Studies in Experimental Autoimmune Disorders. I. Clinical and Laboratory Features of Autoimmunization (Runt Disease) in the Mouse

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THE HYPOTHESIS that autoimmunization, i.e., the production of antibodies within the individual against his own cells and tissues, could be responsible for the development of certain types of hemolytic anemia in man was suggested more than 50 years ago.1,2 This concept was rather slow in development; it was advanced by the systematic finding of previously occult antibodies with the albumin3 and Coombs' antiglobulin4 technics. Nevertheless, a serious defect in the hypothesis that autoimmunization could lead to hemolytic anemia was the lack of an experimental model. Although a severe anemia of the hemolytic variety had been produced by the passive transfer of heteroimmune serum to guinea pigs,5 the definitive demonstration that hemolytic anemia due to antibodies produced by tissues proliferating within the animal's own body was lacking. Several attempts to induce autohemolysis were made utilizing various technics, usually by attempting to modify the antigenic makeup of the red cell, both with and without the use of Freund's adjuvant,6,7 but without notable success.

More recently, as the result of increased activity in the field of transplantation immunity, the possibility arose that some of the manifestations of transplantation reactions could be utilized as an experimental model of autoimmunization. A type of transplantation reaction, termed runt disease, was shown to result from the introduction of an immunologically competent graft into an immunologically neutral host. The immunologically competent graft may be made up of leukocytes,6 splenic cells,8 lymph node cells10 or bone marrow cells.11 The cells of the graft are introduced by various means into the immunologically neutral host, i.e., a newly born animal, a supralethally X-irradiated animal13 or an animal whose genetic relationship to the grafted tissue does not permit the development of an immunologic reaction to it. In the latter situation the host is the offspring (F1 hybrid) of two highly inbred strains of mice.14 These F1 hybrids, because of their genetic constitution, are incapable of rejecting grafts from either parent. However, an immunologically competent graft from either parent may react against the tissues of the hybrid, since the latter contains genes, and therefore antigens, derived from

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both parents. That runt disease (homologous disease,\textsuperscript{15} secondary disease\textsuperscript{16}) is due to an immunologic reaction of graft vs. host has been generally ac-
cepted.\textsuperscript{17,18}

In the present study this graft vs. host reaction in the F\textsubscript{1} hybrid mouse was used as an experimental tool to produce a disease analogous to certain autoimmune disorders of man. This disease in the mouse was characterized by hemolytic anemia with a positive antiglobulin test, leukopenia, thrombocytopenia, splenomegaly, weight loss and alopecia. It could be regularly reproduced in these hybrid mice by the injection of parental strain spleen cells. Induction of the disease was facilitated by sensitization of the donor with tissues of the F\textsubscript{1} recipient or the alternate parent.

MATERIALS AND METHODS

Mice. All mice were obtained from the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine. The following inbred strains were utilized: C\textsuperscript{57L}, A/He, LAF\textsubscript{1}, C\textsuperscript{57B1/6}, BDF\textsubscript{1} and BAF\textsubscript{1}. The mice were housed in an air-conditioned animal room, six to a cage, and were fed water and Fox Chex \textit{ad libitum}. All mice were adults, 8 to 14 weeks of age at the beginning of each experiment. In all cases the donor animals were females and the recipient animals were males. The design of the experimental groups is indicated in Table 1. Groups I, II, and III represent control animals which were used to obtain normal hematologic values for the strains, LAF\textsubscript{1}, BDF\textsubscript{1}, and BAF\textsubscript{1}. Group IV was comprised of LAF\textsubscript{1} animals injected with 266 x 10\textsuperscript{6} isologous spleen cells. Groups V-XVI were injected with parenteral strain spleen cells obtained from either unprepared or sensitized donors.

Splenic Cell Suspensions. The mice were killed by cervical fracture. The spleen was removed and placed in a watch glass containing a small quantity of Ringer’s Solution. A fine scissors was used to mince the splenic tissue into small fragments. The minced tissue was then gently pressed through a 30 mesh tantalum gauze and adherent fragments were washed off the gauze with approximately 0.5 ml of Ringer’s Solution for each spleen processed. Macroscopic clumps of cells were dispersed by gentle pipetting with a Pasteur pipette. If the spleen donor animal had been sensitized, the suspension of cells was then washed twice with large volumes of Ringer’s Solution. Centrifugations were carried out at 500 G in an International Model PR-2 refrigerated centrifuge. The supernatants of these washings were discarded and the cells were resuspended to their original volume of Ringer’s Solution.\textsuperscript{19} Cell counts on the suspensions were made in a standard white blood cell counting hemocytometer chamber using 2 per cent acetic acid as the diluent.

Method of Injection. All injections of spleen cells were made by the intraperitoneal route using a 25–27 gauge hypodermic needle. The recipient animals were injected immediately on processing the splenic tissue.

Methods for Inducing Sensitization

(1) Erythrocytes. Recipient strain erythrocytes were obtained by severing the carotid arteries and jugular veins and bleeding the animal into 1.5 per cent Na-EDTA in 0.75 per cent saline. The cells were washed four times in large volumes of 0.9 per cent saline. Spleen donor animals were injected with 1.0 ml of a 1 per cent suspension of the washed cells by the intraperitoneal route. A course of three weekly injections was given and the spleen donor animals were sacrificed by cervical fracture one week after the last injection of erythrocyte suspension.

(2) Splenic Cells. Sensitization with recipient strain or alternate parental strain spleen cells was done in two ways: (a) by three intraperitoneal injections of 50–100 million cells given at weekly intervals. The donor spleen in this instance was harvested one week after the last intraperitoneal injection; (b) by the injection of 25 million recipient strain
spleen cells mixed with an equal volume of complete Freund's adjuvant (Bacto-Adjuvant, Difco, Lot Number 439139) into the foot pads of both hind limbs of the donor animals. The donor spleen was removed and used 11 or 12 days after the foot pad injection.

Clinical Observations

Each recipient animal was observed at least three times a week and a notation was made of its general appearance, posture and fur condition. They were weighed on a Shadowgraph Scale three times a week. Body temperatures were estimated by the application, with firm, gentle pressure, of a standard clinical thermometer to the abdominal wall in such a manner that the bulb of the thermometer lay enveloped by the animal's skin. Later in the study, rectal temperatures were taken with a fine probe (Cole-Parmer Model Number 8432) leading to a thermocouple device (Thermistor-Thermometer, Yellow Springs Instrument Co., Model Number 8400). At the time of death each animal was autopsied. The spleen was weighed and all tissues were fixed in Zenker's Solution. The histologic observations will be reported in a subsequent paper. Ten normal mice were killed by cervical fracture and their spleens were weighed for the determination of the normal range of spleen size in the mouse.

Laboratory Observations

(1) Peripheral Blood Studies. All blood was obtained from the tail vein. Microhematocrit determinations, leukocyte counts, leukocyte differential counts, direct platelet counts and reticulocyte counts were done by standard methods.

(2) Plasma Electrophoresis. Plasma obtained from the microhematocrit tube was electrophoresed on paper in veronal buffer at pH 8.6 on a Spinco cell run at 4.5 milliamperes for 18 hours. At the end of the run the strips were dried and stained with bromphenol blue. After development in ammonium hydroxide, the strips were scanned with a Spinco Analytrol.

(3) L.E. Preparations. Snapper's technic, with substrates obtained from both mouse and human blood, was used. On several occasions, mouse plasma pre-incubated with an equal volume of freshly prepared guinea pig complement was utilized.

(4) Antiglobulin Tests:
   (a) Preparation and Potency Testing: A rabbit anti-mouse serum was prepared by the method of Jones. Whole mouse serum (Colorado Serum Co., Lot Numbers 12 and 64) was injected into rabbits according to the following schedule: 0.5 ml. of mouse serum was injected subcutaneously between the ears, and three minutes later 0.5 ml. was given intravenously; on each of the next two days 1.0 ml. was given intravenously. Beginning seven days after the first injection, the whole course of injections was repeated. On the fourteenth day after the initial injection blood was obtained from an ear vein and, after clotting, the serum was removed by high speed centrifugation in a cold centrifuge. The rabbit serum was inactivated at 56 C. for 30 minutes. It was then absorbed with washed, packed normal erythrocytes; usually at least two absorptions were necessary. The absorption procedure consisted of the incubation of an equal volume of rabbit serum and thoroughly washed, packed mouse red blood cells at 37 C. for 30 minutes, and then at 4 C. for 90 minutes. The absorbed serum was removed after centrifugation. The presence of antibody was tested utilizing a method of Jandi and Simmons. Washed normal human erythrocytes (0.5 ml. of 50 per cent suspension in saline) were treated with 1 ml. of a 5 micromolar ferric chloride solution; 2.5 ml. of mouse serum, containing 1 Gm. per cent protein was then added to the erythrocyte suspension, and the mixture was incubated at 37 C. for 15 minutes. The red blood cells were washed three times in 0.9 per cent saline. One drop of a 2 per cent suspension of the artificially sensitized red blood cells was mixed with 1 drop of the rabbit anti-mouse serum and centrifuged immediately in a Serofuge (Clay-Adams) for 1 minute. The presence of macroscopic clumps indicated anti-mouse serum activity.
   (b) Technic of the Antiglobulin Test: Mouse blood obtained from the tail vein was suspended in 0.9 per cent saline, washed four times in 0.9 per cent saline and reconstituted
to a 2 per cent erythrocyte suspension. This suspension was centrifuged and the supernatant
discarded. To the button of washed red blood cells was added one drop of freshly
prepared guinea pig complement (Hyland Laboratories) and one drop of rabbit anti-mouse
serum. The mixture was centrifuged in a Serofuge for 1 minute. The presence of either
macroscopic or microscopic agglutinates was considered a positive test. Each batch of
rabbit anti-mouse serum was tested against the red blood cells of various mouse strains
by an identical technic; the anti-mouse serum was found acceptable only when it gave
negative results with all normal red blood cells tested.

(5) Radioactive Antiglobulin Tests. A potent anti-mouse serum was prepared as
described above. The serum was fractionated by the cold ethanol technic of Deutsch,25
and the precipitated gamma and beta globulins were washed twice in ice-cold 25 per cent
ethanol. After washing, the globulins were shell-frozen and lyophilized. The lyophilized
globulins were subsequently dissolved in 0.9 per cent saline to a concentration of about
20 mg/ml. The protein solution was then absorbed with pooled, washed normal
erythrocytes as described above. The final solution gave negative antiglobulin tests with
normal mouse erythrocytes. The absorbed globulins were next iodinated with I\(^{131}\) in
carrier potassium iodide according to the method of Weigle and Dixon.26 Following dialy-
sis the iodinated anti-mouse serum was clarified by centrifugation in the cold. The final
solution had approximately 3 x 10^6 counts per minute per ml., and 95 per cent of the
radioactivity was protein bound. The radioactive antiglobulin test was performed as
described in Section 4 (b), using the iodinated anti-mouse serum. However, after the final
centrifugation the cell mixture was washed four times in large volumes of 0.9 per cent
saline in order to remove any unattached radioactivity. The radioactivity of the cell
buttons was then determined in a well-type scintillation counter which afforded repro-
ducible counts better than 3 per cent. The detector was standardized daily against a
long-lived nuclide, Cs\(^{137}\), and all counts of radioactivity were at least four times back-
ground. Correction for radioactive decay was made in the usual manner, and in addition
a volumetrically diluted aliquot of the original I\(^{131}\)-labeled anti-mouse serum was assayed
daily during the course of the experiments. After the radioactivity of the red blood cells
was measured the cells were lysed with distilled water and the resulting hemolysate
quantitatively transferred to a 1.0 ml. volumetric flask. The optical density of the
hemolysate was determined in a Coleman Jr. Spectrophotometer, using a microcell adapter,
at 540 A.27 An arbitrary "coating index" was determined according to the formula:

\[
\text{Coating Index} = \frac{\text{Counts per minute in RBC button}}{\text{O.D. of hemolysate} \times 1,000}
\]

(6) Preparation of Red Blood Cell Eluates. Eluates were prepared from the red blood
cells of runted animals using the alcohol precipitation method of Weiner.28 Washed
erthrocytes from a variety of mouse strains were incubated for 1 hour at 37 C. with the
eluate and, after thorough washing, a direct anti-globulin test, as described in Section
4 (b), was performed on them. The eluate was also subjected to paper electrophoresis
according to the methods described in Section (2).

(7) Erythrocyte Survival Studies. Isologous mice were given an injection of 5 mg. of
aqueous heparin intraperitoneally one hour before sacrifice. Their blood was collected into
acid-citrate-dextrose solution (A-C-D Solution, Abbott List Number 6761) so that the
resulting suspension contained 10 per cent by volume of acid-citate-dextrose solution.
The red blood cells were labeled with a sodium chromate\(^{51}\) (Chromitope, Squibb List
Number 0590) such that the final concentration of Cr\(^{51}\) was approximately 15-20 \(\mu\text{c.} /\)
ml. of blood. The mixture was incubated for 20 minutes at 37 C., 50 mg. of ascorbic
acid in solution was added and the red blood cells were washed once in a large volume
of 0.9 per cent saline. The labeled, washed red cells were reconstituted to their original
volume with 0.9 per cent saline, and each animal was injected with 0.3 ml. of the Cr\(^{51}\) tagged
erthrocyte suspension. Four days later 20 mm\(^3\) of blood was obtained from the tail vein
and suspended in 1 ml. of 0.9 per cent saline. Similar aliquots of blood were obtained
at 6, 9, 12 and 15 days. Radioactivity of the samples was measured in a well-type
scintillation counter. The lowest limit of radioactivity in the samples was at least four times background. A plot of the radioactive counts was made on semi-logarithmic paper and the T ½ of the labeled erythrocytes was estimated by graphic analysis.

RESULTS

The clinical features of runt disease as observed in this study were: weight loss, lethargy, a characteristic hunched appearance, ruffled fur, facial edema and hypothermia. The degree of weight loss ranged from 10 to 35 per cent of the maximum body weight of the animal (figs. 1 and 2). The onset of weight loss coincided with the appearance of other features of runting and was fairly rapid in its progression. The animals became very lethargic, remaining relatively immobile in their cages, and were passive to manipulation. A characteristic delicate, mincing gait developed which was the result of close apposition of all extremities so that the animal actually appeared "top heavy". The hunched posture resulted in an apparent disappearance of the neck. Facial edema was especially prominent in the periorbital tissues, and, at times, the palpebral fissures were reduced to mere slits. The fur lost its usual glossy appearance and fine texture and became rough and unkempt; partial alopecia, most noticeable in the posterior cervical area, developed. Once seen, the runted animal was unmistakable (fig. 3).

The various clinical and laboratory abnormalities which developed in these animals will be considered separately below. Although the clinical appearance of the runted mouse was independent of its method of induction, several of the combinations shown in table 1 varied somewhat in their laboratory abnormalities.

1. Latent Period. The interval between the first injection of parental spleen cells and the development of the first clinical or hematological sign of dis-

<table>
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<th>21–30</th>
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<td>0</td>
<td>0</td>
<td>4</td>
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<tr>
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<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
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<td>11</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
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<td>4</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>6</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>XIII</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>XIV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>XV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
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<td>8</td>
<td>4</td>
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<td>1</td>
<td>1</td>
</tr>
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<td>0</td>
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<td>2</td>
<td>1</td>
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<tr>
<td>XX</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
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Fig. 1.—Per cent weight loss at death as compared to maximal weight of the animals. The values represent the number of animals in each group.
Autoimmunization (Runt Disease) in the Mouse

Effect of Porenlol Strom
Spleen Cs/I Injection on Weight of The F1 Hybrid

Fig. 2.—Effect of parental strain spleen cell injection on the weight of the F1 hybrid. The changes in weight of representative animals are depicted.

ease was defined as the latent period. This interval varied from 2 to 100 days, depending on the strain combination. LAF1 mice injected with C57L spleen cells obtained from donors sensitized by LAF1 spleen cells (Group VII) rapidly developed signs of runting. On the other hand, the latent period in the combination C57B1/6 spleen into BDF1 was 60 to 100 days. The rapid induction of runting in Group VII appeared to indicate the transfer of highly sensitized spleen cells which were actively forming antibody against host tissues at the time of their injection. The duration of the latent period could
not be correlated with either the mortality rate or life span in any of the groups (fig. 4). Regardless of the latent period, once developed, the disease was almost invariably fatal.

2. Mortality (table 1, fig. 4). The mortality rate exceeded 75 per cent in all combinations studied except in Groups V, X and XI. It should be noted that neither clinical nor laboratory signs of runt disease developed in Groups X and XI. When the A/He donor was sensitized with LAF₁ erythrocytes intraperitoneally (XII), C57L spleen cells intraperitoneally (XIII) or LAF₁ spleen cells in the foot pad with Freund's adjuvant (XIV) the sensitized spleen cells produced a lethal disease.

There was an apparent inverse relationship between the dose of spleen cells administered and the length of the latent period (fig. 5). The type of donor sensitization did not affect this relationship.

3. Body Temperature. The average rectal temperature of 12 normal LAF₁ mice was 99.6 F., with a range of 98–101 F. Hypothermia ensued with the
development of runting and the degree of hypothermia closely paralleled loss of weight (fig. 6). In no instance was fever found.

4. Hematologic Abnormalities (table 2):

(a) Anemia: The normal hematocrit values were: 55 ± 2.7 volumes per cent in the LAF1 mice, 52 ± 7.2 volumes per cent in the BDF1 mice and 50 ± 1.3 volumes per cent in the BAF1 mice. Statistically significant depressions of the hematocrit values (p < .005) developed in groups V, VI, VII, XII, XIII, XIV, XV and XVI. No reduction in the hematocrit values developed in group IV, which consisted of LAF1 mice which had been injected with 266 million isologous spleen cells. No anemia developed in groups IX, X and XI.

(b) Leukopenia: The normal leukocyte values were: 15.9 x 10^3 ± 4.3/mm³
<table>
<thead>
<tr>
<th>Group</th>
<th>Donor</th>
<th>Recipient</th>
<th>Type of Donor Sensitization</th>
<th>Route of Donor Sensitization</th>
<th>Total Dose Donor Spleen Cells (x10^8)</th>
<th>Number of Recipients</th>
<th>Latent Period (Weeks)</th>
<th>Percent Dead</th>
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<td>LAT 1</td>
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<td>I.P.</td>
<td>12 – 600</td>
<td>6</td>
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<td>LAT 1</td>
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<td>Surface Cells</td>
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<td>LAT 1</td>
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<td>Surface Cells</td>
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<td>LAT 1</td>
<td>Surface Cells</td>
<td>Foot Pod Sensitized</td>
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<td>355</td>
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<td>1 / 2 – 16</td>
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Table 1
Fig. 5.—Relationship between dose of spleen cells and latency period of runting syndrome in LAF<sub>1</sub> mice.

in the LAF<sub>1</sub> mice, 11.4 x 10<sup>8</sup> ± 2.8/mm<sup>3</sup> in the BDF<sub>1</sub> mice and 12.6 x 10<sup>8</sup> ± 2.8/mm<sup>3</sup> in the BAF<sub>1</sub> mice. Significant reductions (p < .001) of the leukocyte counts occurred in groups V, VI, VII, VIII, IX, XII, XIII, XIV, XV and XVI. Groups IV, X and XI did not develop leukopenia. The average lymphocyte values were: 88.8 per cent ± 5.7 in the LAF<sub>1</sub> mouse, 82.8 per cent ± 6.5 in the BDF<sub>1</sub> mice and 87.3 per cent ± 6.6 in the BAF<sub>1</sub> mice. The average granulocyte values were: 10.4 per cent ± 5.0 in the LAF<sub>1</sub> mice, 16.3 per cent ± 5.3 in the BDF<sub>1</sub> mice and 12.3 per cent ± 6.7 in the BAF<sub>1</sub> mice. Significant degrees of lymphopenia occurred in groups IV, VI, VII, VIII, IX, XII, XIII, XIV, XV and XVI. The lymphopenia found in group IV was transient and may have represented a nonspecific response to stress. Significant increases in the granulocyte values occurred in those groups which developed significant degrees of lymphopenia, except group IV.

(c) Thrombocytopenia: The normal platelet numbers were: 1.30 x 10<sup>6</sup> ± 0.03/mm<sup>3</sup> in the LAF<sub>1</sub> mouse, 1.20 x 10<sup>6</sup> ± 0.02/mm<sup>3</sup> in the BDF<sub>1</sub> mice and 1.01 x 10<sup>6</sup> ± 0.03/mm<sup>3</sup> in the BAF<sub>1</sub> mice. Thrombocytopenia (p < .001) developed in groups VI, VII, VIII, IX, XII, XIII, XIV, XV. Groups IV, V, X, XI and XVI did not develop significant reductions in their platelet counts.

(d) Reticulocyte Changes: The average normal reticulocyte values were: 3.5 per cent ± 1.7 in the LAF<sub>1</sub> mouse, 1.3 per cent ± 1.2 in the BDF<sub>1</sub> mice and 1.8 per cent ± 0.3 in the BAF<sub>1</sub> mice. Significant degrees of reticulocytosis
Correlation Between Body Temperature and Body Weight Change Following Injection of Parental Strain Spleen Cells into The $F_1$ Hybrid

Fig. 6.—Body temperature changes in runted mice. Normal mice are represented by (X) and the normal range is enclosed in the rectangle.

<p > occurred in groups IV, VI, IX, XII, XIII, XIV and XVI. The reticulocytosis observed in group IV was probably nonspecific, since the maximal response occurred shortly after the third weekly injection of isologous tissue.

(e) Antiglobulin Tests: Over half the animals of groups VI, VII, VIII, IX, XII, XIII, XV, and XVI developed positive antiglobulin tests. One animal in group XIV developed a positive test. None of the animals of groups IV, V, X and XI developed a positive antiglobulin reaction. The finding of a positive antiglobulin test did not necessarily indicate the development of anemia, as can be seen in groups VIII and IX, where the majority of animals had a positive antiglobulin test, but did not develop significant degrees of anemia at any time.

(f) Radioactive Antiglobulin Determination: As can be seen from figure 7 the degrees of erythrocyte coating by the $^{131}$I tagged rabbit anti-mouse globulin serum were in most instances directly related to the degree of anemia. In several animals, red cell sensitization could not be demonstrated by the usual direct antiglobulin method, but the radioactive antiglobulin test showed absorption of an excessive amount of rabbit anti-mouse globulin by the red blood cells.

(g) Red Blood Cell Survival Studies: The T $\frac{1}{2}$ of isologous erythrocytes
labeled with Cr$^{51}$ in the normal LAF, was $14.0 \pm 1.7$ days (mean cell life being 29 days; assuming a Cr$^{51}$ elution rate of 0.8 per cent per day). The T $\frac{1}{2}$ of isologous cells in the various groups of animals with runt disease was $10.0 \pm 2.1$ days (mean cell life 18 days).

(h) L.E. Tests: Repeated searches for L.E. cells were negative in all preparations, regardless of the clinical status of the animal. The addition of guinea pig complement, or the use of human or mouse substrates did not affect these results.

(i) Red Blood Cell Eluates: LAF red blood cells which gave a positive reaction in the direct antiglobulin test were used to prepare eluates. These eluates were tested repeatedly with erythrocytes obtained from various strains of pure-bred mice and were shown to have the following characteristics: (1) antibody activity, (2) antibody specificity in that they reacted only with red blood cells obtained from the contralateral parental strain (i.e., the parental strain which did not serve as a spleen donor) and its related strains, (3) the electrophoretic mobility of a gamma globulin (fig. 8). The results of these studies will be fully described in a subsequent paper.$^{29}$

5. Plasma Electrophoresis. Hypoalbuminemia and an increased concentration of beta globulin were the most frequent abnormalities found in all the groups (table 3). Hypergammaglobulinemia of significant degree developed only in group IX, while hyperfibrinogenemia was found in groups VII, IX and XVI. Plasma alpha globulins were elevated in groups XIII, XV and XVI. None of these electrophoretic abnormalities could be correlated with the hematological abnormalities or the chronicity of the disease. In no instance was hypogammaglobulinemia found.

6. Spleen Weights. Spleens obtained from normal LAF mice had an average weight of $90.9 \pm 6.1$ mg. Splenomegaly was found in all experimental groups. The largest spleens were found in group VII, in which the average spleen weights was $477 \pm 139$ mg. (table 4).

![Fig. 7.—Autoimmune hemolytic anemia in the mouse: radioactive antiglobulin tests.](image)
Table 2.—Hematologic Abnormalities in Runt Disease

<table>
<thead>
<tr>
<th>Group</th>
<th>Hematocrit</th>
<th>Reticulocytes</th>
<th>Leukocytes (x 1000)</th>
<th>Lowest Lymphocytes</th>
<th>Highest Granulocytes</th>
<th>Platelets (x 10⁶)</th>
<th>Per cent Positive Antiglobulin Tests</th>
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<tr>
<td>I</td>
<td>55</td>
<td>2.7</td>
<td>3.5</td>
<td>15.9</td>
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Table 3.—Plasma Electrophoretic Abnormalities in the Runted F₁ Hybrid

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<tr>
<th>Group</th>
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<th>P</th>
<th>Alpha %</th>
<th>P</th>
<th>Beta %</th>
<th>P</th>
<th>Gamma %</th>
<th>P</th>
<th>Fibrinogen %</th>
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<td>—</td>
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DISCUSSION

1. The Graft vs. Host Reaction—Runt Disease

The laws governing transplantation phenomena have been formulated by Snell after his extensive experiments with pure-bred strains of mice. These are: (1) grafts exchanged between genetically identical individuals will grow, (2) grafts exchanged between genetically different individuals will not grow, (3) grafts applied from either pure-bred parent to the F₁ hybrid will grow, and (4) grafts donated by the F₁ hybrid to either pure-bred parent will be rejected. Therefore, the offspring of two pure-bred mice is incapable of rejecting grafts from either parent because it contains transplantation antigens derived from each parent. Conversely, either parent will reject a graft from the hybrid animal because of the presence of transplantation antigens foreign to either one of the parents. If a graft containing immunologically active cells is implanted into a host incapable of rejecting it, either because of its genetic constitution or due to other factors, a disease results. This disease is an immunological disorder characterized by the formation of antibodies by the graft against the host, antibody production being stimulated by the antigens present in the host and not present in the graft. This phenomenon has been observed following the treatment of X-irradiated animals with homologous bone marrow or spleen injections ("secondary" disease, wasting disease, homologous disease), or in the newborn animals given homologous lymphoid tissue (runt disease) and in the F₁ hybrid injected with parental spleen cells (runt disease). In each of these situations the common factor present in the host animal is its inability to reject the graft, either because its antibody forming system has been destroyed by X-irradiation, or because of the acquisition of immunologic tolerance to the graft, as in the newborn animal, or because of its genetic makeup, as in the F₁ hybrid. Even though the basic mechanism is identical in each of the above instances, the resultant disorder
varies depending on the mode of induction and the type of animal studied. For example, Defendi et al. observed severe dermatitis in newborn rats injected with homologous spleen cells, whereas the dermatologic manifestations of runt disease in the F1 hybrid of this study were minimal. Whatever the variations, the several syndromes reported to occur in immunologically neutral recipients following the administration of immunologically competent cells have all been ascribed as due to an immunologic reaction of graft vs. host.

Runt disease can easily be induced in newborn mice by the injection of relatively small numbers of spleen cells. In weanling animals, 4–5 weeks of age, the disease has been induced by the injection of parental spleen cells into the F1 hybrid. Cole found that a relatively small dose (33 million) of parental spleen cells (A/He) will induce a high degree of mortality due to runt disease in 10 month old LAF1 mice. In our studies this phenomenon has been demonstrated in adult mice (8–14 weeks of age), but much larger doses of spleen cells were required. In the young adult LAF1 mouse it was not possible to produce runt disease with doses of spleen cells comparable to those used by Cole. From the genetic standpoint such a dosage phenomenon should not exist, and it would appear that an age dependent factor is responsible for this effect. It is possible that the host's reticuloendothelial system, which changes with age, could account for these differences.

Table 4.—Splenomegaly in Runt Disease

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight of Spleen at Time of Death (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>90.9 ± 6.1</td>
</tr>
<tr>
<td>V</td>
<td>151 ± 184</td>
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<tr>
<td>VI</td>
<td>208 ± 174</td>
</tr>
<tr>
<td>VII</td>
<td>477 ± 139</td>
</tr>
<tr>
<td>VIII</td>
<td>127 ± 109</td>
</tr>
<tr>
<td>IX</td>
<td>174</td>
</tr>
<tr>
<td>XII</td>
<td>207</td>
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<td>XIII</td>
<td>196 ± 131</td>
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<td>225</td>
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<td>XV</td>
<td>270 ± 116</td>
</tr>
<tr>
<td>XVI</td>
<td>196 ± 79</td>
</tr>
</tbody>
</table>
In these experiments a small number of animals developed the full-blown disease from which they recovered spontaneously. This effect was not confined to any particular experimental group, but was found sporadically. Long term follow-up (10-12 months) of these animals disclosed recovery of their normal growth pattern, with retention of the ruffled fur. The hematologic abnormalities disappeared, but the positive antiglobulin test persisted in several cases for some weeks after the hematocrit and leukocyte values had returned to normal. Two of these animals relapsed after six months, developed typical runt disease with anemia and a positive antiglobulin test and died. This seems to indicate that in some of these animals grafted lymphoid tissue may lie dormant for lengthy periods of time and then, for an unknown reason, become re-activated and produce disease. Observation of the apparently recovered animals is continuing in an attempt to assess the fate of the transplanted cells. Simonsen\textsuperscript{24} has observed a similar recovery from runt disease in mice treated with homologous lymphoid tissue and has interpreted this finding to indicate the acquisition of immunological tolerance by the grafted cells to the host.

2. Analysis of Results

(a) General Features. In the present study the injection of parental spleen cells into the F\textsubscript{1} hybrid caused a characteristic syndrome which developed after a variable latent period, depending upon the type of donor spleen cells used and the recipient strain. The animals became hunched, developed ruffled fur and splenomegaly, lost weight, became hypothermic and usually died. They were also found to have hemolytic anemia, a positive antiglobulin test, leukopenia, thrombocytopenia and abnormal plasma electrophoretic patterns. L. E. preparations were consistently negative.

The graft responsible for the induction of this disorder consisted of a lightly homogenized suspension of splenic tissue obtained from the parents of the male LAF\textsubscript{1}, BDF\textsubscript{1} and BAF\textsubscript{1} mice (female A/He, C57L, C57B1/6). The homogenate was introduced intraperitoneally in all instances. Two types of spleen donors were used: those which were not prepared and those which were sensitized with either splenic cells or erythrocytes. The spleen cells used to sensitize the donors were obtained either from a parent or from the recipient strain. In some instances, sensitization of the donor was accomplished by the injection of a mixture of spleen cells in Freund’s adjuvant into the footpads. The injection of nonreactive (isologous) splenic tissue into LAF\textsubscript{1} mice was nontoxic and produced neither clinical nor hematologic changes. It was found that the disease-producing potential of the graft was dependent upon the number of cells injected, the donor strain used, and the method of sensitization. As can be seen from table 1, the injection of unsensitized A/He spleen in varying doses was ineffective in producing disease in the LAF\textsubscript{1} recipient. This finding confirms the previous observation that A/He tissues evoke a stronger immunologic response than those derived from the C57L strain,\textsuperscript{85} and suggests that the antigens of the LAF\textsubscript{1} mouse derived from the C57L parent are only weakly antigenic to the A/He strain. In general, sensitization
of the donor heightened the reactivity of the graft against the host, so that the disease appeared and evolved more rapidly. The most potent sensitizing technic was that which employed Freund's adjuvant. This effect might be expected, since the recipient was grafted with cells already producing antibodies against its own tissues. The injection of C57B1/6 splenic tissue into the BDF<sub>1</sub> induced runt disease with a very severe anemia as well as the most marked degree of thrombocytopenia. It should be noted that the latent period in this group was relatively long, indicating the lack of correlation between the rapidity of onset of disease and the severity of the hematologic abnormality. Furthermore, the length of the latent period could not be correlated with the mortality rate in the various groups (fig. 4).

An invariable feature of runt disease, as observed in the present experiments, was weight loss. A considerable number of animals developed weight loss (at least 10–20 per cent of their original body weight) within 1–4 days after the injection of spleen cells. In a few of these animals, the weight loss was progressive and death ensued rapidly. However, the majority of these mice regained and surpassed their pre-injection weight, although ruffling of the fur persisted. After a variable period, these animals again lost weight. This secondary loss of weight was a constant sign of impending death. The causes of the weight loss in the affected animals are probably related to anorexia and lethargy which resulted from the underlying transplantation disease.

The earliest signs of runt disease in these animals were the hunched posture and ruffled fur. Once developed, ruffled fur persisted even though the animal gained weight and was apparently normal in all other respects. Other workers have demonstrated the development of severe dermatitis as the result of graft vs. host reactions in rats<sup>31</sup> and mice.<sup>36</sup> The skin changes occurring after the injection of homologous spleen cells into newborn rats are characterized by a massive infiltration of the dermis by reticulum cells. These investigators have postulated that such cells are derived from the graft. The phenomenon of ruffled fur may be due to a similar mechanism. The long-term (10–12 months) survivors of our study developed large circumscribed areas of complete alopecia, usually about the neck and shoulders (fig. 9). The denuded skin showed a desquamative dermatitis, which became generalized just prior to death in two of these survivors. It is possible that the great majority of mice died too soon to develop marked skin changes. Whether or not the age of the affected mouse is a factor in the development of skin changes remains to be studied.

The degree of hypothermia was directly related to the amount of weight lost, and was most marked just prior to death. The reduction of body temperature may have been due to starvation, stress or an endocrinological abnormality. Reduction in body temperature has been observed in man following large dose adrenocortico steroid therapy.<sup>37</sup>

(b) Hematologic Manifestations. The interval between the injection of the graft and the onset of anemia was variable; anemia appeared rapidly in those combinations which quickly developed the outward manifestations of runt disease, and more slowly in animals who developed those signs after a longer latent period. The anemia was usually of mild degree except in group XV
AUTOIMMUNIZATION (RUNT DISEASE) IN THE MOUSE

Fig. 9.—Alopecia in a runted mouse.

(C57B1/6 spleen into BDF1), in which severe anemia developed. Some of the mice of this group had hematocrit values of 10 to 20 volumes per cent as compared to the normal value of 52 volumes per cent.

Red blood cell survival studies done with Cr11-tagged isologous cells demonstrated a moderate reduction in life span. This finding indicated the presence of an extracorpuscular factor which was responsible for the shortened life span of the injected isologous erythrocytes. This humoral cytotoxic factor was most probably an antibody produced by the graft, since it was possible
to demonstrate a positive antiglobulin test in the majority of the sick animals. Early in this study numerous attempts to demonstrate erythrocyte sensitization met with failure, and only when complement was added to the reaction mixture were positive tests obtained. It has been known for many years that the demonstration of in vitro immunological reactions involving mouse tissues requires the addition of complement.\(^3\) Winn\(^4\) has recently shown that the neutralization of lymphoma cells by mouse antiserum was enhanced by complement, and that the degree of tumor cell neutralization was dependent on the dose of complement employed. Perhaps our finding of only a slight to moderate reduction in red blood cell life span was related to the low levels of complement present in the mouse. A lethal disease was not invariably associated with the development of either anemia or a positive antiglobulin test in the groups with a short latent period (group VIII), indicating that the mechanism of erythrocyte sensitization may be different from that involving nucleated cells.

More direct quantitation of the degree of erythrocyte sensitization was obtained by the use of the I\(^{131}\) labeled rabbit anti-mouse serum. Studies with this reagent demonstrated the following: (1) a correlation between the degree of anemia and the degree of absorption of the labeled antiglobulin serum by the sensitized red cells, (2) demonstration in some instances of erythrocyte coating by this method when the usual antiglobulin test was negative. This method of revealing and quantitating red cell sensitization appears to be promising.

Further characterization of the erythrocyte coating substance was obtained by a study of red blood cell eluates. These eluates were found to have antibody activity and specificity. Paper electrophoresis showed them to be gamma globulins (fig. 8). In the indirect antiglobulin test the erythrocyte coating gamma globulin demonstrated immunological specificity with the red cells of the contralateral parental strain.

Although increased hemolysis was evident in the runted mice, jaundice was not observed in these animals. The most probable explanation is the extremely rapid clearance of bilirubin by the rodent liver.\(^4\) Porter found jaundice in his study of homologous bone marrow disease in the rabbit only when fungal infection of the liver was present, even though hemolytic anemia with spherocytosis and a positive direct antiglobulin test was demonstrated.\(^4\)

The demonstration of a positive direct antiglobulin test in these mice indicates the production of a univalent anti-erythrocyte antibody by the grafted spleen tissue. This would indicate the presence of substances in the red blood cells of the host which are antigenic to the graft. Homologous tumor grafts have been shown to induce the development of hemagglutinating antibodies directed against the red blood cells of the tumor donor in mice\(^4\) and rats.\(^4\) Similar antibodies have been demonstrated following the application of a skin homograft in mice and in one human case.\(^4\) The production of these antibodies appears to be independent of the presence of donor erythrocytes in the graft. The extensive studies in mice by Gorser have shown a clear relationship between these erythrocyte antigens and transplantation antigens.\(^4\) He showed that the same gene determines H-2 transplantation
antigens as well as the red blood cell antigens. These antigens also have been linked to certain skeletal deformities of the mouse, allowing for their easy recognition. The role of the hemagglutinating antibodies in the destruction of homografts has been disputed and considered coincidental and not instrumental in the rejection of the graft. Gorer and his colleagues, however, have demonstrated cytotoxic properties of these antibodies in a variety of systems. Our finding of hemolytic anemia in mice whose red blood cells are sensitized by antibody would lend support to Gorer's contention that anti-erythrocytic antibody plays a role in the homograft reaction. Present work in our laboratory indicates that humoral antibodies may indeed play an active role in the homograft reaction.

Hemolytic anemia with a positive antiglobulin test as the result of a graft vs. host reaction has been observed previously in other species. Simonsen induced runt disease with severe hemolysis and a positive antiglobulin test by injecting embryonic chicks with a variety of homologous tissues, including whole blood. Splenomegaly was a striking feature and was evidently the result of proliferation of the grafted cells within the host. Serial passage of splenic material obtained from these chicks caused the disease in chick embryos after nine passages. Pionelli and Brooke showed that X-irradiated rabbits treated with homologous bone marrow developed runt disease accompanied by hemolytic anemia and a positive antiglobulin test. These results indicate the formation of antibody by the marrow graft against the recipient's red blood cells.

The most consistent hematological abnormality found in the runted animal was leukopenia. The finding of leukopenia was almost universal in the animals which developed weight loss, regardless of the ultimate outcome of the disease. In those instances in which the initial weight loss was regained by the animal the leukopenia disappeared, and when loss of weight reappeared the leukopenia also recurred. A rapid loss of weight was usually accompanied by severe leukopenia (700–2,000 WBC/mm³). In general, the leukopenia was due to lymphopenia, while the absolute granulocyte number remained relatively constant.

A reduction in the thrombocyte count was a less constant feature in these animals, and was most pronounced in the BDF1 mouse injected with C57B1/6 spleen cells (group XV). In this group, the most marked degree of anemia was also present. These animals were noted to bleed freely during blood collection procedures. Although leukocyte and platelet antibodies were not determined in this study, it would appear reasonable that an immunological mechanism similar to that responsible for red cell destruction was operative against these two formed elements of the blood.

3. Analogies to Autoimmune Disease in Man: Theories of Human Autoimmunization

The clinical disease produced in these animals as the result of the action of an acceptable, immunologically competent graft against its host bears many similarities to certain autoimmune diseases of man. The development of
multiple disorders including hemolytic anemia, positive antiglobulin tests, leukopenia, thrombocytopenia and splenomegaly is reminiscent of abnormalities frequently encountered in systemic lupus erythematosus (SLE) and related disorders. The term “autoimmune” has been used to indicate the formation of antibodies within the host which are active against his own tissues without reference to the nature of the antibody producing cells. In a strict sense, runt disease is caused by the reaction of homologous tissue against the host, whereas in the autoimmune diseases of man it has been considered that autologous tissues are responsible for the formation of the antibodies. However, an alternative view is that the antibody forming tissues in the autoimmune states of man are genetically deranged (and thus “homologous”) in such a way that they produce antibody against host tissues, yet cannot be rejected by the host. This interpretation of the autoimmune disorders of man thus makes them highly analogous to runt disease in the mouse.

The autoimmune theory has been widely discussed in recent years and an important consideration often raised has been the failure to detect and characterize an antigen responsible for the formation of auto-antibodies. Previous attempts to produce autoimmune diseases in experimental animals have centered on the alteration of normal body constituents so as to make them auto-antigenic. Such experiments, with the notable exception of autoimmune thyroiditis in rabbits, have almost uniformly met with failure. The data in this paper suggest that autoimmunization need not imply a change in normal body components, but rather a change in the antibody forming apparatus of the individual. Thus, under these circumstances, normal, unchanged tissues, e.g., red blood cells, would become antigenic to the aberrant lymphoid system. This would preclude the characterization of a supposed antigenic change by these red cells. It is conceivable that in SLE, in which multiple antibodies are found, the entire body may serve as the antigen.

Another criticism of the autoimmune concept has been the failure by many workers to demonstrate antibody specificity, particularly in autoimmune hemolytic anemia. It should be pointed out, however, that painstaking serological analysis of serum or eluate in these cases has shown specificity in a considerable number of them. The demonstration of specificity of antibody against normal red cell antigens further suggests that changes in red blood cell components are not necessary to provoke autoimmunization. The mapping of antibody specificity in pure bred mice is not a difficult problem, since the genetic makeup of these animals is well known. However, in dealing with a highly outbred population such as man, in which antigens are broadly distributed, this task may become almost impossible because of the presence of so-called “public antigens.” Furthermore, the heterogeneity of man may result in a high degree of antigenic specificity for each individual, thus further complicating the problem.

The mechanisms which would permit the appearance of abnormal antibody forming cells in an individual are not known. Two possibilities are (1) the implantation of maternal lymphoid cells during fetal life and (2) an alteration of the host’s own lymphoid cells. Maternal lymphoid cells might pass through the placenta and localize within the fetus. The fetus,
because of its immunological immaturity, would be tolerant of these cells. Tolerance of the transplanted cells to the fetus might occur in a manner similar to the mechanism responsible for the spontaneous recovery in runt disease as described in the present experiments. Later in life, the tolerance of these maternal cells to the individual could be lost, with their resultant multiplication and the production of antibodies to a variety of normal tissues, in a manner similar to the relapse of runt disease.

The second mechanism, in which an alteration of the host’s own lymphoid cells occurs, may take place by mutation. Such a mutation could occur spontaneously or as a result of viral infection, chemicals or radiation. The alteration of these cells would probably involve a gene deletion, so that they would be tolerated by the individual, yet be capable of forming antibody against autologous tissue. This or a related mechanism may well be the basis for the common development of autoimmune hemolytic anemia in chronic lymphocytic leukemia as already suggested by Green and by us in previous publications.

To recapitulate, runt disease in the experimental animal may be thought of as analogous to systemic autoimmune diseases in man. The experimental disease results from a graft vs. host reaction, the graft being immunologically active and the host being immunologically neutral. Autoimmune disease of man may be the result of the reaction of an aberrant lymphoid system vs. normal tissue antigens, the lymphoid tissue being immunologically active and the patient being incapable of defending himself against it.

**Summary**

The graft vs. host reaction (runt disease) was studied in LAF1, BDF1 and BAF1 mice. The disease was induced by the injection of parental splenic tissue into adult recipients.

The F1 hybrid recipients developed weight loss, hair changes, dermatitis, hypothermia and splenomegaly. The laboratory features included hemolytic anemia with a positive antiglobulin test, leukopenia, thrombocytopenia and abnormalities of their plasma electrophoretic patterns.

Certain similarities between runt disease and autoimmune diseases of man were suggested, and their significance was discussed.

**Sommario in Interlingua**

Le reaction graffo contra hospite (morbo secundari) esseva studiate in muses LAF1, BDF1, e BAF1. Le morbo esseva inducite per le injection de tissu splenic parental in recipientes adulte.

Le recipientes hybrida F1 disveloppava perdita de peso, alterationes de capillatura, dermatitis, hypothermia, e splenomegaly. Le aspectos laboratorial includeva anemia hemolytic con positivitate del test de antiglobulina, leukopenia, thrombocytopenia, e anormalitates del plasma in le configurationes electrophoretic.

Certe similitudes inter le morbo secundari e morbos autoimmun in humanos es signalate. Lor signification es discutite.
REFERENCES

active proteins from various animal sera by ethanol fractionation techniques. Methods in Medical Research 5:284, 1952.


35. Snell, C. D.: Personal communication.


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A report of two cases of acquired auto-immune hemolytic anemia associated with bone marrow aplasia, studied by tracer technics with Fe59 and Cr51. Control of the hemolytic process by steroid therapy was associated with improvement in marrow function in each case. It is suggested that the antibody in these two cases was active against red cell precursors in the marrow as well as against circulating red cells.—R. M. H.


Some patients with disseminated lupus erythematosus show in their serum a substance producing a precipitating reaction with desoxyribonucleic acid. This reaction can be evidenced by the ring-test, the Ouchterlony technic and immunoelectrophoresis. These sera produce no precipitation if ribonucleic acid is used. The precipitating substance reacts as well with purified DNA of various origin. It appears not to be an histone, likely it is an antibody. The L.E. phenomenon persists after absorption of lupus sera on DNA.—G. M.


L.E. sera giving a precipitin reaction with DNA also give a positive complement fixation reaction. This fact is a definite point against the histone hypothesis and is an argument for the antibody nature of this substance.—G. M.
Studies in Experimental Autoimmune Disorders. I. Clinical and Laboratory Features of Autoimmunization (Runt Disease) in the Mouse

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