A CYTOCHEMICAL STUDY OF THE L.E. BODIES OF SYSTEMIC LUPUS ERYTHEMATOSUS

II. PROTEINS*,‡

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PLATES 62 AND 63

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The cytochemical observations on the nucleic acid of the L.E. bodies of systemic lupus erythematosus presented in the foregoing communication (1) have drawn attention to the presence of protein, of unusual kind and/or amount, in these bodies. The protein of the L.E. bodies manifested itself indirectly through the more marked interference of its basic groups with the binding of basic dyes by nucleic acid. The investigations presently reported were undertaken in an attempt to gain information by more direct means on how the constitution of the protein moiety of the L.E. body differs from that of the type of nucleus which becomes transformed under the influence of the lupus factor. Data contributing toward a characterization of the protein of L.E. bodies might enable inferences to be drawn regarding the pathogenesis of the L.E. change.

Before considering the material of the L.E. body, it is germane to recall the main protein components known to be present in normal nuclei. Proteins constitute from about 75 to 95 per cent of the dry weight of normal nuclei isolated in non-aqueous media (2, 3). The nuclear proteins, in contrast to the constancy of DNA, vary markedly in amount and nature in different cell types and in different physiological conditions (4–9). Lymphocytes (including thymic lymphocytes) and probably also mature granulocytes are among the cell types characteristically having the lowest total protein contents, which amount to from 74 to 79 per cent of the dry nuclear weight (2–4) and thus give lower protein: DNA ratios. (2.7, (2); 4.6; (3)). Lymphocyte nuclei from different species, including man, are alleged to be "practically identi-

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cal" in quantitative distribution of their constituents (10). Associated with DNA as integral components of the somatic chromosome are the histones, and a higher protein or proteins designated "residual chromosomal protein" (11). In addition, the nucleus contains larger amounts of other incompletely characterized proteins which may make up nearly 50 per cent or more of the nuclear dry weight (12). Among these is a lipoprotein (13) and saline-soluble globulin (14).

In the present work the alterations in the L.E. phenomenon were found to affect both the total amount of nuclear protein, and the histone fraction.

Materials and Methods

L.E. cells and free bodies in preparations made by the dried substrate technique of Davis *et al.* (15), the "ring" method of Snapper and Nathan (16), or the atabrine procedure of Lee (17) were compared with lymphocytes in control preparations. The dried-substrate preparations were incubated with lupus serum for 5, 10, 20, and 30 minute intervals. Sera and substrate cells were the same as those described in (1). As in the foregoing study of this series (1), methanol-fixed Wright's stained smears were mapped to permit accurate identification and relocation of cells in the subsequent treatments. Except when specified, free L.E. bodies rather than phagocytosed inclusions were studied to avoid the secondary events of intracellular digestion.

For quantitation of the "total protein" in nuclei and in L.E. bodies, their binding of the anionic dye naphthol yellow S (C.I. 10), the dipotassium salt of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid) at pH 2.7, as elaborated by Deitch (18), was measured microspectrophotometrically. Owing to the wide separation of their spectral absorption maxima, it has been shown (18) that the concentration of naphthol yellow S (absorption maximum at 435 mμ) can be measured concurrently with that of the Feulgen dye complex (absorption determined at 568 mμ) in the same locus, the naphthol yellow S being used as a counterstain. Visualization of the Feulgen stain permitted accurate identification and delimitation of the area of the L.E. bodies and nuclei measured. The Feulgen reaction was performed as noted in reference 1. The cytochemical Millon reaction suggested by Pollister and Ris (19) and Pollister (20), as modified by Rasch and Swift (21) was also used to give an indication of the concentration of the total protein. The extinction of the colored complex was measured at 490 mμ. Histones were detected by means of the alkaline fast green technique of Alfert and Geschwind (22). Methanol-fixed preparations, some of which were postfixed in 10 per cent neutral formalin, were stained as prescribed in the diaminotriphenylmethane dye fast green FCF at pH 8.1–8.2, after extraction of nucleic acids, usually with hot trichoracetic acid, and in one instance with both ribonuclease and deoxyribonuclease (see reference 1). Both methanol and formalin fixation permitted adequate fast green staining for histones (see reference 8) and gave comparable preparations. The optical density of the bound fast green was determined microphotometrically at 625 mμ. For the demonstration of arginine residues the classical histochemical Sakaguchi reaction was modified somewhat to give more stable and reproducible color. In this procedure, slides were allowed to react for 3 minutes with an alpha naphthol in 70 per cent alcohol and sodium hypochlorite mixture alkalinized with sodium hydroxide; they were then dehydrated rapidly in 95 per cent and absolute alcohol or tertiary butyl alcohol, transferred to xylene, and mounted in oil. The color intensity was measured at 510 mμ, within a day after performing the reaction.

The microspectrophotometric apparatus, procedures and calculations used in this study were those referred to in the first communication in this series (1).

RESULTS AND COMMENTS

"Total" Protein.—

That the diminished basophilia of the nucleic acid in L.E. bodies, as compared with that of nuclei from which they are derived, is due primarily to the interference of increased numbers of competing protein basic groups has already been indicated (1) by the fact that acetylation of ϵ-amino groups effected an increase of methyl green uptake by DNA in L.E. bodies to approximate or somewhat exceed that of lymphocyte nuclei, affording evidence of the greater influence of such basic groups in the L.E. body. Direct determination of the protein of the L.E. body was then sought to verify and extend these data and to ascertain whether, if the increased activity of protein basic groups were indeed the result of their increased number, this was due to an actual increase of the quantity of protein in the formation of the L.E. body.

Naphthol Yellow S Binding.—

The acidophilia of proteins affords a means for their quantitative chemical and cytochemical estimation. It has been clearly demonstrated by Deitch (18) in model experiments and microspectrophotometric measurements that the anionic dye naphthol yellow S (flavianic acid) can bind the available ϵ-amino groups of lysine, the guanidyl groups of arginine, and the imidazole of histidine residues in fixed protein stoichiometrically and reproducibly. Measurement of the concentration of naphthol yellow S thus provides a measure of the number of protein basic groups in any site in fixed cells. Like all staining with acid and basic dyes which depends upon a salt-like union, the binding of naphthol yellow S to protein is subject to inhibition by competing anionic ions or groups, electrostatic or steric hindrance, covering of receptor groups by other combinations, etc. Therefore the presence of nucleic acid limits the number of basic groups free to bind naphthol yellow S, and when the concentration of naphthol yellow S in protein is measured in a body in the presence of nucleic acid, only those "available" protein basic groups not preempted by combination with or otherwise blocked by the DNA are demonstrable. Owing to the difficulty in determining the geometrical limits of any body or nucleus to be measured with the naphthol yellow S stain alone, it has not been found feasible to measure the amounts of dye in any body which was not also stained with the Feulgen technique to make its limits clearly visible under the microspectrophotometric microscope at 568 mμ. For this reason the relative amounts of naphthol yellow S recorded in the data are representative only of available protein basic groups rather than the potential total number, and when hydrochloric acid was used in the Feulgen hydrolysis, only in the non-histone proteins which survive acid extraction. Since it has previously been shown that hydrochloric acid extraction did not affect the methyl green binding of L.E. bodies (1), acid extractable protein was considered to be negligible in them.

The amounts of Feulgen dye and of naphthol yellow S bound in free L.E. bodies and in nuclei of control lymphocytes were measured successively in the

same object. In one group of experiments L.E. bodies derived from lymphocytes were measured in preparations made by the method of Davis *et al.* (15) and incubated with serum containing the lupus factor for 5 and for 30 minute periods. The results, given in Table I and Text-fig. 1, confirm the fact that the lupus transformation entails no loss of Feulgen-revealed DNA. In the conversion of the lymphocyte nucleus to an L.E. body, however, there is a more than two-fold increase (actually about 136 per cent in the experiment charted) in the mean naphthol yellow S binding capacity. After 30 minutes of incubation of substrate nuclei in lupus serum, the L.E. bodies show a small (13 per cent) decline in their mean values for Feulgen-stainable DNA, and a greater decrease

TABLE I

Mean Amounts of DNA and Protein as Determined by the Feulgen Technique and Naphthol Yellow S Binding

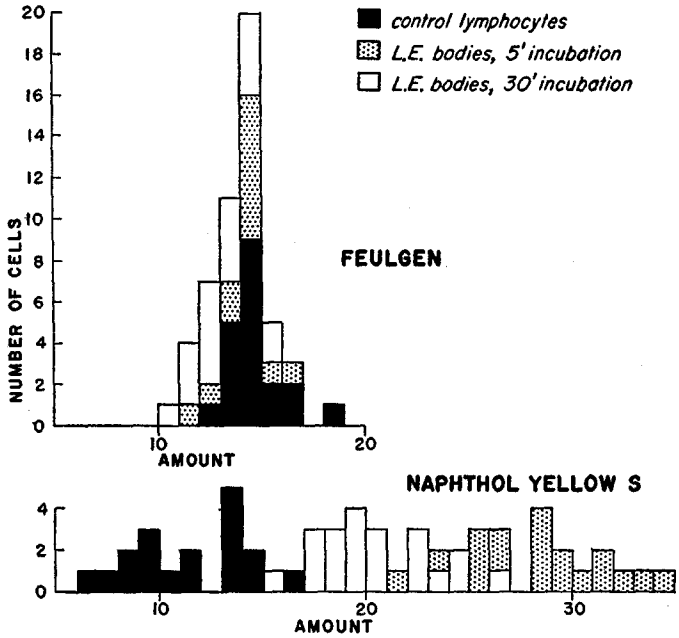
Relative quantities of nuclear nucleoproteins in lymphocytes and free L.E. bodies derived from lymphocyte nuclei. The stability in amount of DNA (Feulgen) and marked rise of protein basic groups (NYS) occurring in the L.E. transformation are noteworthy. Older L.E. bodies appear to lose protein and possibly some DNA.

	Lymphocytes	L.E. bodies	
(No. Measured)	(20)	(20)	(20)
Incubation time	—	5 min.	30 min.
Feulgen	14.6 ± 0.3	14.7 ± 0.3	13.4 ± 0.4
Naphthol yellow S	12.0 ± 0.8	28.4 ± 0.7	20.6 ± 0.7
$\frac{\text{Naphthol yellow S}}{\text{Feulgen}}$	0.82	1.93	1.54

(about 30 per cent) in their binding of naphthol yellow S, as compared with the mean values for bodies measured after 5 minutes of incubation. It is not apparent to what extent this is attributable to actual loss of DNA and protein, piossibly in an autolytic process, or to differences in sampling or technical dscrepancies. With the Feulgen-DNA values serving as a standard of reference, these changes are also expressed in the naphthol yellow S:Feulgen ratios, (Table I) which indicate relatively less naphthol yellow S stainable protein per DNA in the 30 minute specimens, suggesting a more rapid protein than DNA loss from the older L.E. bodies.

Another set of concurrent measurements of Feulgen-DNA and naphthol yellow S binding was made of L.E. bodies derived from leukemic lymphocytes in the atabrine type of preparations described by Lee (17). These were compared with the values determined in control lymphocyte nuclei. In L.E. preparations of this kind, enlarged nuclei may be found in which the vitreous or 'smoky' homogenization of nuclear contents characteristic of the L.E. alteration has not proceeded to completion, and in which some remnant of structure

can be discerned. Such bodies, which represent an intermediate stage in the L.E. transformation have been designated "pre-L.E." bodies, and are probably analogous to the "pre-L.E. cell" of Stich *et al.* (23) derived from the polymorphonuclear leukocyte. The Feulgen and naphthol yellow S dye bound in these have been compared with the values obtained from fully matured L.E. bodies. These data are shown in Table III and Text-fig. 2, from which it is



TEXT-FIG. 1. Histogram showing the distribution frequencies of the relative amounts of DNA (Feulgen) and protein basic groups (NYS) in lymphocyte nuclei, early L.E. bodies and late L.E. bodies derived from lymphocytes. The DNA is relatively unchanged; the NYS binding is markedly increased in the L.E. bodies.

evident that in the L.E. change there is a marked and progressive increase in the relative numbers of available protein basic groups revealed by naphthol yellow S, up to about 157 per cent more in the L.E. body than in the lymphocyte nucleus.

These values, and the greater volume of L.E. bodies as compared with nuclei, suggest that the L.E. change entails an increase in the amount of protein.

Millon Reaction.—

The results of the Millon reaction further attest to the fact that an increase in protein is entailed in the L.E. change. The cytochemical Millon reaction for histone plus nonhistone protein as determined in the present essay, in which similar objects

similarly treated were compared, affords a relative measure of the tyrosine residues in protein. The Millon reaction is complex (21) and is thought to depend upon the formation of nitrosomercurial chromophores with tyrosine and tryptophane, of which only the tyrosine complex is significantly measurable at 480 mμ. The Millon reaction is independent of those ionic and electrostatic factors concerned in acid and basic dye binding and affords a useful check on such data as they relate to proteins.

Table II and Text-fig. 2 indicate that the average L.E. body has about 159 per cent more tyrosine chromophore after the Millon reaction than the average lymphocyte nucleus. Figs. 1 to 5 illustrate these protein tests in various types of L.E. preparations.

TABLE II

Mean Amount of Protein as Determined by the Million Reaction

Increase in the relative amounts of tyrosine residues of L.E. bodies derived from lymphocyte nuclei as compared with nuclei of control lymphocytes. These data are taken to indicate an augmentation in the quantity of protein in the L.E. body.

	Lymphocytes	L.E. bodies
Incubation time	—	15 min.
(No. measured)	(20)	(20)
Millon	2.2 ± 0.2	5.7 ± 0.4

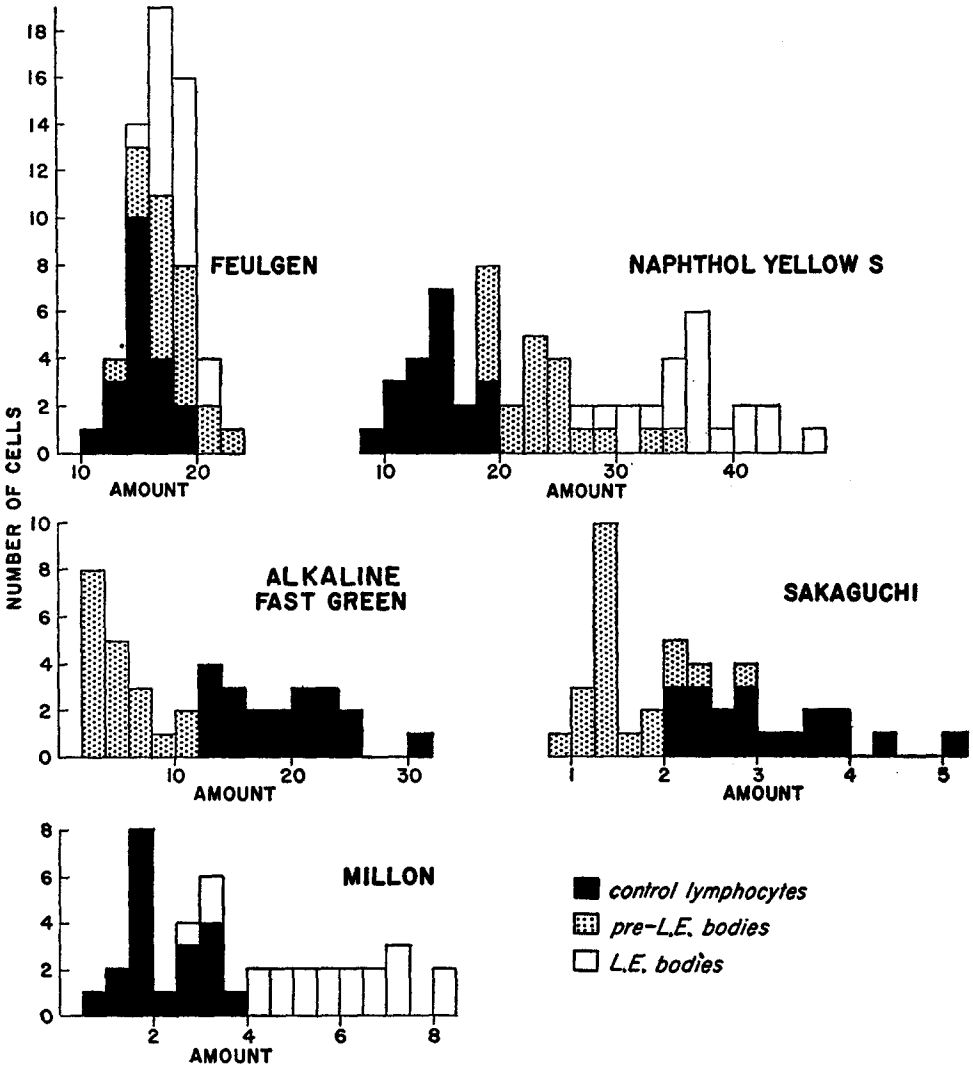
$$\frac{\text{L.E. Body}}{\text{Lymphocyte}} = 2.59$$

From these data it is inferred that in the transformation of nuclei to L.E bodies there is an augmentation of protein and hence increase in the amount of anionic dye bound by protein basic groups.

Histones.—

Histones are strongly basic nuclear proteins which are presumably bound to DNA by salt-like linkages as histone deoxyribonucleates (4, 12, 24, 25). They are found in definite quantitative ratio to DNA. The histones may constitute from about 25 per cent (10) to 30 per cent (2) of the nuclear dry weight of thymic lymphocytes; about 35 per cent of their nuclear proteins are histones. Histones extracted by dilute acids from nuclei, chromosome, or nucleohistone preparations appear to be heterogenous (4, 26); a fraction with high lysine content appears to be more easily dissociable from combination with DNA and more soluble (26).

Basic proteins such as histones can be selectively and quantitatively demonstrated *in situ* with the method of Alfert and Geschwind (22). By virtue of their high isoelectric point such basic proteins can bind the anionic dye fast green at alkaline pH (8.1–8.3). Since, under these conditions, this dye is incapable of competitively displacing the nucleic acids, the latter must be extracted prior to staining. A modification of the Feulgen procedure proposed by Bloch and Godman (27), which insures



TEXT-FIG. 2. Histogram showing the distribution frequencies of the relative amounts of DNA (Feulgen), protein basic groups (NYS), tyrosine residues of protein (Millon), histones (alkaline fast green) and arginine residues (Sakaguchi) in lymphocyte nuclei, transitional forms (pre-L.E. bodies), and L.E. bodies derived from lymphocytes. The increase of protein basic groups (NYS) and protein tyrosine (Millon) in pre-L.E. and L.E. bodies indicate an ingress of protein into the altered nucleus. Concomitantly, there is a fall to ultimate disappearance in the L.E. body of histone and arginine residues.

retention of protein during the Feulgen hydrolysis and staining, permits revelation successively of DNA, then histones in the same body. In this way it has been shown that in normal nuclei histones are invariably localized only wherever DNA is found, and has precisely the same distribution (27). The binding of fast green to histones at high pH, employing these methods, has been shown to (27) follow Beer's law, and to be valid for cytophotometric determination of relative amounts of trichloracetic acid–precipitable histones in nuclei. Alkaline fast green binding, since it depends primarily on the formation of salt-like union of an anionic dye to the positively charged amino and guanidyl groups of histones is subject to competitive interference effects from anionic ions and groups, and steric or other masking, like that affecting all acid and basic dye binding. The major fraction or fractions of lymphocyte histones, precipitable by trichloracetic acid and less readily dissociable from DNA, owe their basicity chiefly to a high content of arginine residues (27, 28). The arginine residues may be revealed *in situ* by some modification of the histochemical Sakaguchi method, a procedure relatively uninfluenced by charged competing groups or macromolecular screening. It therefore serves as a useful check on the alkaline fast green method for histone. Its usefulness after the attainment of a stable color for the quantitative cytochemical estimation of arginine has been attested to in the careful study of McLeish *et al.* (29).

In L.E. preparations of various kinds stained with the alkaline fast green technique, L.E. cell inculsions, *i. e.* the phagocytosed L.E. bodies, although stained by the Feulgen reaction, were almost invariably devoid of fast green stainable histone (Figs. 6, 7, 11). In the free unengulfed bodies there was diffuse, faint coloration or complete absence of visible dye (Figs. 9 to 11, 14). In contrast, unaffected nuclei showed intense fast green coloration sharply localized to the chromatin. L.E. bodies derived from polymorphonuclear leukocyte nuclei early in the course of the L.E. transformation, as evidenced by the polymorphous lobate nuclear form and by persisting granular cytoplasm, were irregularly faintly stained for histones, or often quite unstained (Figs. 11 to 13). Some lobes of the same transformed nuclei were sometimes unstained, while others retained a diffuse color. "Pre-L.E." bodies, *i. e.* those swollen altered nuclei in the course of transformation in which some remnant of chromatin structure was visible, were generally stainable for histones, more faintly than unaffected nuclei. Microphotometric measurements of the amount of dye bound in these bodies as compared with that bound by histones in control lymphocyte nuclei were made to determine the total amount of dye in the bodies and to what extent a mere dilution effect resulting from increased volume could account for the apparent diminution of fast green concentration. The results, given in Table III and Text-fig. 2, indicate a striking decrease in the amount of fast green bound to histones very early in the L.E. transformation. The change effected by lupus factor on the substrate nuclei of leukocytes entails the swelling and dissolution of chromosomal structure, and a loss of histones or some change which renders them more susceptible to preparative loss in the

subsequent histochemical processing, or else a masking of their stainable groups by some substance not normally present in nuclei. The diminution by half of arginine residues revealed by the Sakaguchi reaction in pre-L.E. bodies, and their subsequent disappearance (Table III; Text-fig. 2; Fig. 8) would argue in favor of the hypothesis that histones are actually lost or rendered susceptible to loss in the L.E. transformation of nuclei. The failure of acid extraction to alter binding of methyl green by the DNA of L.E. bodies (Table IV of reference 1) is taken as further evidence that histones as such are absent from these bodies.

TABLE III

Mean Amounts of DNA and Protein in L.E. Bodies Derived from Lymphocytes

Increase of protein basic groups (NYS) with concomitant decline in amount to ultimate virtual disappearance of histones (alkaline fast green) and arginine residues (Sakaguchi) in the course of the development of free L.E. bodies derived from lymphocytes.

	A Lymphocytes	*B* "Pre L.E." bodies	*C* L.E. bodies	Ratios	
				B/A	*C/A*
(No. Measured)	(20)	(20)	(20)		
Feulgen	15.4 ± 0.4	18.0 ± 0.5	17.7 ± 0.4	1.17	1.15
Naphthol yellow S	14.5 ± 0.6	23.8 ± 1.1	36.5 ± 1.1	1.64	2.54
Alkaline fast green	19.0 ± 0.4	5.5 ± 0.6	*	0.29	—
Sakaguchi	3.1 ± 0.2	1.5 ± 0.1	*	0.48	—

* Extinctions below measurable limits.

DISCUSSION

It is obvious, from the results of the cytochemical tests, that the nucleoprotein complex of the hematoxylin body differs profoundly from that of normal and other varieties of pathologically altered nuclei, and that the principal changes involve the protein moiety. An initial event in the L.E. transformation of susceptible nuclei appears to be an increase in total protein content by more then twofold, in spite of an apparent loss of histone. The occurrence of an increase in mass has also been shown by interferometric microscopy (30). It is reasonable to suppose that this protein is normally foreign to nuclei and enters the nucleus from without, possibly from the plasma. The origin and perhaps the nature of the protein which gains access to the nuclei should be further elucidated histochemically by tracing the distribution in substrate cells of fluorochrome or radioiodine-labelled plasma fractions obtained in various ways, by means of fluorescence microscopy or radioautography. It is tempting to speculate that this protein which enters the L.E. cell is the lupus factor of the γ-globulin serum fraction, but the evidence for this concept has yet to be sought.

It is consistent with the data to postulate that this protein becomes associated

with DNA and is therefore mainly responsible for the observed depression of methyl green binding (1). As a consequence it may be that histone is displaced or detached from its combination with DNA, and is thereby rendered more vulnerable to degradation or loss in subsequent preparative manipulations.

The primary events in the pathogenesis of the L.E. phenomenon have received several explanations. Among these the concept of the liberation of an intracellular deoxyribonuclease (31, 32) has been doubted for reasons discussed in the first communication of this series (1). The occurrence of an antigen antibody reaction involving the nucleoproteins of the nucleus has received some experimental support (33–36) and is in no way inconsistent with the data presented here.

The observations recorded by Inderbitzin (37) may be of interest in relation to the cytochemical findings. This author found that cells "morphologically identical" with L.E. cells were produced from normal leukocytes by exposure to the sodium salt of a polyvinyl alcohol polysulfonic acid ester (PVAS, Roche) in the presence of whole serum or gamma globulin, especially when the mixture was heated to 58°C. Although other polyacid esters, such as heparins, failed to reproduce this effect, the hypothesis was entertained that a pathological combination of "heparinoids" with gamma globulin could effect the L.E. transformation. However, the identity of the forms described by Inderbitzin (37) with true L.E. cells is questionable. Further insight into the possible actions of heparin or heparin-like polyacids on the nuclear constituents can be gained from the interesting experiments of Anderson and Wilbur (38) and Roberts and Anderson (39) on nuclei isolated in sucrose. They found that the addition of heparin to isolated rat cells and nuclei resulted in nuclear swelling and the release of a gel from the nucleus. They postulated that heparin effected a displacement of histone from nucleohistone by combination as histone heparinate; the released DNA, still highly polymerized, was supposed to combine with other protein to form a viscous expanding gel which was Feulgen-positive and stainable with basic dyes. It is interesting that the nuclei of leukocytes failed to show heparin damage under the conditions set by these authors.

While the cytochemical studies on L.E. bodies afford no evidence on the possible role of heparin-like substances, the changes brought about by the L.E. factor on the nucleoprotein constitution of substrate nuclei seem to parallel, at least in some part, those believed by Anderson *et al.* (38, 39) to occur in heparinized preparations. The loss of nuclear structure, the apparent displacement and fugacity of histone in L.E. bodies, and the occurrence of an expanded DNA-protein material in the course of the L.E. transformation finds many points of consonance with the changes described as occurring after the action of heparinoids. The metachromasia of L.E. bodies (1) might conceivably be related to the presence of polyacid materials of these types. However, identity of appearances or even of constitution does not imply that a similar pathogenetic process is involved. It is, nevertheless, important to investigate further the action of polyacid ester–protein compounds on nuclei.

The cytochemical tests used give some information concerning the nature of

the protein in the L.E. body. There is evidently a higher protein of the non-histone type, which in its capacity to inhibit basic dye binding of associated DNA resembles the "residual chromosomal protein" (8) rather than those intranuclear proteins responsible for the functional enlargement of nuclei in some types of secreting cells (5, 6). A difference in composition between the protein of the L.E. body and that of the lymphocyte is shown by the effect of acetylation on methyl green binding to its associated DNA (see Table II and Text-fig. 1 of reference 1). Treatment with acetic anhydride at room temperature for 2 hours, which resulted in a modest rise of methyl green binding by lymphocytes, produced a much more drastic increase in L.E. bodies, whose uptake of methyl green surpassed or overshot that of acetylated lymphocyte nuclei, although both L.E. bodies and lymphocytes had approximately the same Feulgen-DNA content. Acetylation, under the mild conditions employed, is known to involve only the ϵ-amino groups of lysine residues but not the basic groups of the other dibasic residues (18, 40). The dye binding data are explainable by assuming that relatively few lysine residues are present to inhibit methyl green staining of DNA in lymphocyte nuclei. In contrast, the protein in L.E. bodies, as judged from the more marked effect of mild acetylation, is much richer in lysine relative to arginine for, as shown by the Sakaguchi test, it is poor in arginine. By measuring the binding of naphthol yellow S in the same bodies before and after mild acetylation, in the absence of nucleic acid, a ratio of the lysine basic groups to those of arginine and histone may be obtained and compared with that of other proteins, which might afford a useful means of qualitative characterization. However, the technical handicaps already mentioned make this information rather difficult to obtain. It is evident that in its greater number of lysine residues and paucity of arginine, the protein of the L.E. body differs from that present in normal lymphocyte nuclei; these results can be explained by postulating a loss of histone and addition of an unusual protein.

Klemperer (41) and Gueft and Laufer (42) have drawn attention to the importance of this nuclear change in the pathogenesis of the morbid alterations seen in the tissues in systemic lupus erythematosus. From this point of view the new nucleoprotein material constituting the L.E. or hematoxylin body may be thought of as undergoing a secondary evolution, in which aggregation, further addition, or change of constituents, and gradual loss of DNA occur, eventuating in extracellular proteinaceous deposits which have been equated by some (42) with the fibrinoid material of systemic lupus erythematosus.

SUMMARY

Microspectrophotometric comparison of lymphocyte nuclei and free L.E. bodies which are derived from them show a more than twofold average increase

in the latter of protein basic groups revealed by naphthol yellow S binding, and tyrosine residues demonstrated by the Millon reaction. The alkaline fast green method for histones and the Sakaguchi reaction for arginine residues suggest that there is a marked loss and ultimate disappearance of histones in the course of formation of the L.E. body from a nucleus. It is postulated that the L.E. transformation entails influx of protein normally foreign to the nucleus, displacement of histone from combination of DNA, and association of the DNA with the new protein which is then responsible for masking the anionic groups of DNA.

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EXPLANATION OF PLATES

PLATE 62

FIG. 1. Field showing an L.E. cell, a polymorphonuclear leukocyte (*P*) and a red blood corpuscle (*r*) in a dried substrate preparation (15). The nucleus of the original phagocyte (arrow) is itself beginning to undergo the lupus change. × 900. Wright's stain. Orange filter (Corning).

FIG. 2. The same field as that shown in Fig. 1, to show distribution of protein basic groups. The original L.E. inclusion, which stains less intensely than the altered nucleus of the original phagocyte (arrow) may have undergone some intracellular digestion. × 900. Naphthol yellow S stain. Blue filter (Corning).

FIG. 3. The same field, to show concentrations of protein tyrosine residues (Millon chromophore). × 900. Millon reaction. Blue green filter (Wratten no. 75).

FIG. 4. Free L.E. bodies derived from granulocyte nuclei in a dried substrate preparation (15), early in the course of the lupus transformation. The cytoplasm of the cells is adherent to the swollen lobate nuclear bodies, which show a higher concentration of tyrosine residues. × 900. Millon reaction. Blue green filter (Wratten No. 75).

FIG. 5. Two L.E. bodies (arrows) and polymorphonuclear leukocytes, showing the greater intensity of Millon chromophore revealing tyrosine residues in the L.E. bodies. Millon reaction. × 900. Blue green filter (Wratten No. 75).

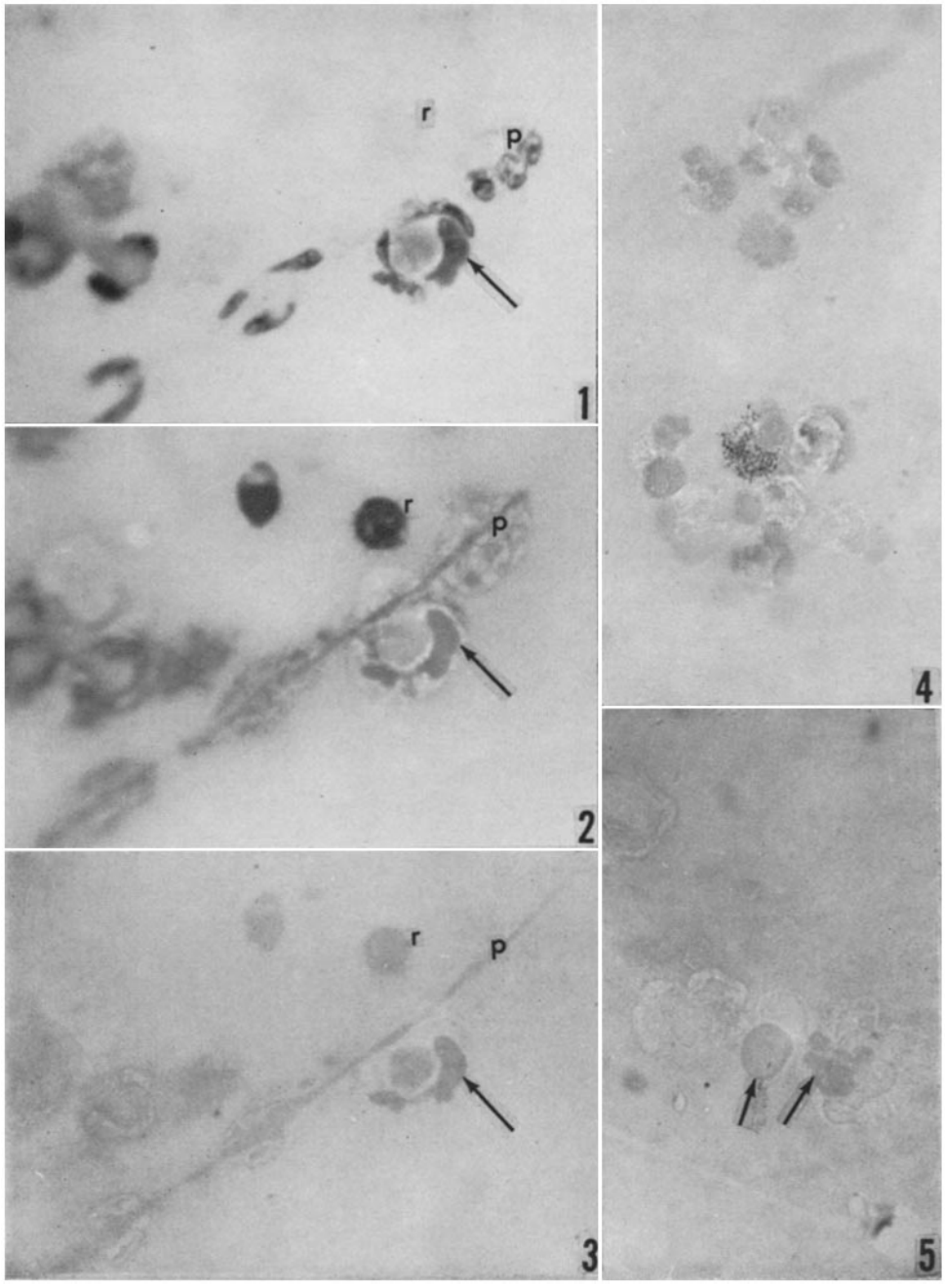

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PLATE 63

FIG. 6. Three typical L.E. cells in a "ring" preparation (16) with characteristic inclusions of engulfed L.E. bodies (arrows). The other elements in the field are red blood corpuscles. Wright's stain. × 460.

FIG. 7. Same field as that of Figure 6 to show distribution of basic protein of the histone type. The chromatin of the phagocyte nuclei is well stained; the L.E. inclusions are diffusely and faintly colored, or quite unstained. The red blood corpuscles, because of the basicity of their hemoglobin, stain moderately. Alkaline fast green method. × 460. Red filter (Corning).

FIG. 8. Three polymorphonuclear granulocytes surrounding a large (? compound) L.E. body to form a rosette in a dried substrate preparation (15), stained to show distribution of arginine residues. These are most concentrated in intact nuclei and appear virtually uncolored in the L.E. body. Modified Sakaguchi method. × 900. Blue green filter (Corning).

FIG. 9. Lymphocyte nuclei and pre-L.E. bodies in an atabrine preparation (17) stained to show basic proteins of the histone type. The specimen has been mounted in oil of matched refractive index (1.568) and examined at full numerical aperture and optimal diaphragm opening. Alkaline fast green method for histone. × 1300. Red filter (Corning).

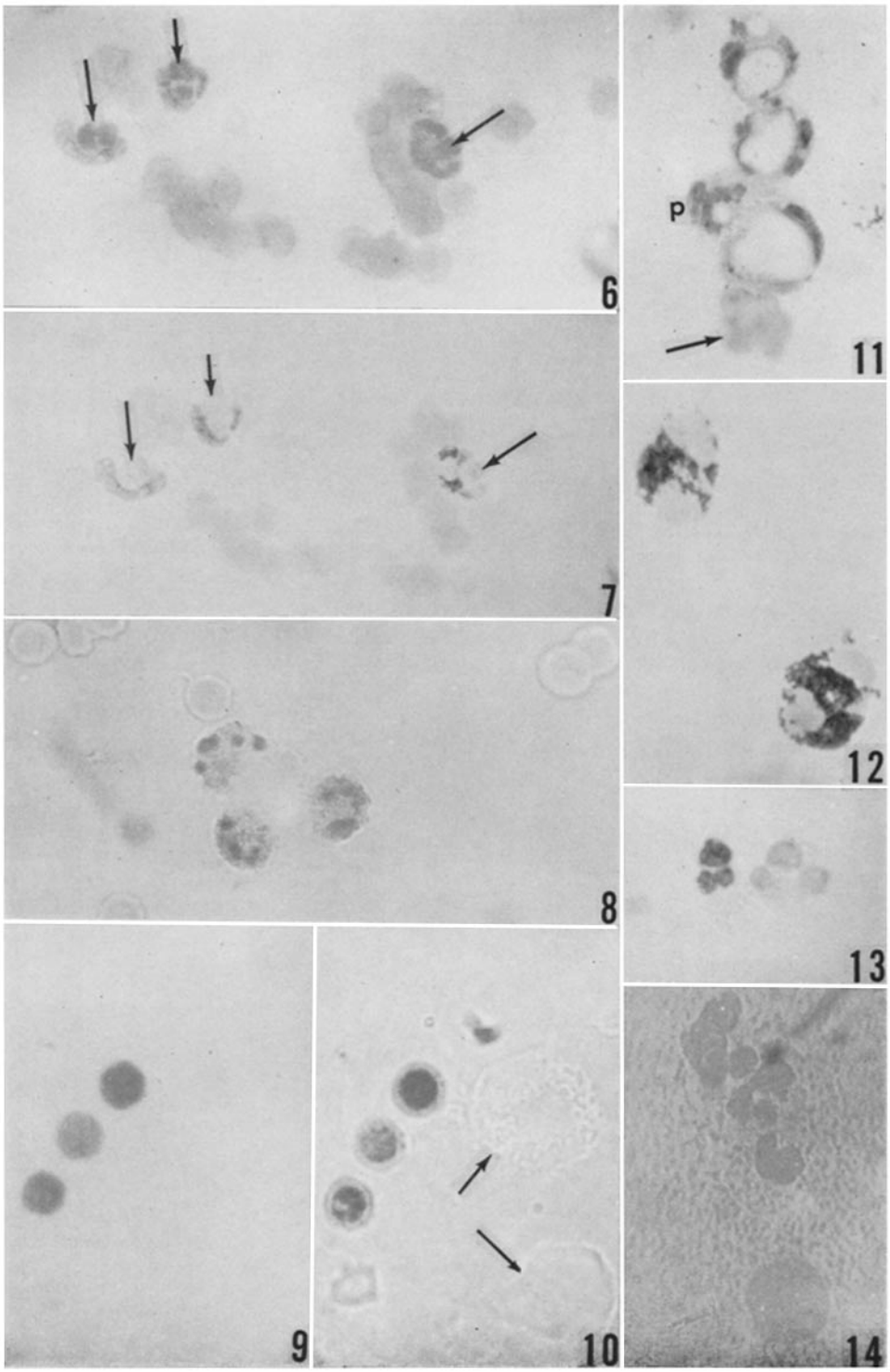
FIG. 10. Same field as that shown in Fig. 9, examined in oil of mismatched refractive index (1.400) and reduced numerical aperture and diaphragm opening to reveal the contours of the unstained L.E. bodies (arrows) invisible in Fig. 9. Alkaline fast green method for histone. × 1300. Red filter (Corning).

FIG. 11. Three typical L.E. cells, a free L.E. body (arrow), and a polymorphonuclear leukocyte (*p*) in a dried-substrate preparation (15) stained to reveal histones. Chromatin of nuclei is well-stained; the inclusions of the L.E. cells are unstained; the free L.E. body (arrow) is diffusely and rather faintly colored. Alkaline fast green method for histone. × 900. Red filter (Corning).

FIG. 12. Two eosinophiles in a dried substrate preparation (15) whose nuclei exhibit the swelling and loss of structure characteristic of the early stages of the L.E. transformation. These nuclei stain diffusely and rather faintly for histone. The eosinophile granules, which contain a strongly basic arginine-rich protein of high isoelectric point, are intensely stained in the alkaline fast green procedure. Alkaline fast green method. × 1300. Red filter.

FIG. 13. A normal polymorphonuclear nucleus at left, and a polymorphonuclear nucleus showing the swelling and homogenization of the early phases of the L.E. phenomenon, at right. The intense stainability of histone in the chromatin of the normal nucleus is contrasted with the faint diffuse staining of the altered nucleus. Ring preparation (16), alkaline fast green stain. × 900. Red filter.

FIG. 14. Free L.E. bodies in dried substrate preparation (15) almost uncolored with the fast green method for histones, mounted in oil of mismatched refractive index (1.452) and examined at reduced diaphragm aperture to show their contours. The group at top is faintly stained; the lower body is unstained. Alkaline fast green. × 1300. Red filter.



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