from hemi-castrated cretins. It would seem, therefore, that either rapid depletion of gonadotrophin from these cells had occurred or that a slight, but sustained, secretion of hormone stimulated a very sensitive residual gonad. The increased sensitivity of testes and secondary sex organs of thyroidectomized animals(19,20) and of ovaries of thiouracil-fed rats(21,22) to gonadotrophins seems to support the latter possibility.

The various peripheral effects of hypothyroidism upon target-organ responses to pituitary hormones(19-22) must be considered in interpreting our data. On the other hand, the effect may be central, so that the imbalance of the pituitary-thyroid axis in the cretin, induced by thiouracil administration, affects other pituitary-target-organ axes and thereby mechanisms through which compensation normally occurs are either hampered or facilitated.

Summary. Degree of compensatory hypertrophy of various glands in the cretin rat was compared with that in euthyroid controls. Per cent renal compensatory hypertrophy in the cretin of either sex was equal to that of the normal animal. Compensatory responses of certain endocrine viscera, however, are dramatically affected. Such responses appear to be greatly lessened in adrenals, virtually abolished in thyroids, and markedly increased in testes and ovaries of cretin rats. Correlations of various glandular responses with pituitary cytology are considered.

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Latex Agglutination Test for Disseminated Lupus Erythematosus.* (24195)

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Evidence has been presented which suggests that the LE cell factor reacts specifically with materials of nuclear origin. Holman and Kunkel(1) and Friou(2), using immunofluorescent technics, have demonstrated localization of γ globulin from sera of patients with disseminated lupus erythematosus (DLE) onto whole nuclei and nucleoprotein. Robbins *et*

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al.(3) found that some DLE sera fixed complement with whole nuclei, nucleoprotein, and desoxyribonucleic acid (DNA). Meischer has reported on agglutination of DNA and nucleoprotein-coated sheep cells by DLE sera (4). Preliminary reports indicate that addition of soluble DNA to some DLE sera induces precipitate formation(3,5). By a modification of the F II latex fixation test(6), polystyrene latex particles could be "coated" with calf thymus nucleoprotein. Latex particles so treated were agglutinated by the majority of DLE sera tested. This report describes our experience with this procedure.

Materials and methods. Polystyrene latex particles (diameter size "0.81 μ") were obtained from Dow Chemical Co. An aqueous stock suspension of the particles was prepared as described by Singer and Plotz(6). Glycine-NaOH buffered saline (pH 8.2) served as diluent of all test materials, unless otherwise stated. The constituents were present in the following molarities: NaCl - 0.15 M, Glycine - 0.1 M, NaOH - .0025 M. pH adjustment was made with Na_2CO_3. Calf thymus nucleoprotein was prepared by extraction of thymus glands with 1 molar NaCl and reprecipitation 3 times with 0.15 M NaCl(7) and was stored in 1 molar NaCl at 4°C. The nitrogen (Kjeldahl) concentration was 42.2 mg %. Phosphorus concentration(8) was 15 mg %. The nucleoprotein moved as a single component in electrophoretic studies (moving boundary technic) and sedimented as a single component with ultracentrifugation (Spinco model E). Nucleoprotein-latex was prepared by the following method: (a) the 1 molar salt solution of nucleoprotein was diluted 1:6.9 with distilled water, making the salt concentration isotonic, (b) 24 ml of the above were added to 76 ml of buffered saline and centrifuged at 15000 rpm for 30 minutes (International centrifuge, model PR-2 with high speed attachment) (c) the supernatant from above was mixed with 2 ml of the stock latex solution and, without incubation, centrifuged at 15000 rpm for 30 minutes. The supernatant was discarded. The packed latex was dispersed in 100 ml of buffered saline and centrifuged again under same conditions, (d) the washed packed latex was dispersed in 200 ml of buffered saline by vigorous shaking. This suspension constituted the nucleoprotein latex reagent. Plain latex reagent was prepared by washing 2 ml of stock latex solution with 100 ml of buffered saline followed by dispersion in 200 ml of the saline, as above. Sera were obtained from our patients. Patients listed as DLE had clinical syndromes compatible with that disease and at some time had had positive LE cell preparations. Patients with clinical and laboratory features suggestive of DLE but lacking positive LE cell preparations were designated probable DLE. Sera were stored at −20°C. One ml of serum was serially diluted in 1 ml portions of buffered saline in 12 x 75 mm test tubes, the first tube in 1:2 dilution. One ml of nucleoprotein latex reagent was added to each tube and the contents mixed by agitation. The tubes were incubated at 37°C 1 hour and centrifuged at 2000 rpm 10 minutes. Agglutination of latex particles was judged macroscopically with light shaking. The tubes were then incubated overnight in a 37°C water bath, after which they were centrifuged again under same conditions and reinterpreted. Agglutination in a dilution of 1:4 or greater after the second centrifugation was considered positive. All sera showing agglutination of nucleoprotein latex were tested with plain latex reagent. (A few sera, notably sera from patients with rheumatoid arthritis, agglutinated plain latex.) Positive and negative control sera were included in each study. The degree to which various materials could inhibit the agglutinating property of a DLE serum was determined by inhibition studies. Varying amounts of test materials in volumes of 0.5 ml were added to 0.5 ml of a diluted DLE serum. Dilution of serum was such that the ratio of the previously determined agglutination titer to the dilution was 10. (A serum with a nucleoprotein latex titer of 1:128 was diluted 1:12.8.) After incubation at 37°C for 30 minutes, 1 ml of nucleoprotein latex reagent was added and agglutination determined as above. LE cell preparations were made by modification of the technic described by Davis and Eisenstein(9). The criterion for positivity employed was presence of polymorphonuclear leukocytes containing hematoxylin

TABLE I. Sera Which Demonstrated Nucleoprotein Latex Agglutination.

Diagnosis	Nucleoprotein latex titer*	No. of patients	Pos. LE prep. on same serum
DLE	4	5	3
	8	1	1
	16	3	3
	32	6	6
	64	1	1
	128	1	1
	256	2	2
Probable DLE	4	1	0
	64	1	0

* Titers expressed as reciprocals of serum dilutions.

bodies. Rosette formation and nucleophagocytosis were not considered as evidence for a positive test.

Results. Sera were obtained from 24 patients with documented DLE, 6 patients with probable DLE, 4 patients with discoid LE, 53 patients with rheumatoid arthritis, and 84 patients with a variety of diagnoses. Table I lists all sera which demonstrated agglutination of nucleoprotein latex but no agglutination of plain latex. (Not listed are sera of 3 patients with rheumatoid arthritis, 1 patient with multiple myeloma, and 1 patient with chronic renal disease, all of which agglutinated both nucleoprotein latex and plain latex reagents.)

Sera which failed to agglutinate nucleoprotein latex are noted in Table II. Inhibition studies were performed with highly polymerized preparation of DNA, ribonucleic acid (RNA) desoxyribose, and histone—all of calf thymus origin.§ (See Materials and methods). These studies are summarized in Table III. Small amounts of DNA were inhibitory, and this inhibition was abolished or diminished by prior incubation of DNA with desoxyribonuclease (DNase). Sodium versenate (EDTA) prevents enzymatic action of DNase by binding divalent cations.

Table IV summarized absorption experiments with 2 DLE sera. Two ml of the test sera were incubated for 12 hours (37°C) with packed latex sediment of 100 ml of both nucleoprotein latex and plain latex reagents. The supernatants after absorption with nucleoprotein latex were negative in LE cell tests. After absorption, serum B.L. no longer agglutinated nucleoprotein latex and the titer of serum J.B. was reduced from 1:32 to 1:2.

Discussion. Agglutination of nucleoprotein latex appeared to be specific for DLE. The test correlated closely with the results of LE cell preparations on the same sera. Of particular interest were the consistently negative results with rheumatoid arthritis (RA) sera. Incidence of positive LE cell tests in RA patients remains highly controversial. The divergence of opinion on this point results, at least in part, from the following difficulties: 1. many different technics with varying degrees of sensitivity have been employed for recognition of the LE phenomenon, 2. criteria for positivity are subject to individual interpretation, and 3. with present limitations of clinical identification, confusion of diagnosis between RA and DLE continues. Although we have made no systematic study of the incidence of positive LE cell tests with RA sera, our experience with technics that avoid leukocyte trauma suggests that, if sufficiently rigid criteria are utilized, LE cells are rarely found in diseases other than DLE.

Absorption of DLE sera with nucleoprotein latex removed the agglutination property and the LE cell factor. The present data suggest (1) that the serum factor which induces LE cell formation is the same as that which ag-

TABLE II. Sera Which Failed to Agglutinate Nucleoprotein Latex. (Miscellaneous group includes cases with hepatitis, cirrhosis, myocardial infarction, various neoplasia, gastrointestinal hemorrhage, and chronic renal and pulmonary diseases.)

Diagnosis	No. of patients	Pos. LE prep. on same serum
DLE	5	0
Probable DLE	4	0
Discoid LE	4	0
Rheumatoid arthritis	50	Not done
Degenerative joint disease	10	*Idem*
Psoriasis and arthritis	3	"
Acute rheum. fever	4	"
Penicillin sensitivity	2	"
Dermatomyositis	1	0
Scleroderma	1	0
Periarteritis	1	0
Miscellaneous	62	Not done

§ Nutritional Biochemicals, Cleveland, Ohio.

TABLE III. Inhibition Studies with DLE Serum (J.B.).

Test materials	Degree of nucleoprotein latex agglutination: Tube No. 1	2	3	4	5	6	7	8	9	10	Inhibiting conc.
No added material*	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	
	Concentration test materials (mg/cc)										
	2.0	1.0	.5	.25	.1	.05	.02	.01	.005	.002	
DNA	—	0	0	0	0	0	+	3+	3+	3+	.05
" + DNase†	—	0	0	+	+	2+	3+	3+	3+	3+	.5
" + EDTA‡ + DNase	—	0	0	0	0	0	2+	3+	3+	3+	.05
" + EDTA‡	—	0	0	0	0	0	+	3+	3+	3+	.05
RNA	3+	3+	3+	3+	3+	3+	3+	—	—	—	No inhib.
Desoxyribose	3+	3+	3+	3+	3+	—	—	—	—	—	*Idem*
	Concentration histone (mg N_2/cc)										
	.7	.28	.14	.07	.02	.01	.005	—	—	—	
Histone§	4+	3+	3+	3+	3+	3+	—	—	—	—	No inhib.

* Diluted DLE serum, in absence of added materials, demonstrated strong agglutination.
† 2 mg of DNA incubated with .1 mg of DNase for 30 min. (37°C) $MgSO_4$ .003 molar.
‡ Sodium ethylene diamine tetraacetic acid conc. .25 mg/ml. EDTA alone did not influence agglutination.

TABLE IV. Absorption of DLE Sera with Plain Latex and Nucleoprotein Latex (See Text).

Patient	Serum conditions	Nucleoprotein latex agglutination: Reciprocals of dilution 2	4	8	16	32	LE prep.
B.L.	Unabsorbed serum	3+	3+	3+	2+	±	+
	Plain latex absorbed serum	3+	3+	3+	2+	0	+
	Nucleoprotein latex absorbed serum	0	0	0	0	0	0
J.B.	Unabsorbed serum	4+	3+	3+	2+	+	+
	Plain latex absorbed serum	4+	3+	3+	2+	+	+
	Nucleoprotein latex absorbed serum	+	0	0	0	0	0

glutinates nucleoprotein latex particles, and (2) that this factor reacts primarily with DNA.

Some properties of the LE cell factor suggest that it may be part of an immunologic reaction with specificity for nuclear constituents. At present, this remains speculative, and there is no evidence that the factor is responsible for the diverse manifestations of DLE.

Summary. Polystyrene latex particles, after treatment with calf thymus nucleoprotein, were agglutinated by some DLE sera. The agglutinating property of DLE serum in most cases paralleled the results of LE cell tests. DNA in small amounts inhibited nucleoprotein latex agglutination.

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