl green and the Feulgen reaction in succession on the same normal and pycnotic nuclei from material fixed in Carnoy's fluid. The orthodox HCl-Feulgen technique was employed in this case, and the sections were again pretreated with ribonuclease to exclude this step as a variable between the two procedures. Independent evidence indicates, however, that the Feulgen/methyl green ratio of Carnoy-fixed mammalian nuclei is not affected by ribonuclease.

In the formalin series a total of 36 normal and 46 pycnotic nuclei were mapped, and measured three times in succession; in the Carnoy series 21 normal and 20 pycnotics were mapped and measured for methyl green and Feulgen only. Three individual values for normal, and one for pycnotic, formalin-fixed nuclei were omitted from the computation of the Feulgen/fast green ratios. The reason was that these values differed by more than 100 per cent from the mean of the population and fell far outside its range of variation. The nuclei omitted belonged to the lower range of diploids in terms of Feulgen dye and showed no peculiarities with respect to methyl green staining; however, they exhibited very low fast green

TABLE I

Ratios between average optical densities at the different wave-lengths used for measurements of normal and pycnotic nuclei (means ± S.E. of 10 determinations).*

		Normal	Pycnotic	Difference in multiples of its S.E.
Formalin-fixed	Feulgen $\frac{E_{510}}{E_{485}}$	2.32 ± 0.03	2.45 ± 0.02	3.6
	Methyl green $\frac{E_{650}}{E_{600}}$	2.52 ± 0.03	2.56 ± 0.02	1.1
	Fast green $\frac{E_{600}}{E_{560}}$	3.03 ± 0.10	3.15 ± 0.14	0.7
Carnoy-fixed	Feulgen $\frac{E_{560}}{E_{500}}$	2.04 ± 0.03	2.13 ± 0.03	2.1
	Methyl green $\frac{E_{650}}{E_{600}}$	2.66 ± 0.02	2.62 ± 0.04	0.9

* Example: Carnoy-fixed, methyl green stained normal nuclei were measured at 650 mμ; pycnotics were measured at 600 mμ and their optical densities were multiplied by the factor 2.66.

staining. Bloch and Godman (1955a) also encountered occasional nuclei which, for reasons presently unknown, give a very weak histone reaction.

2) Comparison of normal and pycnotic nuclei revealed extreme differences in staining intensity, falling far outside the range in which valid absorption measurements in one spectral region can be made. Consequently the relatively less dense normal nuclei were measured using wave-lengths at, or close to, peak absorption, while the pycnotic nuclei were measured at wave-lengths farther away from the absorption peak. The optical densities of the latter were then multiplied by an appropriate conversion factor.

The procedure consisted of the following steps: absorption curves for each type of chromatin, each reaction and each fixative were determined on one or two nuclei. Two appropriate wave-lengths were then chosen and the exact ratio between them was established by measuring samples of ten normal whole nuclei at both wave-lengths. To ascertain the extent of possible shifts in the absorption curves of pycnotic chromatin, these same ratios were also measured on pycnotic nuclei sectioned sufficiently thin to reduce optical densities to a range below 1.0. For this purpose approximately 2 μ paraffin sections of Carnoy fixed material and 0.5 μ sections of formalin-fixed material embedded in methacrylate were used. The results of the calibration measurements are given in Table I.

The differences between these ratios for normal and pycnotic nuclei are numerically small in all cases, even in the one instance where it is statistically highly significant. The differences in stainability between normal and pycnotic chromatin turned out to be much larger than could be accounted for by such small curve shifts. In other words "distributional error" (*cf.* Ornstein, 1952) or other errors giving rise to deviations from Beer's law could not have affected the results appreciably. Serious errors due to the effect of stray light on high optical densities were avoided by the device of measuring normal and pycnotic nuclei at different wave-lengths; all measurements on mapped nuclei fell thereby within the range of 16 to 61 per cent transmission. Our microspectrophotometer, similar in princi-

ple to that described by Pollister (1952), was used in conjunction with the monochromator of a Beckman B spectrophotometer with 0.2-mm. slit width. Transmitted light was measured in microscopic images projected onto the cathode of a 1P21 photomultiplier tube.

Carnoy-fixed 3 μ sections of ovary were also stained by the Millon procedure for tyrosine (Pollister and Ris, 1947) for cytological study. One such slide was photographed, subsequently stained by the regular Feulgen procedure and the same area rephotographed for detailed comparison of the distributions of protein and DNA in the same cells. Examples of this comparison are given in Figures 3 a,b, and 4 a,b.

Results

Occasional divisions are seen in normal follicle cells. Using Feulgen-DNA content per nucleus as a measure of the degree of ploidy (*cf.* Swift, 1953) it is obvious that the majority of follicle nuclei are diploid. Occasional tetraploid DNA values probably do not represent permanent tetraploid cells such as those commonly found in mammalian liver, but are pre-prophasic values which one would expect to find in a mitotically active tissue.

Cell degeneration leading to pycnosis may start in resting diploids as well as in cells with the tetraploid DNA content. The process of pycnosis in this tissue takes a very similar course to that described in Ries-Gersch (1953) and it is almost invariably accompanied by fragmentation of the nuclear material (karyorrhexis): the nuclear substance breaks up into two or several clumps as it condenses, or sometimes into one large clump and a cluster of small granules (see Figures 2, 3b, 4b). Simple photometric determinations can only be done on the larger ones of these masses; in many cases, therefore, not all the nuclear substance of a pycnotic cell was included in the measurement. The appearance of normal and pycnotic cells with the histone reaction is shown in Figures 1 and 2. The Feulgen and methyl green picture is essentially similar, but does not correspond to the appearance obtained by application of the Millon reaction: this can be seen by comparing Figures 3a and 4a to Figures 3b and 4b. The Millon-protein picture shows a great deal of condensation, probably by expulsion of fluid since vacuoles are often encountered. The nucleohistone as visualized by either Feulgen, methyl green or the fast green reaction condenses in an even more striking manner. This process is aptly described as a dissociation ("Entmischung") of the cellular nucleoprotein complexes by Ries-Gersch (1953, page 367).

In Figure 5 individual nuclear DNA contents in arbitrary Feulgen units at peak absorption are plotted against volume, and means and standard errors for normal diploid and pre-prophasic (tetraploid) cells are given. The pycnotic masses have lost volume much more rapidly than DNA content and therefore possess a higher concentration of DNA. As noted by Leuchtenberger (1950) their increased Feulgen density is due to the latter factor and not to any increase in the average amount of DNA. In this particular case the 23 per cent decrease in the average DNA content of pycnotic masses can probably be accounted for by the nuclear fragmentation discussed above, and by the fact that in many instances only part of the nuclear material was of a measurable size. Such fragmentation does not always accompany pycnosis: in autolysing guinea pig kidney slices, many nuclei be-

PLATE I

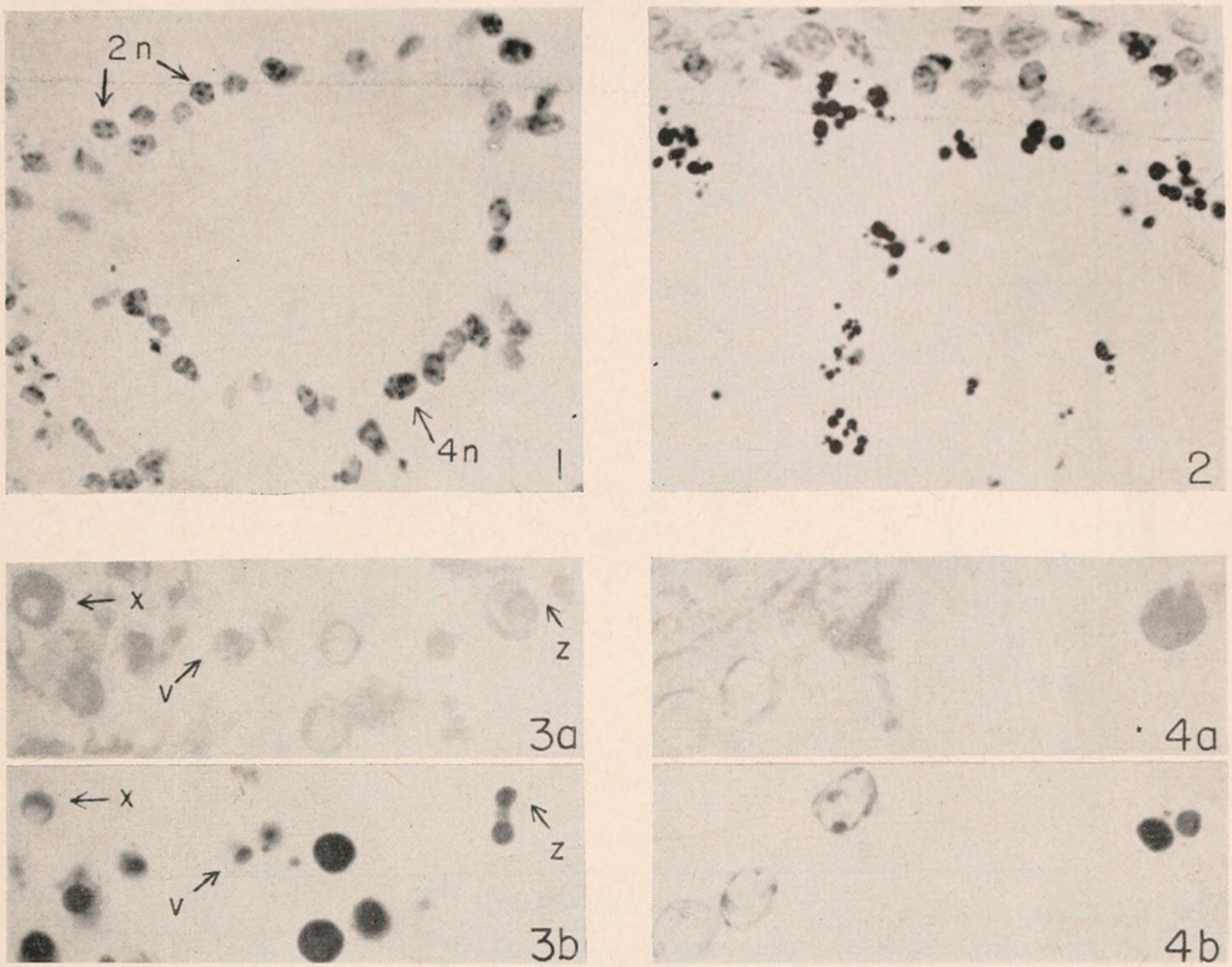

FIGURE 1. Nuclear histone staining (fast green at pH 8) of normal follicle cells surrounding a young oocyte. Formalin, 10 μ, × 570.

FIGURE 2. Nuclear histone staining of pycnotic masses in the antrum of an old follicle. Formalin, 10 μ, × 570.

FIGURES 3 AND 4. Distribution of Millon-protein (a) and Feulgen-DNA (b) in the same follicle cells. Carnoy, 3 μ, × 1100. Figures 3a and b pycnotic nuclei; figures 4a and b normal cells at the left, a degenerating cell at the right. Compare identical nuclei, as identified by arrows, in 3a and 3b. (Photomicrographs by Dr. Walter Plaut.)

come pycnotic by uniform shrinkage without fragmentation and lose none of their DNA during a period of 24 hours autolysis (Roslansky and Alfert, unpublished).

In Table II the Feulgen/fast green and Feulgen/methyl green ratios of the formalin series are given in the first two columns. These data indicate a striking change in the fast green stainability of histone and a small change in the methyl green stainability of DNA. The ratios are lowered, because of a relative increase in acidophilia of histone, and in basophilia of DNA. Put in other terms, there seems to be more histone and more methyl green stainable DNA in the pycnotic nuclei, per unit Feulgen-DNA content, than was present in the normal nuclei from which they were derived.

The methyl green effect is exactly opposite to that described by Leuchtenberger (1950) for pycnotic tumor nuclei: those had an increased Feulgen/methyl green

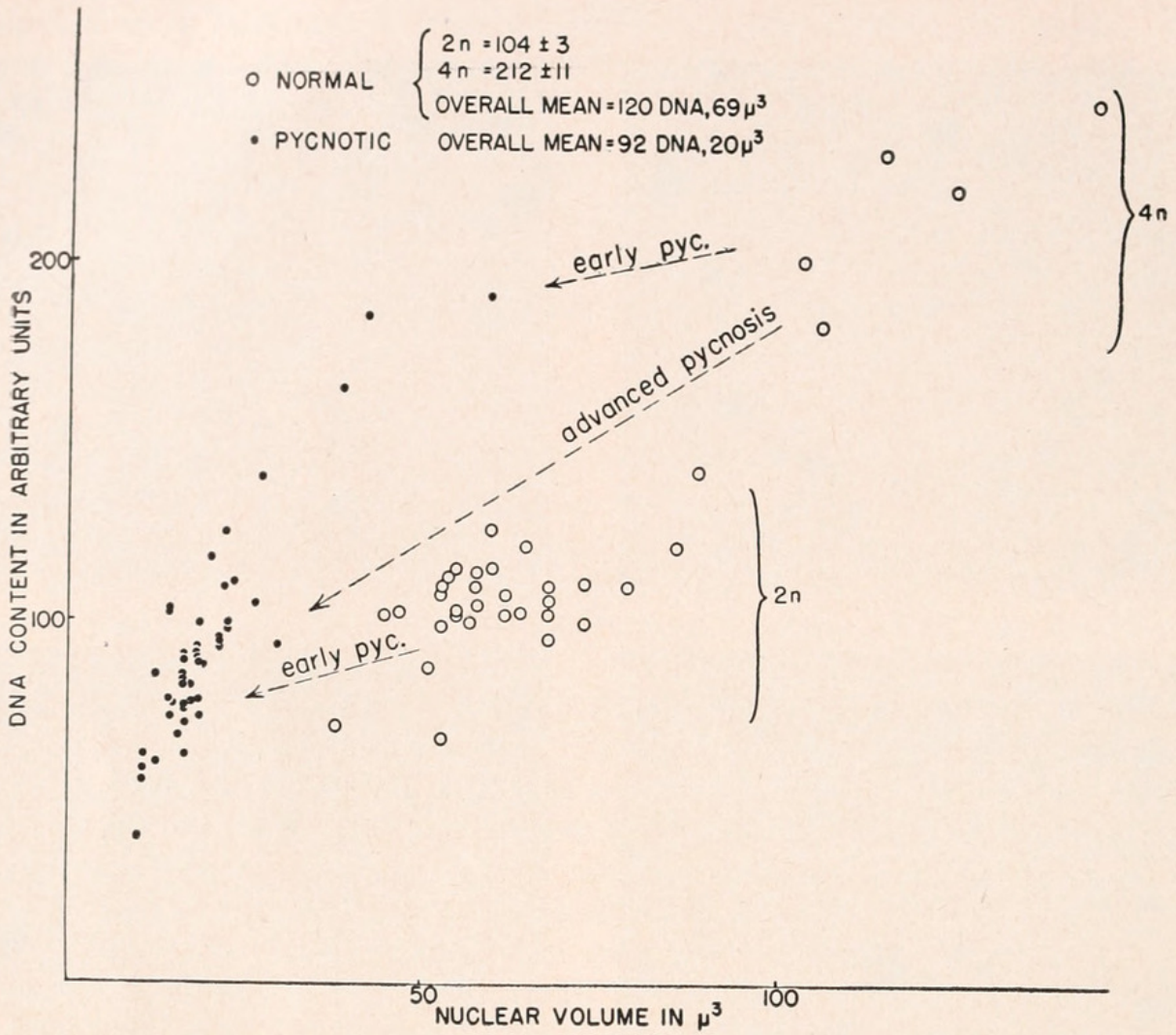

FIGURE 5. The relations between DNA content and volume in normal and pycnotic chromatin of guinea pig follicle cells fixed in formalin.

ratio, *i.e.*, relatively decreased methyl green stainability. In order to see to what extent different types of fixation affect the Feulgen/methyl green ratio, the second series of measurements was performed on nuclei fixed in Carnoy's fluid. These data are presented in the third column of Table II. It was again found that the pycnotic nuclei have a lower Feulgen/methyl green ratio, and that the staining difference was even more pronounced after Carnoy than after formalin fixation. These cases therefore appear to represent a basically different pattern of pycnosis than that described for tumor nuclei.

It is also of interest to compare the difference between the staining capacity of both types of chromatin after Carnoy and formalin fixation in terms of average amounts of dye bound. The compositions of the measured nuclear populations with respect to polyploidy were similar: the formalin data are given in Figure 5; in the Carnoy series 19 diploid nuclei had a mean Feulgen dye content of 55 ± 1.7, and two tetraploids averaged 105; the over-all average was 59, while that of twenty pycnotic masses was 46, a 22 per cent reduction compared to the normals. The complete data for such a comparison are given in Table III.

It can be seen that the Feulgen values of both normal and pycnotic nuclei are about twice as high, after formalin fixation, as they are in Carnoy-fixed material. On the other hand the methyl green contents of pycnotic masses are almost identical

TABLE II

Means ± S.E. of the ratios of optical densities obtained by Feulgen, methyl green and fast green staining. Numbers of determinations in parentheses

	Formalin fixation		Carnoy fixation
	Feulgen* / Fast green***	Feulgen* / Methyl green****	Feulgen* / Methyl green****
Normal nuclei	1.50 ± 0.07 (33)	1.15 ± 0.02 (36)	1.58 ± 0.02 (21)
Pycnotic masses	0.60 ± 0.01 (45)	0.95 ± 0.03 (46)	0.96 ± 0.02 (20)
% change in pycnosis	− 60	− 17	− 39

* Calculated in terms of E_{510}, ~ 51% of peak absorption at 570 mμ.
** Calculated in terms of E_{560}, peak absorption.
*** Calculated in terms of E_{600}, ~ 53% of peak absorption at 635 mμ.
**** Calculated in terms of E_{650}, peak absorption.

after the two types of fixation, while the methyl green stainability of normal nuclei is substantially greater after formalin.

DISCUSSION

The results obtained by the three staining methods employed in this investigation will be discussed separately in the succeeding paragraphs.

a) The Feulgen reaction

Measurements of amounts of Feulgen dye were undertaken in order to obtain a standard against which the other two staining reactions could be compared. The average DNA content of pycnotic nuclei (or nuclear fragments) is lower than the average of normal nuclei. The data plotted in Figure 5 clearly show, however, that the largest of the pycnotic nuclei, although they fall into the same size range as normal diploid nuclei, have a markedly higher than diploid DNA content; in all probability these relatively large pycnotic masses result from the degeneration of normal tetraploid cells. The origin of the more frequent small pycnotic masses is less easy to establish: they might either represent early stages of degeneration of normal diploid cells, or more advanced stages of degeneration of tetraploids. The possible origins of the pycnotic nuclei are indicated by the arrows in Figure 5.

TABLE III

Average amounts of DNA in arbitrary units after different fixations

		Carnoy	Formalin	Apparent increase over Carnoy-fixed
DNA content in Feulgen units	Normal	59	120	103%
	Pycnotic	46	92	100%
DNA content in methyl green units	Normal	37	53	43%
	Pycnotic	48	49.5	3%

As indicated above, the degrees of nuclear degeneration in this tissue cannot be easily seriated because karyorrhexis accompanies the degenerative process right from its onset.

The amount of Feulgen dye per nucleus differs by a factor of two in the formalin and Carnoy series. It has been previously known that Feulgen intensity can vary greatly, depending on the type of fixative used, and the conditions under which the reaction is performed (Sibatani and Naora, 1952). These factors have been discussed by Swift (1955). It must again be emphasized that these differences in Feulgen intensity are due to intrinsic factors related to the availability of aldehyde groups for the Schiff reagent, and cannot be explained on the basis of the "distributional error" in cytophotometry (*cf.* Ornstein, 1952). Although the absorption curves of Feulgen-stained nuclei differ somewhat after Carnoy and formalin fixation (the absorption maxima being located at 560 mμ and 570 mμ, respectively), this difference, or any other slight distortion of the curve, is far too small to account for a 100 per cent difference in staining intensity. In the present context it is important to note that, whatever factor might be responsible for the staining difference between the Carnoy and formalin series, it has affected both normal and pycnotic nuclei to the same extent (see Table III).

b) Methyl green staining of DNA

The Feulgen measurements after either fixation demonstrated that the pycnotic masses averaged 22–23 per cent less DNA than the normal follicle nuclei. Any decrease in the Feulgen/methyl green ratio indicates a relative increase in basophilia of DNA. Accordingly, each unit of DNA in pycnotic nuclei is able to bind more methyl green than the same quantity of DNA in normal nuclei. This effect is difficult to explain on the basis of the hypothesis, proposed by Kurnick (1950), that methyl green combines only with highly polymerized DNA. However, in the present writer's opinion, there exists little evidence for such a specificity of methyl green when used in ordinary histological procedures. The reasons for this opinion were given in detail in a previous paper (Alfert, 1952). Observations made at that time were in good agreement with the present data and supply a reasonable explanation for the increased basophilia of pycnotic chromatin.

It was formerly noted that intensity of methyl green staining depended on the degree of protein interference, *i.e.*, competition of basic groups of certain nuclear proteins with dye molecules for nucleic acid phosphate groups. Agents which abolished or combined with protein amino groups (acetylation, Van Slyke reaction, formalin treatment) at the same time enhanced the methyl green stainability of DNA. In the former experiments acetylation or Van Slyke reaction had raised the methyl green stainability of Carnoy-fixed mouse pancreas nuclei between 40 per cent and 61 per cent (Alfert, 1952; Table III, p. 153); in the present series (Table III) the dye-binding capacity of normal follicle nuclei is 43 per cent greater after formalin than after Carnoy. It therefore appears that methyl green stainability is partially inhibited in normal follicle nuclei by protein interference, and that most of this inhibition can be removed by formalin treatment. The pycnotic degeneration may by itself have resulted in a physical separation of the nucleoprotein complexes, leading to an unmasking of stainable groups of DNA. Consequently formalin treatment would have no further effect on the dye-binding capacity of

pycnotic chromatin. As the data in Table III indicate, almost identical methyl green values were obtained for pycnotic nuclei after either fixation.

This hypothesis can be put somewhat differently in terms of the Feulgen/methyl green ratios listed in Table II. (One can then make the comparison *within* each series of measurements instead of *between* two series of independent samples.) After Carnoy fixation there is a large difference between the Feulgen/methyl green ratios of normal and pycnotic nuclei, because methyl green staining is partially inhibited in the former and uninhibited in the latter. This difference is much smaller in the formalin-fixed series, because formalin had the effect of "equalizing" the staining capacities of normal and pycnotic chromatin by removing at least a large fraction of the staining inhibition present in normal nuclei only.

The protein fractions responsible for staining inhibition, and which may be dissociated and perhaps lost during pycnosis, seem to consist of non-histone proteins. It is known from the work of Mirsky and Ris (1951) that a variable fraction of nuclear DNA may be associated with non-histone protein. Such a condition could be reflected in differences between the Feulgen/methyl green ratios of different nuclear types if one assumes that methyl green cannot displace non-histone proteins, but is capable of competing with histones for binding sites on the DNA molecule. The present fast green data, still to be discussed, justify at least the second part of the foregoing assumption.

In the previous investigation (Alfert, 1952) Feulgen/methyl green ratios of mouse tissues fixed in Carnoy's fluid were determined at different wave-lengths from the ones used here for guinea pig follicle cells. If the data are recalculated for the same wave-lengths, reasonable agreement is obtained: in terms of peak extinctions, the formerly found ratio of 2.47 becomes 1.78 against the present one of 1.58 (Table II). In mouse embryonic nuclei (Swift, 1953) and plant nuclei (Alfert, 1952) very different Feulgen/methyl green ratios have been observed.

c) Fast green staining of basic proteins

The changes in fast green stainability that occur in pycnosis are of the same type as those discussed in connection with methyl green, but more extreme: per unit of DNA the basic proteins bind more dye in pycnotic than in normal nuclei. Basic proteins have thus not been lost, but appear to have become highly concentrated, and even increased, during pycnosis.

In case of the methyl green stainability of DNA, the observed increase in dye binding of pycnotic chromatin could safely be attributed to a mere increase in basophilia, since the concurrent Feulgen measurements demonstrated that the actual amounts of DNA per nucleus had not increased. The fast green measurements are more difficult to interpret. The decreased Feulgen/fast green ratio could have one of two reasons (or a combination of both): 1) either the actual *amounts* of stainable groups have increased in pycnotic nuclei, or 2) the amounts have not changed but their ability to bind acid dye has increased (similar to the increased basophilia of DNA). Until an independent method for the determination of basic proteins in these nuclei becomes available it will not be possible to distinguish with certainty between these alternatives. An actual increase in the amounts of fast green stainable groups could occur by degradation of non-histone

proteins into acid and basic residues and subsequent loss of the acid portions only. At present there is no evidence that this actually occurs, but until it is excluded the explanations for the observed change in fast green stainability must of necessity be speculative.[1]

In previous applications of the fast green method for histones it was found that nuclei can undergo wide physiological variations in size and protein content without apparent change in the number of stainable histone groups (*cf.* Alfert and Geschwind, 1953; Alfert, Bern and Kahn, 1955). There are, however, differences in the relative histone stainability among different nuclear types; sperm cells, especially, which are known to contain very basic proteins, stain correspondingly stronger than somatic nuclei. Staining artifacts, due to interference by acid protein, have also been described by Bloch and Godman (1955a) in model systems designed to test the fast green-histone procedure. More recently, Bloch and Godman (1955b) have observed parallel deviations of Feulgen/methyl green and Feulgen/fast green ratios during "differentiation" of formerly rapidly dividing embryonic cells. In that case these ratios increased together as the nuclei grew in size and protein content; in the present case, both ratios dropped as the nuclei condensed. However, changes in these ratios do not always occur simultaneously: the macro- and micronuclei of the ciliate *Tetrahymena pyriformis* have identical Feulgen/methyl green but different Feulgen/fast green ratios (Alfert and Goldstein, 1955). At present it is impossible to decide in which of these instances actual changes in the amount or character of the basic proteins occur, and which result from variable degrees of staining interference by non-histone protein fractions.

Conclusions

Acid and basic staining methods which depend on salt formation between dye and substrate ions are at least under certain conditions subject to artifacts due to competing ions (*cf.* Swift, 1953). There is strong evidence that such a mechanism is responsible for the difference in methyl green stainability between normal and pycnotic chromatin. The model experiments of Bloch and Godman (1955a) demonstrate that the same effect could also be responsible for the change in fast green stainability of histone. A hypothesis which agrees with the known facts, but which will require eventual analytical confirmation, can be construed as follows:

In normal chromatin DNA, histones and non-histone proteins are intimately combined (*cf.* Mirsky and Ris, 1951; Bernstein and Mazia, 1953). The nature of this complex is such that only a fraction of the acid groups of DNA and of the basic groups of histones are available for dye binding. The pycnotic degeneration leads to a dissociation of this complex that is reflected in the structural changes which accompany pycnosis: nucleohistone condenses to a greater extent than the remaining protein fractions. This dissociation leads to the unmasking

[1] This possibility was subsequently tested by comparing histone content after pycnosis in two types of nuclei which, in the normal condition, had been found to contain equal amounts of histones but greatly different amounts of total protein. Mouse kidney slices were allowed to autolyse and histones were measured in pycnotic nuclei of collecting ducts and of proximal convoluted tubules. Both types of nuclei exhibited identical increases in histone stainability over the normal condition.

of stainable groups in both moieties of the nucleohistone. A similar mechanism has previously been invoked by Kelley (1939) who observed differences in toluidine blue basophilia among resting, dividing and necrotic nuclei and with whose observations the present data agree.

The main interest of the data presented here lies in the possibility that they may furnish an indirect clue to some physical properties of nucleoprotein complexes in normal nuclei, and provide a starting point for further investigations. The apparent existence of widely different patterns of pycnotic degeneration is also noteworthy.

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Summary

1. Quantitative changes in the staining capacity of nuclear DNA and basic proteins during pycnosis of guinea pig follicle cells were investigated by photometric techniques. The great density of these pycnotic nuclei requires special precautions during photometric analysis.

2. The results show that amounts of measurable DNA, in terms of Feulgen dye, decrease during pycnosis, probably because of progressive nuclear fragmentation. At the same time the methyl green basophilia, per unit Feulgen-DNA, is greatly enhanced. Basic proteins show an even greater relative increase in their capacity to bind acid dye during pycnotic degeneration.

3. These data are interpreted to indicate that a dissociation of the normal nucleoprotein complexes occurs during the degenerative process; this leads to unmasking of charged groups which had previously been unavailable for combination with dye ions.

4. The pattern of pycnosis in the present material appears to differ from that previously described for tumor nuclei.

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