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THE COMPOSITION OF THE LE AND HEMATOXYLIN BODIES OF SYSTEMIC LUPUS ERYTHEMATOSUS *

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The hematoxylin stained bodies of systemic lupus erythematosus which were recognized by Gross,[1] and Ginzler and Fox[2] were correctly believed by these authors to be derived from altered nuclei. So unique to systemic lupus are the changes leading to the formation of these bodies and aggregates that they have been regarded as pathognomonic of this disease, and serve as additional criteria for its anatomic diagnosis. With the discovery of the LE cell phenomenon[3-6] it became obvious that the hematoxylin bodies were the counterparts in the tissues of the LE bodies formed *in vitro*. Histochemical characterization of these bodies,[7,8] which appeared to indicate that they contained partially depolymerized deoxyribonucleic acid (DNA), led to the concept that a depolymerase (DNASE) was activated in systemic lupus.[7,9-13] However, serum levels of DNASE were not found to be significantly elevated in this disease,[11,14] and it was hypothesized[11-13] that depolymerization of DNA in the LE cell was due to release of an intracellular DNASE from an intracellular inhibitor of DNASE by an action initiated by the circulating LE factor of the blood. It has since been proposed that the LE factor itself permits entrance of serum protease into the cytoplasm which in turn releases the intracellular deoxyribonuclease from its inhibitor.[13,15]

While most interest centered about the state of DNA in the hematoxylin body, it was understood by Klemperer,[9] and Gueft and Laufer[10] that the body was a nucleoprotein complex, possibly containing still other substances. The importance of the protein moiety was emphasized

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by the suggestion of Gueft and Laufer[10] that the fibrinoid substance in systemic lupus erythematosus was the residue of degraded nucleoprotein of the hematoxylin bodies from which some or all of the stainable DNA had disappeared.

Many of our conceptions about the pathogenesis of the tissue changes occurring in systemic lupus erythematosus have thus depended upon histochemical analysis of the hematoxylin bodies. In the work of Klemperer and co-workers,[7] the state of nucleic acid in the hematoxylin bodies was inferred from microspectrophotometric measurement of the relative degrees of Feulgen reaction and methyl green staining. Interpretation of methyl green staining of DNA has since been somewhat complicated by the demonstration that it is influenced by factors other than the degree of "polymerization" of DNA, particularly by protein interference. The protein component of the hematoxylin body has barely been characterized thus far, but it was regarded primarily as a residue of nuclear origin by Klemperer,[9] and by Gueft and Laufer.[10]

The recent elaboration of specific and quantitative staining methods for proteins has provided additional means for studying the composition of the hematoxylin and LE bodies. Their composition was therefore reinvestigated with the objects of assessing the cause of the previously observed depression of methyl green binding by DNA in the hematoxylin bodies, and of gaining additional information on their protein components.

Materials and Methods

Tissues from 3 fatal cases of systemic lupus erythematosus were available for study.* Sections of kidney tissue from one of these, fixed in Carnoy's fluid, were used for microspectrophotometric measurement. The remaining tissues, which had been fixed in formalin, were washed to remove unbound formaldehyde, and were examined after successive procedures. Abundant LE bodies (the free nonphagocytosed material) were obtained from LE preparations made according to the "ring" method of Snapper and Nathan,[16] the method of Davis and Eisenstein[17] in which dried leukocytes, concentrated in buffy coat, serve as substrate cells, and the method of Lee,[18] in which atabrine (quinacrine) is used to prepare leukocytes for the action of the LE factor of the serum (Figs. 10 and 11). Smears were then fixed in methanol or in 10 per cent neutral buffered formalin. Sections stained with hematoxylin and eosin, or smears of LE preparations stained with Wright's solution were photographed or mapped in order to permit

* Tissue for study was generously contributed by Dr. Henry Michelson of the University of Minnesota, and Dr. Harlan Firminger of the University of Kansas.

reidentification of the same objects in all subsequent procedures. Slides were then destained in 70 per cent ethyl alcohol acidified with trichloroacetic acid prior to the application of other methods.

Methyl green staining was performed in a phenol-glycerine solution at pH 4.2, according to the specifications of Pollister and Leuchtenberger.[19] The sections were then treated in one of two ways. In one group, after removal of the methyl green with alcohol, the free basic groups of the protein were blocked by acetylation in pure acetic anhydride for two hours at room temperature. They were then restained with methyl green and the areas previously studied were rephotographed or remeasured. The conventional Feulgen technique was then carried out on some of these preparations. After removing the methyl green, the other group of slides was stained with a modified Feulgen reaction method for DNA[20] in which one normal trichloroacetic acid replaced hydrochloric acid in the hydrolysis and in the preparation of the Schiff reagent. After rephotographing or remeasuring the same fields, the nucleic acids were extracted with 5 per cent trichloroacetic acid at 90° C. for 15 minutes. The slides were then stained for histone with the anionic dye fast green FCF at pH 8.0-8.2, according to the directions of Alfert and Geschwind.[21] Quantitative cytophotometric determinations of "total" protein were made on free LE bodies in LE preparations, by means of the binding of naphthol yellow S (C.I. 10) (flavianic acid) to protein basic groups, as elaborated by Deitch,[22] and the cytochemical Millon reaction of Pollister and Ris[23] as modified by Rasch and Swift.[24] The Sakaguchi reaction for arginine, modified to yield a more stable color by rapid dehydration after reaction in an alkalinized alphanaphthol and hypochlorite mixture, was used to detect arginine residues. The utility of the Sakaguchi reaction for cytophotometry has been established by McLeish and co-workers.[25]

A microspectrophotometric apparatus[26] incorporating some of the modifications proposed by Pollister[27] and Moses[28] was employed with a tungsten ribbon-filament light source powered from a battery-buffered system designed to improve voltage stability. Radiation of desired wavelength was isolated with a Bausch and Lomb diffraction grating monochromator. The methyl green color was measured at 633 mμ, the Feulgen complex at 568 mμ, naphthol yellow S at 435 mμ, the fast green at 625 mμ, the Millon complex at 490 mμ, and the Sakaguchi reaction at 510 mμ. A constant plug of 3.5 μ diameter was circumscribed for photometric measurement of LE bodies, and a plug of six tenths or less of the short diameter of whole nuclei in sections. Measured values are reported in arbitrary units of apparent amounts or

concentrations of dye per body or nucleus. Amounts were calculated by multiplying extinction by area in the case of LE bodies, and extinction by $\frac{D^2}{F} \times \frac{d_b}{d_a}$ in the case of nuclei in sections, where D is the plug diameter, F the fraction of the nuclear volume included in the plug, and $\frac{d_b}{d_a}$ the ratio of the longer to the shorter diameter of the nucleus. The units of any set of determinations are not necessarily comparable with those obtained in other series of measurements; ratios, however, can always be compared from experiment to experiment.

Observations

Nucleic Acids

Deoxyribonucleic acid, which is principally responsible for the basophilia of cell nuclei, is among the most stable of the nuclear components. It is cytochemically demonstrable by its marked absorption of ultraviolet light at about 2,600 Å, by the Feulgen reaction given by its deoxypentoses, and by the affinity of its phosphate groups for basic dyes.

Methyl Green Staining

Although most basic dyes exhibit no such specificity of staining, the cationic dye methyl green has been known to cytologists for many years as a selective and reproducible nuclear stain.[19] It has been shown that under certain conditions, methyl green (heptamethylpararosanilin) can combine selectively and even stoichiometrically with available phosphoryl groups of polymerized DNA[19,29-31] and it has therefore been employed for cytophotometric measurement of DNA in nuclei.[19,32-35] Depolymerization of DNA was found to reduce its binding of methyl green *in vitro*.[29,36] This fact was thought to explain the microspectrophotometric depression of methyl green stainability observed in cytologic material under various conditions,[19,33,35-38] assuming intracellular alteration of DNA "in the nature of depolymerization."[7] Depression of methyl green binding by hematoxylin bodies of systemic lupus erythematosus as compared with normal nuclei was thus interpreted to mean that in the transformation of nuclei in this disease, DNA became depolymerized.[7-10] However, it is known that various factors, including the presence of cations,[30,31,39] and especially associated proteins, effectively interfere with methyl green binding by nucleic acids.[40] Methyl green appears to be more sensitive to such interference than other basic dyes.[40] The influence of these factors must be accounted for before it is possible to assess cytochemically the state of polymerization of molecular configuration of DNA in tissues.

To evaluate the possible effect of protein interference on the methyl green binding by DNA in hematoxylin and in LE bodies, the methyl green staining in the same bodies was photographed or measured microspectrophotometrically before and after acetylation of protein basic groups. Acetylation is one of the ways of blocking the positively charged groups of proteins which compete with basic dyes for the available anionic binding sites of the nucleic acid.[22,40-42] Figures 6, 7, 12 and 13 illustrate some intensification of methyl green staining of hematoxylin and LE bodies after acetylation.

Microspectrophotometric comparison of the amounts of DNA revealed by methyl green in methanol-fixed lymphocytes and LE bodies in an atabrine LE preparation,[18] before and after acetylation, is shown in Table I. After acetylation of control lymphocytes, the amount of methyl green bound to DNA is augmented by about 6 per cent. In lupus bodies, however, blocking of protein basic groups by acetylation

TABLE I

Mean Amounts of DNA in Arbitrary Units as Determined by Methyl Green Binding and the Feulgen Technique

	Blood		Kidney	
	Lymphocytes	Lupus bodies	Normal nuclei	Hematoxylin bodies
Expressed as:	Amounts	Amounts	Amounts	Concentrations
No. measured:	20	20	30	15
Methyl green:	16.7±0.5	11.9±0.3	15.5±0.47	0.15±0.14
Methyl green after acetylation:	17.8±0.8	23.1±0.6	22.3±1.5	0.47±0.02
Feulgen:	19.9±0.4	21.3±0.5	17.8±0.5	0.43±0.02
$\frac{\text{Feulgen}}{\text{Methyl green}}$:	1.19	1.79	1.15	2.8
$\frac{\text{Postacetyl Me. gr.}}{\text{Methyl green}}$:	1.06	1.94	1.43	3.26
$\frac{\text{Feulgen}}{\text{Postacetyl. Me. gr.}}$:	1.12	0.92	0.80	0.90

Successive measurements of: (1) methyl green binding capacity and the Feulgen reaction of DNA in free LE bodies derived from leukemic lymphocytes in an "atabrine" LE preparation, and comparable control nuclei of such lymphocytes; (2) the same hematoxylin bodies and normal renal tubular epithelial nuclei. Total amounts have not been calculated for the hematoxylin bodies because their irregular shapes make calculation of their volume difficult and inaccurate; only the extinctions (concentrations) are given. The ratios of both experiments may be compared, but the amounts in arbitrary units are comparable only within a given experiment (i.e., lymphocytes and LE bodies).

Note that acetylation of protein effects a greater rise of capacity to bind methyl green in nucleic acid of LE and hematoxylin bodies, and revises the Feulgen:methyl green ratios to the same general range as those of nuclei.

effects almost a twofold average rise in the ability of their DNA to bind methyl green. The ratio of the amount of methyl green bound after acetylation to that capable of being bound before acetylation gives us a measure of the DNA phosphate masked or pre-empted by combination with protein, and from these it would appear that about half of the stainable sites of DNA in lupus bodies were masked by protein, while less than one tenth of the nucleic acid phosphate was pre-empted in this way in the lymphocyte. Very similar ratios were obtained from measurement of LE preparations made by other methods.[43]

The results of such comparison of methyl green binding by normal renal tubular epithelial nuclei and hematoxylin bodies in kidney tissue fixed in Carnoy's fluid, from a case of systemic lupus erythematosus, are tabulated in Table I. The DNA of normal kidney epithelial nuclei exhibits a 43 per cent average increase in methyl green uptake following elimination of competing protein basic groups by acetylation. This larger rise, contrasted with only a 6 per cent increase in lymphocyte nuclei after this treatment, is a reflection of the smaller total amount of protein in the latter. In hematoxylin bodies in tissue, acetylation effects a more than threefold augmentation of the concentration of methyl green in the same bodies.

The methyl green binding capacity of hematoxylin bodies in tissues which had been fixed in formalin for long periods remained almost unchanged after subsequent acetylation, a result attributable[41,44] to the irreversible combination of formaldehyde with amino, imino and guanido groups. Formalin fixation thus resembles acetylation in its effect on methyl green binding.

Comparison of Methyl Green and Feulgen Staining

The Feulgen reaction for DNA is relatively insensitive to those changes in the state of DNA or in its relation to proteins that affect methyl green uptake. Measurements of the Feulgen reaction therefore provide a standard of reference with which other staining properties of DNA such as methyl green binding can be compared. For many types of nonproliferating cells, the ratio of the amount of methyl green-stained DNA to the amount of Feulgen-revealed DNA is nearly constant and happens to be around 1.0. In the data reported by Klemperer and co-workers,[7] the ratios of the Feulgen to methyl green reactions in hematoxylin bodies in tissue were found to be greatly enhanced as compared with normal nuclei, ranging from about 2.0 to 8.0, owing to depression of methyl green binding. These high ratios, which were at

that time interpreted as indicative of depolymerized DNA, may now be regarded as having been due, in some part, to protein interference. The methyl green binding of LE or hematoxylin bodies after acetylation of their protein, compared with the concentration of DNA measured in the same bodies by the Feulgen technique (i.e., the Feulgen: postacetylated methyl green ratio) may be expected to indicate whether there is any significant residual decrease in methyl green binding which cannot be accounted for by the presence of associated protein. The results, which are given in Table I, reveal that the Feulgen:postacetylated methyl green ratios of LE bodies and lymphocyte nuclei, and hematoxylin bodies and renal tubular epithelial nuclei alike are between 0.8 and 1.1 and thus tend to approach 0.1. It is therefore concluded that there is no significant decline of methyl green staining of DNA in lupus bodies which cannot be accounted for by protein interference, and that the DNA of the LE or hematoxylin body is not depolymerized or altered in state in a manner which is detectable cytochemically.

Comparison of the amounts of Feulgen-revealed DNA of lymphocyte nuclei and of whole nonphagocytosed LE bodies, each of which apparently originated from lymphocyte nuclei, make it clear that in the transformation of the leukocyte nucleus to the free LE body there is no appreciable loss in the total amount of DNA. After such bodies have been engulfed by polymorphonuclear leukocytes, evident loss of stainable material ultimately occurs. The reduction in the Feulgen stainability of hematoxylin bodies presumed to be of longer standing has previously been noted.[9,10]

PROTEIN

The foregoing data on nucleic acid stainability have drawn attention to the protein moiety of the nucleoprotein material which constitutes the hematoxylin or the LE body. The extent of its interference with methyl green uptake by the nucleic acid in these bodies suggests that their protein components differ both in kind and amount from those normally present in nuclei.

Proteins of the Normal Nucleus

From about 75 per cent (in lymphocytes) to 95 per cent (in liver cells) of the dry mass of normal nuclei isolated in nonaqueous media is made up of protein.[45,46] In contrast to the constancy of the DNA complement of each nucleus, the dry mass may vary considerably in amount and in kind of different cell types of the same organism, and in the same cell types in different physiologic and pathologic states.[47-54]

Closely associated with DNA in all somatic nuclei are the strongly basic histones, which presumably exist in the nucleus as salt-linked histone deoxyribonucleates,[47,54,55] and are present in definite quantitative ratio to DNA in any cell type.[20,47,55] They are less active metabolically than other nuclear proteins, as judged by isotopic turnover rates.[47] Also associated with DNA as components of the somatic chromosome, are higher proteins which have been designated "residual chromosomal protein."[53] The amounts of "residual" proteins depend upon cell type and metabolic activity.[47,52,53] Liver and kidney cell nuclei, for example, have more such protein than lymphocyte nuclei.[47,52] Protein of the "residual" type is particularly effective in interfering with methyl green binding to the DNA with which it is associated, and this fact is reflected in the higher Feulgen:methyl green, and postacetylated methyl green:methyl green ratios of kidney nuclei as compared with those of lymphocyte nuclei (Table I). In addition to those named, the normal nucleus also contains other, hitherto little-studied proteins, presumably not chromosomal, among which there is a soluble globulin and a lipoprotein.

Proteins of the LE Body

"Total" Protein. Quantitative measurement of the changes in amount of protein resulting from the LE transformation necessitates comparison of the protein content of LE bodies, each of which is known to be derived from a whole nucleus, with that of the type of nuclei from which they originate. Such cytochemical determinations were made on lymphocyte nuclei and free LE bodies resulting from transformation of lymphocyte nuclei in atabrine preparations.[18]

Stoichiometric binding of flavianic acid (naphthol yellow S, the dipotassium salt of 2,4-dinitro-1-naphthol-7-sulfonic acid) to *available* basic (ε-amino, guanido and imidazole) groups of the dibasic protein residues provides the basis for their quantitative determination, and hence an index of the total amount of protein.[22] The concentration of Feulgen-revealed DNA can be measured in the same object concurrently with that of naphthol yellow S, due to the wide separation of the respective absorption peaks (435 mμ and 568 mμ) of the colored complexes.[22] This was done in these determinations in order to visualize and delimit the objects, since visual definition with naphthol yellow S alone proved insufficiently accurate. Since nucleic acid was not extracted prior to staining, the relative amounts of naphthol yellow S measured in these experiments represent only those protein basic groups not pre-empted by combination with DNA, rather than the

potential total demonstrable number. Microspectrophotometric measurements of such material show that more than a twofold increase in naphthol yellow binding capacity occurs in the LE transformation from lymphocyte nuclei, and there is a change in the naphthol yellow S:Feulgen ratio of from 0.82 for lymphocytes to 1.93 for LE bodies.[56]

These values point to an increase in protein basic groups and suggest that the LE change involves an actual increase in the amount of protein. The results of the cytochemical Millon reaction for tyrosine residues of the protein further indicate that this is the case. There are more than twice as many Millon-reactive tyrosine residues in the LE bodies than in the parent lymphocyte nuclei.[56] Interferometric measurements have also revealed that a more than twofold gain of dry mass is entailed in the transformation of nuclei to LE bodies.[57] From these data it is concluded that an actual augmentation in the total amount of protein occurs in the LE transformation of nuclei.

Histones. Owing to their high isoelectric point, strongly basic proteins like histones (which are rich in arginine and lysine residues) can be selectively and quantitatively stained *in situ,* after removal of nucleic acids, by the anionic dye fast green FCF at alkaline pH.[20,21] A modification of the Feulgen procedure, in which the molar substitution of trichloroacetic acid for hydrochloric acid insures retention of protein during hydrolysis and staining, permits the successive demonstration of DNA and then histone in the same body.[20] Utilizing this method, it has been shown that histone is present in all normal and pyknotic chromatin, and has precisely the same microscopic distribution as DNA.[20,21] Some results of the application of these techniques to the hematoxylin bodies of the tissues are shown in Figures 4 and 9. Neither in these nor in the inclusions of typical LE cells (Figs. 14 and 16) can stainable histones be detected. The free unengulfed LE bodies in LE preparations of different kinds are diffusely and relatively faintly stained (Fig. 15), or sometimes quite unstained for histones. Some of the bodies early in the course of transformation show irregular diffuse staining of some parts, and absence of staining in others. Microspectrophotometric measurements of the histone in bodies very early in the course of their evolution from lymphocytes confirm the visual impression of a striking decrease of stainable histone in them, while the concentration of stainable histone in fully formed LE bodies is below accurately measurable limits.[56] The LE change involves a dissolution of chromatin structure and either a loss of histones or some change which renders them vulnerable to preparative loss, or else a masking of their stainable groups. The Sakaguchi reaction for arginine

residues is not affected by those electrostatic factors which influence acid and basic dye binding, and affords a useful check on the fast green technique. Thus, the marked diminution of arginine stainability of LE and hematoxylin bodies as compared with nuclei suggests that an actual loss of histones occurs in the formation of LE or hematoxylin bodies. In contrast to the strong PAS stainability of hematoxylin bodies in tissue, the LE bodies fail to color after the PAS reaction; neutrophil cytoplasm is well stained.

Discussion

The cytochemical method of analysis affords a practicable and feasible approach to an examination of the composition of hematoxylin and LE bodies, in spite of the relative paucity of chemically specific colorimetric reactions available. Through knowledge of the composition of these bodies, and of the ways in which this differs from that of the nuclei from which they take origin, it was hoped to gain some insight into a pathogenetic process operative in systemic lupus erythematosus.

Previous cytochemical interpretations[7-9] of the lupus bodies did not take into account those factors, other than the state of polymerization of DNA, that influence methyl green binding to nucleic acid. Indeed, the effects of fixation,[31,51,59] molecular size,[59] pH and ion competition,[39,51] and the presence of protein[30,40,51,59] on the uptake of basic dyes by nucleic acid have only recently been given sufficient attention, especially as they relate to methyl green staining.

Knowledge of these variables provides us with a means of controlling some of them, and in this way probing into the state of the nucleoprotein complex and the relationship of DNA to protein in various cell states.[51,52] The data presently reported indicate that the impairment of methyl green binding to nucleic acid in both hematoxylin and LE bodies is due to interference by competing protein which is not present in nuclei from which such bodies may form. The restoration of the Feulgen:methyl green ratio of LE and hematoxylin bodies, after destruction of the basic groups of competing protein, to values approaching those of nuclei would indicate that there is no cytochemically detectable change in the state of polymerization or molecular configuration of the DNA in these bodies. Moreover, there is no loss of Feulgen-demonstrable DNA in the original LE transformation.

Inquiry into the quantity and kinds of protein associated with the nucleic acid in the LE body confirms the suggestion of the data provided by methyl green binding that the conversion of nuclei to LE

bodies entails a marked increase in protein and hence in competing protein basic groups. Nonhistone protein, such as chromosomal "residual protein," has been found to be more effective than histone in depressing methyl green stainability of DNA in interphase nuclei.[51] This fact indicates that the masking action of protein with respect to methyl green stainability of nucleic acid phosphate groups is not solely a property of the number of positively charged groups on the protein. Similarly, in the LE body, despite the apparent loss of histone, an unusual protein of higher type becomes linked to DNA and masks more than half of its methyl green stainable anionic sites.

In the formation of the LE body, protein normally foreign to the nucleus enters into it. This would appear to be an initial event.[57] The resulting nucleoprotein mass differs markedly in composition from that in the original nucleus. The data permit one to hypothesize that this protein, at least in part, effects a displacement of histones from their usual linkage with DNA. This would result in their susceptibility to degradation, loss or recombination and masking. The foreign protein itself becomes associated with DNA in such a manner as to interfere with methyl green staining. There is no evidence from the cytochemical data to support the view that the LE phenomenon primarily affects the DNA molecule.

Previous investigations[10] of the protein of the hematoxylin bodies were concerned with their contribution to the formation of fibrinoid material and hyaline thrombi. The hematoxylin bodies and the material remaining after hot trichloroacetic acid extraction were shown to give positive reactions with a modification of the Millon reaction developed for cytochemical use.[23] It was concluded, therefore, that these substances contained tyrosine residues. They were also found to be readily digested by trypsin at pH 7.3. On the basis of their staining with the aniline blue-orange G mixture at pH 3.0, it was inferred that the bodies also contained a basic protein,[10] at first believed to be histone. According to White,[60] histone and globin in purified state and in tissues from which the nucleic acids had been extracted by trichloroacetic acid, bind orange G selectively from the dye mixture. However, any protein possessing basic residues may bind anionic dyes such as orange G at low hydrogen ion concentration. Specific characterization of a protein which stains with orange G under these conditions would now seem to be of questionable validity.

Gueft and Laufer[10] stated that there was no difference in optical density of the Millon stained hematoxylin bodies before and after use of the mercuric sulfate-sulfuric acid reagent in the technique developed

by Pollister and Ris[23] for detection of total and nonhistone protein. However, no detailed data on this point were published by Gueft and Laufer.[10] The observations with the Millon reaction, and those presently reported indicating failure of hematoxylin bodies to bind fast green at pH 8.1, would seem to show that histone is absent from this protein moiety. The other characteristics of the protein residue, such as insolubility in hot trichloroacetic acid or the sulfuric acid Millon reagent, its digestibility by trypsin, and its relative acidophilia, are shared by most nonhistone, nonprotamine proteins, including the "residual chromosomal protein" of Mirsky and Ris.[53] These, however, cannot be considered as specific or even characteristic qualities of any class or group of proteins.

The view of Gueft and Laufer[10] that the "protein constitution of the hematoxylin body does not differ from that of the normal nucleus" is surprising, and cannot be accepted on the basis of present evidence. Indeed, in the failure to demonstrate stainable histone alone, the protein of the hematoxylin body differs profoundly from that of all normal and pathologic nuclei thus far studied.

Further indications of the origin and nature of the incurrent protein found in the LE body have recently been provided by the use of fluorescent labeled antibodies for histologic localization. Mellors and co-workers,[61] and Vazquez and Dixon[62] have localized human gamma-globulin by this means in the inclusions of LE cells, and have noted its absence from normal nuclei. Friou, Finch and Detre[63] have observed that fluorescent antibodies to globulin derived from patients with systemic lupus, but not others, become localized in nuclei when applied to normal mouse tissues. It is of interest that gamma globulin has been localized in the fibrinoid alterations of arterioles and glomeruli in lupus.[64] Moore, Weisberger and Bowerfind[65] have claimed that the protein and carbohydrate material of hematoxylin bodies in lymph nodes are derived from intracytoplasmic bodies which develop in plasma cells in the nodes.

The nature of the reaction of the protein with the nuclear constituents remains unknown. Any explanation of the LE phenomenon must take into account the fact that substrate cells must have been previously traumatized. It is also necessary to take into consideration the rapidity with which the phenomenon occurs[57] and its lack of specificity with respect to species and cell type.

The chemical constitution of the free LE body cannot be regarded as identical with that of the LE cell inclusion, which may have undergone intracellular digestion by the polymorphonuclear leukocyte. For this

reason, in order to gain some insight into the primary pathogenetic effects of the lupus factor on cell nuclei, the free LE body has been used for most of the examinations in this study.

While there can be little doubt that the hematoxylin body is derived from the LE body, histochemical studies make it obvious that substances added to and dissipated from the hematoxylin body in the course of its sojourn in the tissues may alter its composition secondarily. For example, LE bodies produced *in vitro* have undiminished content of DNA; they are metachromatic[43] and fail to react after the PAS procedure. Moreover, older hematoxylin bodies in tissue apparently often have reduced Feulgen stainability; they are not metachromatic, and they react strongly with the PAS test.[10,65] The original nucleoprotein material (LE body) resulting from the action of the lupus factor on susceptible nuclei thus undergoes further changes in the tissue. These appear to be characterized by aggregation of the bodies, the addition of extracellular protein and of PAS-reactive carbohydrate.[10,65] There is also gradual loss of DNA[10] and possibly of other materials. Gueft and Laufer,[10] and Klemperer[9] believe that such hematoxylin bodies may appear as extracellular proteinaceous deposits from which nucleic acids have disappeared. These may then constitute the fibrinoid material of systemic lupus erythematosus.

Summary

LE bodies formed *in vitro*, and hematoxylin bodies from the tissues of 3 cases of systemic lupus erythematosus were examined cytochemically. Binding of methyl green by the DNA of these bodies was compared with control nuclei and found to be depressed, with consequent elevation of their Feulgen:methyl green ratios. Destruction of competing basic groups of the protein associated with DNA in these bodies increased methyl green uptake by 2 or 3 times, and restored the Feulgen:methyl green ratios to values similar to those of control nuclei. The amount of DNA was found to be undiminished in the conversion of a lymphocyte nucleus to an LE body. These data would indicate that the DNA of LE and hematoxylin bodies was not demonstrably depolymerized, and that a protein of a kind or amount not normally present in nuclei was linked to the DNA of these bodies.

The formation of LE bodies was found to entail large increases of protein demonstrable by means of naphthol yellow S (flavianic acid) binding and the Millon reaction. At the same time histones, normally linked to DNA in nuclei were found to be apparently diminished or lost. It is postulated that in the LE transformation there was an

influx of protein normally foreign to nuclei, a displacement of histone from its combination with DNA, and an association of the DNA with the new protein.

The nucleoprotein mass formed in such a pathogenetic process appeared to undergo further changes in the tissues, giving rise to more mature hematoxylin bodies.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Skin biopsy, showing hyaline thrombi and hematoxylin bodies, indicated by arrows, in dilated capillary vessels of the upper corium. The hematoxylin stained smudges in some of the hyaline material are noteworthy. Hematoxylin and eosin stain. × 300.

FIG. 2. Identical field stained with methyl green for DNA, illustrating methyl green binding capacity of hematoxylin bodies. Methyl green stain. × 300. Red filter.

FIG. 3. Identical field after application of TCA-Feulgen procedure. The same bodies are stained by the Feulgen reaction. × 300. Yellow-green filter.

FIG. 4. Identical field after the alkaline fast green technique for histone. The hematoxylin bodies and smudges fail to stain; their locations are marked by arrows. × 300. Red filter.

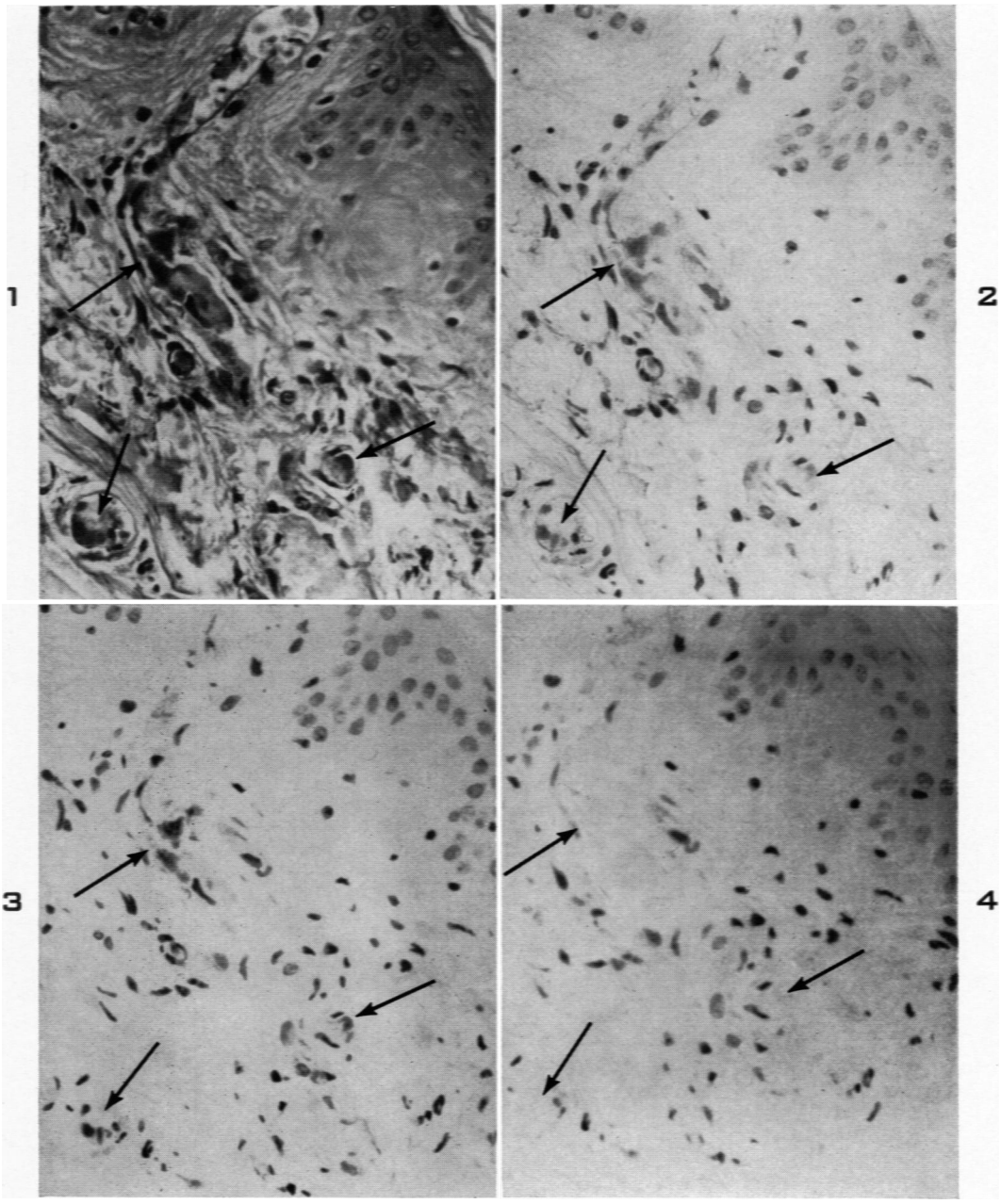
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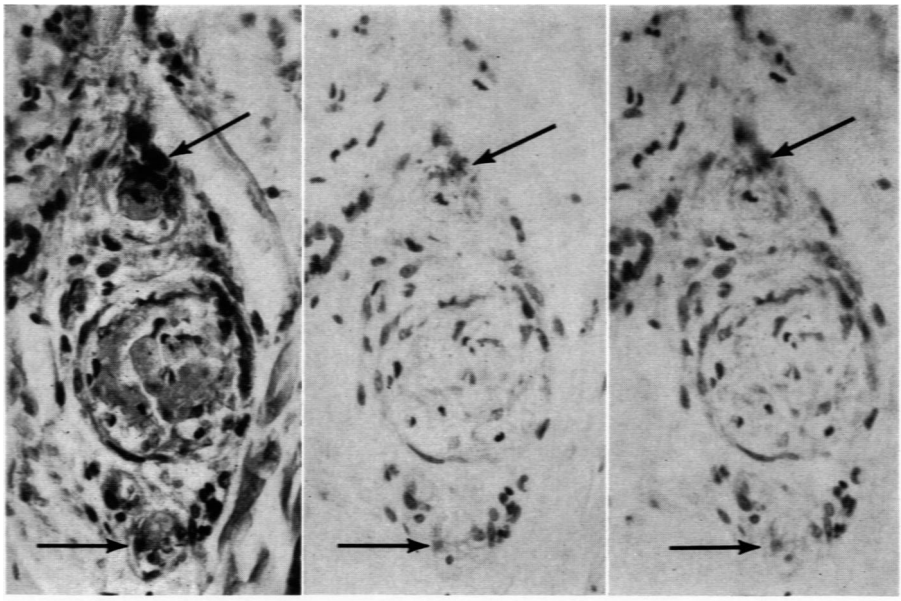
FIG. 5. Skin biopsy. Hyaline thrombi and hematoxylin bodies, the latter indicated by arrow, and smudges in vessels of the corium. Hematoxylin and eosin stain. × 300.

FIG. 6. Identical field, stained for DNA with methyl green stain. × 300. Red filter.

FIG. 7. Identical field, restained with methyl green after acetylation. Some increase in the intensity of staining of the hematoxylin bodies is evident after the elimination of potentially competitive protein basic groups. Methyl green stain. × 300. Red filter.

FIG. 8. Lymph node. Large aggregate or packet hematoxylin body in medullary channel of lymph node in which there is extensive necrosis. TCA-Feulgen procedure. × 450. Yellow-green filter.

FIG. 9. Same hematoxylin body after alkaline fast green technique for histone, showing failure to stain. The sharp outlines of the body in some areas are caused by difference of refractive index between tissue and medium. Alkaline fast green stain. × 450. Red filter.



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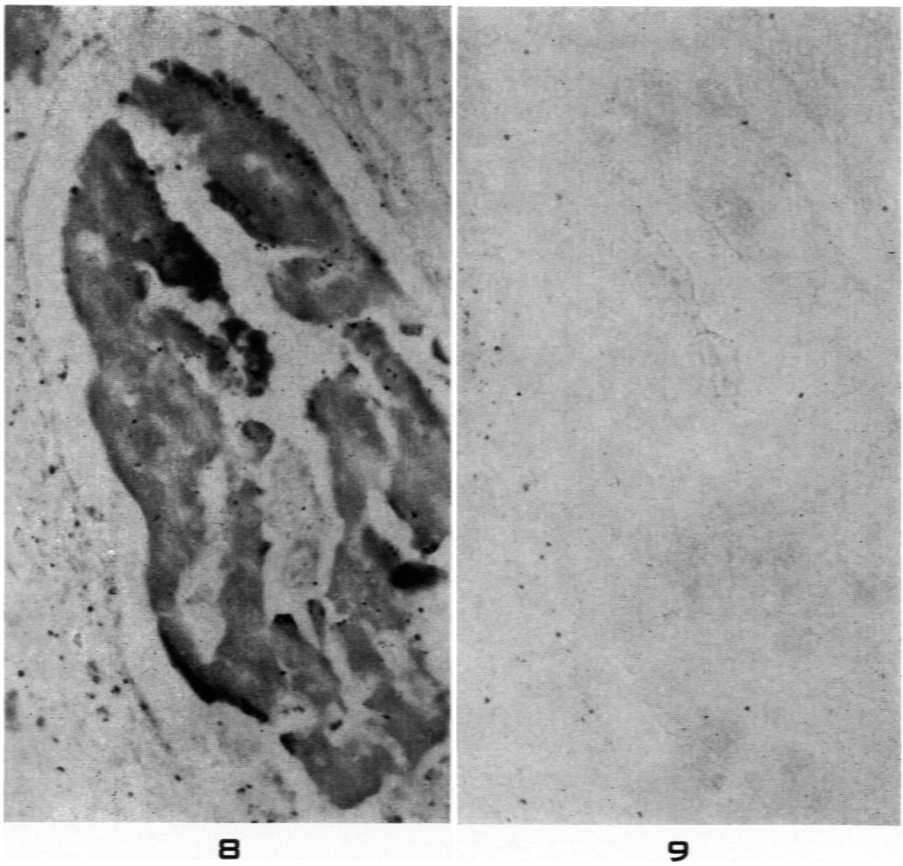

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FIG. 10. LE preparation from normal human buffy coat made by the dried substrate method.[17] All of the LE bodies shown in the field have been engulfed by phagocytes. Wright's stain. × 900.

FIG. 11. LE preparation from buffy coat of a patient with lymphocytic leukemia, made by the atabrine method.[18] The converted bodies are enlarged and pale. Such bodies are susceptible to phagocytosis on the addition of viable polymorphonuclear leukocytes. Wright's stain. × 900.

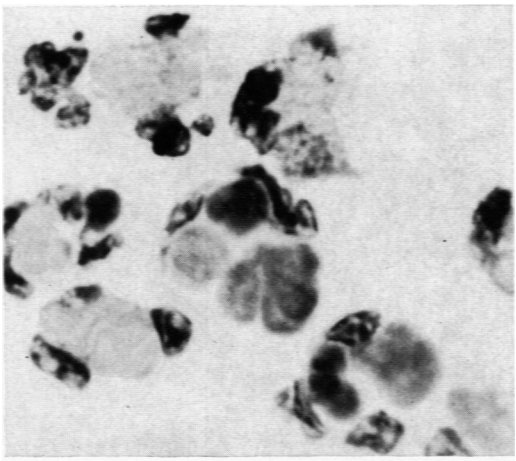
FIG. 12. Free LE bodies (arrow) and LE cell inclusion, in a dried-substrate preparation, stained with methyl green for DNA. × 920. Red filter.

FIG. 13. Same field as that shown in Figure 12, after acetylation and restaining with methyl green. Intensification of staining is evident, especially in the LE bodies. × 920. Red filter.

FIG. 14. LE cells and polymorphonuclear leukocytes in a dried substrate preparation, stained with alkaline fast green method for histones. The nuclear chromatin stains well; the LE inclusions (arrows) are unstained. × 920. Red filter.

FIG. 15. A relatively intact polymorphonuclear leukocyte (upper) and a leukocyte (lower) the nuclear lobes of which have undergone the early changes of the LE transformation. The fast green stainability of the latter is considerably diminished. Alkaline fast green method. × 920. Red filter.

FIG. 16. Polymorphonuclear neutrophils, an eosinophil and two LE cells in a dried substrate preparation, stained with the alkaline fast green method for histones. The chromatin of the nuclei and basic protein of the eosinophil granules are colored; the LE inclusions fail to stain. × 920. Red filter.



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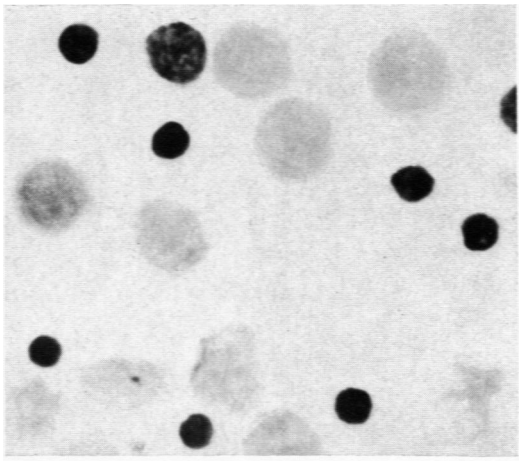

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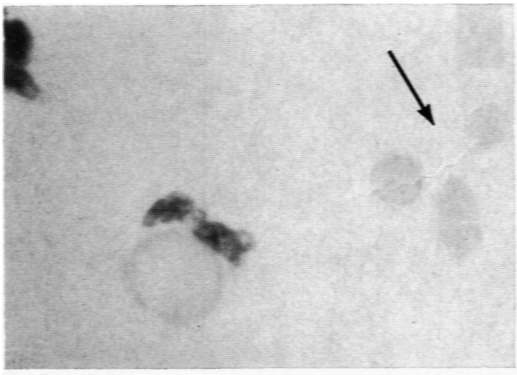

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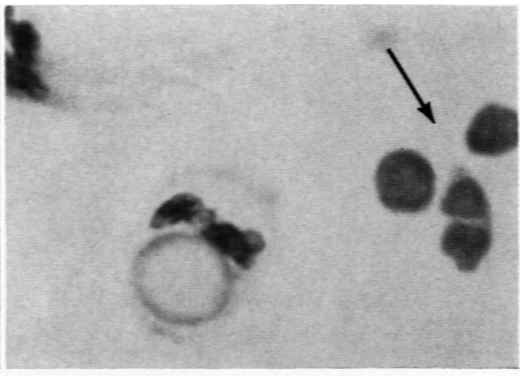

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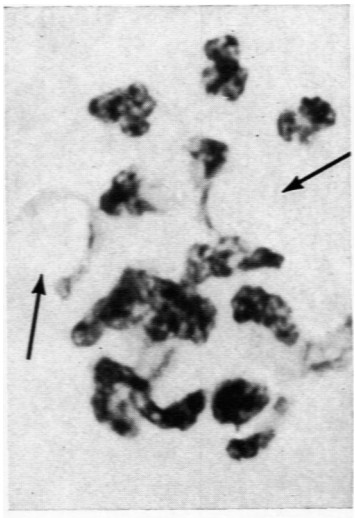

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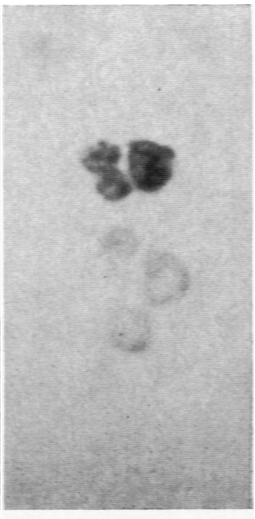

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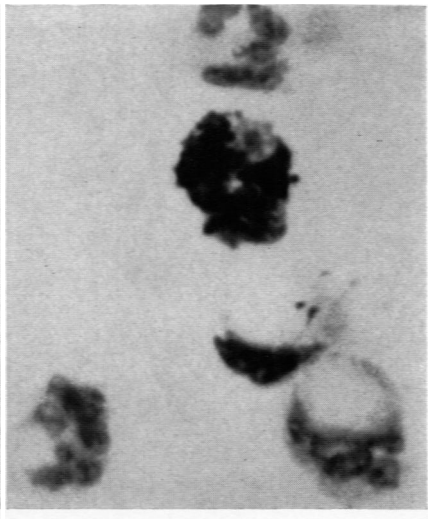

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