HYDRALAZINE DISEASE IN THE GUINEA-PIG AS AN EXPERIMENTAL MODEL FOR LUPUS ERYTHEMATOSUS*

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In 1954 Dustan et al. (1) reported that some patients receiving hydralazine for the treatment of hypertension developed clinical and laboratory findings characteristic of lupus erythematosus. Since that time many investigators have attempted to produce a lupus erythematosus-like illness by feeding animals hydralazine. Unfortunately, such studies have been the subject of much dispute.

Comens (2) was the first to report positive results. He produced in dogs a syndrome characterized by L.E.-like cells, thickening of the basement membranes of renal glomeruli, anemia, leukopenia, and aberrations in serum proteins. His evidence also suggested that manganese deficiency might play an important role in the genesis of the hydralazine syndrome (3). Dubois (4) and Earl (5) were unable to confirm these findings while Gardner (6) found L.E.-like cells and noted glomerular basement membrane thickening but felt that the latter was within normal limits for the dog. Zingale et al. (7) reported false positive Wassermann reactions in two dogs as well as elevated beta globulins in similar experiments. They did not find L.E.-like cells or histopathological changes compatible with lupus erythematosus.

Pigs (8) and rats (9) also have been used in hydralazine feeding experiments, but no lupus erythematosus-like syndrome has been produced. Siguier et al. (10) used the guinea-pig in an effort to reproduce this lupus-like syndrome. Their positive data included classical L.E. cells in four out of 15 animals and L.E.-like cells in three others, leukopenia and anemia, elevated alpha-2-globulins without hypergammaglobulinemia, and anorexia, apathy, and weight loss leading to death within two to four months. Histological examination of the organs was not performed.

We decided to reinvestigate this problem because of the urgent need for an experimental model for lupus erythematosus. Any clue to the mechanism by which hydralazine produced a syndrome, clinically and pathologically almost identical to lupus erythematosus, would be of obvious value. The guinea-pig was selected for our investigation not only because of Siguier's recent work but also because of the experiments by Carrera et al. (11) which showed that guinea-pig leukocytes were very sensitive to the action of human L.E. serum.

We also wanted to test a new hypothesis, namely, the role of riboflavin deficiency in the genesis of the hydralazine syndrome and lupus erythematosus. The anti-malarials atabrine and chloroquine are very effective in treating lupus erythematosus. The chemical configurations of riboflavin, atabrine, and chloroquine are quite similar to one another (Fig. 1). If weanling rats are made riboflavin deficient (12) and are fed atabrine, they will grow normally. Atabrine substitutes for riboflavin in the flavin-containing enzymes because of its structural similarity. In vitro, however, atabrine inhibits flavin-containing enzymes (13, 14) by irreversibly combining with the protein moiety of the enzyme—again because of its structural similarity.

Hydralazine is somewhat similar in structure to the above-mentioned compounds (Fig. 1). It was thought that if hydralazine, which produces a lupus erythematosus-like illness in humans, were fed to riboflavin-deficient guinea-pigs, it might be easier to produce the lupus-like disorder.

A third experiment was suggested by the observation that mesantoin and related (anti-convulsant medications can cause a lupus-like illness (15, 16). Mesantoin (Fig. 1) was given to normally fed and riboflavin deficient guinea pigs in an effort to enhance the production of this syndrome.

METHODS

Four experimental designs were used:

Experiment A. All animals were fed guinea-pig chow, supplemented twice weekly with raw cabbage. Nineteen animals were given hydrala-
The compounds of interest in hydralazine syndrome and lupus erythematosus are shown in Figure 1. Note the similarity in the chemical structures of atabrine, chloroquine, and riboflavin. Hydralazine bears less resemblance to these compounds. The hydantoin ring of mesantoin is similar to one of the rings of the riboflavin molecule.

Experiment A. Nineteen guinea-pigs were treated with propylene glycol and 23 were housed under the same conditions to serve as controls. Five of the third group received propylene glycol as a specific control for the mesantoin treated group.

Experiment B. All animals were placed on a riboflavin-deficient diet. Seventeen animals were given hydralazine and eight served as controls. Another 17 animals received mesantoin in propylene glycol while eight control animals were given propylene glycol.

Experiment C. All animals were fed guinea-pig chow supplemented twice weekly with raw cabbage. Seventeen received hydralazine as in the above experiments and eight served as controls. In addition all 25 animals were irradiated with ultraviolet light from a Westinghouse FS40T12 fluorescent sun lamp. The lamp, housed in an Alzac aluminum reflector, was placed 12 inches above the animal’s back. The exposure began at five minutes a day and was gradually increased to a maximum of two hours daily, six days a week. The guinea-pig’s hair was removed first by shaving and then by a chemical depilatory, Nair®, prior to irradiation.

Experiment D. All guinea-pigs were placed on a riboflavin-deficient diet and exposed to ultraviolet irradiation as outlined above. Seventeen guinea-pigs were fed hydralazine and eight served as controls. All guinea-pigs used in this last experiment were females whereas previously there was an equal distribution of males and females. In addition, genetically heterogenous guinea-pigs purchased from local farms were used in the first three experiments. These animals had a high incidence of chronic pneumonitis. Consequently, in the fourth experiment, albino guinea-pigs of the inbred Hartley strain were used because they are very resistant to the development of pneumonitis. All animals weighed between 300–350 g at the start of the experiments.

Hydralazine hydrochloride was dissolved in distilled water and given orally by syringe once daily seven times a week. Mesantoin was dissolved in propylene glycol and administered the same way. In Experiment A, the hydralazine dose was 10 mg/Kg for the first three months. The results described by Siguier et al. were not obtained, so the dose was increased slowly to 300 mg/Kg during the subsequent six months. The mesantoin dose was 10 mg/Kg throughout both Experiments A and B.

In Experiments B, C, and D, the hydralazine dose was 100 mg/Kg. All animals were bled before the experiments were begun and their sera frozen and stored at −20° C. Blood was obtained at weekly to monthly intervals.
intervals for L.E.-cell tests, white blood counts, hematoctrit, blood urea nitrogen, and serum protein electrophoresis. Urinalyses were done every two weeks, and the organs were examined histologically when the animals died.

L.E.-cell tests were performed by the methods of Snapper and Nathan (17), Zinkham and Conley (18), and Lachmann (19). In Snapper and Nathan's method, guinea-pig leukocytes served as substrates in the rings. In Lachmann's method, guinea-pig leukocytic nuclei served as substrates, and guinea-pig white cells served as the phagocytic component.

We also performed induction tests using these identical procedures with potent lupus serum acting on guinea-pig leukocytes. Carrera's method (11) was also employed for this purpose. In some of the induction tests human leukocytes served as the phagocytic element. The purpose of these procedures was to determine what morphologic changes are actually induced in guinea-pig leukocytes by the action of lupus serum, and the ease with which they are produced.

In order to detect anti-nuclear factors in the sera of the treated guinea-pigs, Coon's immuno-fluorescent technic was employed. The method of Calabresi (20) was modified for this purpose: a smear was made with normal guinea-pig blood and fixed in absolute methanol for 15 minutes. A few drops of the test animal's undiluted serum were incubated in a moist petri dish at 37° C. for 30 minutes. Then the slide was washed under running tap water for 20 minutes, followed by air drying. Fluorescein conjugated rabbit anti-guinea-pig gammaglobulin was placed on the same area. The slide was kept in a moist petri dish for 30 minutes at room temperature and then washed under running water again. The preparation was viewed with a Leitz fluorescence microscope employing a HBO 200 Osram lamp.

One looks for nuclear fluorescence without cytoplasmonic fluorescence. If more than 50% of the nuclei fluoresce with a typical yellow-green color, the test is considered positive. As a control, smears were incubated with fluorescein conjugated antiseraum alone as well as with non-fluorescein conjugated rabbit anti-guinea-pig gammaglobulin prior to incubation with the fluorescein conjugated antiseraum. In the first instance there was no non-specific nuclear fluorescence. In the second, the nonfluorescein conjugated anti-serum blocked production of nuclear fluorescence.

Friou's nucleohistone "lupus globulin" test also was employed (21), as was Calabresi's original technic using human blood smears in an effort to detect anti-nuclear factors in the guinea-pigs' sera.

Serum protein electrophoresis was performed in a Spinco-Durrum cell at pH 8.6 in Veronal buffer.

Post-mortem examination of the organs was performed. Alternate kidney sections were stained with hematoxylin and eosin and periodic acid Schiff technic.

The riboflavin deficient diet was modified slightly from that of Reid (22). The composition of the diet is given in Table 1. The animals adapted themselves to the diet quite readily if it was introduced gradually. Riboflavin levels were determined in the diet, feces, and urine of two animals by Dr. Lester Hankin of the Connecticut Agricultural Station using the L. casei microbiological method. The urine was collected in brown bottles covered with foil and kept acid with 10 ml of 6 N hydrochloric acid. The riboflavin content of the diet was found to be 0.08 γ/gram. The animals ate 30-40 grams of diet per day. The urinary excretion of riboflavin was 0.007 γ/ml with a total of 25 ml excreted per day by one animal and 100 ml by the second. The fecal content of riboflavin in the first animal was 15.3 γ/gm feces per day (total feces 1.98 gm/day) and 5.91 γ/gm feces per day in the second animal (total 2.63 gm/day). The clinical appearance of the riboflavin deficient animals was one of apathy, roughening of the hair coat, and loss of weight over a two-week period leading to death. There was no dermatitis. If 200 γ/day of riboflavin was given orally to the guinea-pigs after they began to lose weight, they responded immediately with a sustained weight gain. They became active and regained their appearance of well-being. Figure 2 shows a typical response. Using these clinical signs, weight curves, and response to riboflavin administration as criteria, it appeared that 60% of the treated animals in Experiments B and D became riboflavin deficient.

RESULTS

Negative results can be summarized as follows:

(1) The mesantoin treated animals showed no abnormalities.

(2) The riboflavin deficient diet, ultraviolet irradiation, and use of female animals did not seem to enhance the production of hydralazine disease.

(3) There were no significant abnormalities in the white count, hematoctrit, urinalysis, blood urea nitrogen, or serum protein electrophoresis.

(4) Chronic wasting disease secondary to hydralazine, described by Siguer et al., was not seen.

The following positive results were obtained:

(1) Seventeen per cent of the animals receiving hydralazine had L.E.-like cells and/or showed the presence of anti-nuclear activity when their blood or serum was appropriately tested.

(2) In 17% of the animals minimal thickening
of the renal glomerular basement membrane occurred, but no other organs showed histological changes compatible with lupus erythematosus.

(3) The most striking positive findings were in animals receiving hydralazine for at least six months.

In Table 2 the results of the four experiments are summarized.

In Experiment A, an epizootic characterized by clinical and pathological evidence of pneumonitis killed 15% of all the animals in the first six weeks. Thereafter, only a rare animal death could be attributed to pneumonitis. Before Experiment A was begun, serum protein electrophoresis was performed on all the animals. Many of them showed an alpha-2 globulin peak as high as Siguier et al. had found after their animals had received hydralazine. The gammaglobulin fraction rose in our animals as they contracted pneumonitis and subsequently died. At the conclusion of Experiment A, almost every animal had histological evidence of a chronic granulomatous pneumonitis even though some appeared clinically healthy during the course of the experiment.

Because of these findings any changes in the serum protein electrophoresis were disregarded. Therefore, Hartley strain guinea-pigs, which are resistant to this type of infection, were used in the last experiment. There were no changes in serum protein electrophoresis and no evidence of pneumonitis when the experiment was terminated after three months.

The clinical course of the guinea-pigs in these experiments was different from that reported by Siguier et al. (10). Our animals did not exhibit a chronic wasting disease. Most of them grew normally and appeared well until three or four days before they died. During the terminal episode they developed acute weight loss, apathy, and seizures. A few animals who were on very high doses of hydralazine, 150—300 mg/Kg per day, maintained a uniform weight before expiring in an identical fashion.

Experiment A lasted for nine months and gave the greatest number of positive findings. Experiments B, C and D lasted for only three months. The reason for this difference is that riboflavin deficient guinea-pigs do not survive very long if riboflavin is not made available to them. It was hoped that if riboflavin deficiency were an important factor in the genesis of this syndrome, the period of survival would have been sufficient to allow its production.

The L.E. cell tests were characterized by the
given orally while the animals were still on the diet on the weight of guinea pigs and the response to subsequent administration of riboflavin. At this point, they began to lose weight over a 10-14 day period. If riboflavin was not supplied at this time, the animals would die. When riboflavin was given orally while the animals were still on the diet, a prompt and sustained weight gain occurred.

In order to see if a species difference might account for the failure of guinea-pig antinuclear factor to react with human and calf nuclear material, the following reverse experiment was performed. Human lupus serum was incubated on smears from rabbit, dog, hamster, and guinea-pig blood. Specific nuclear fluorescence was seen in all cases except when guinea-pig blood was used. In order for human lupus serum to exert antinuclear activity against guinea-pig white cell nuclei, it was necessary to isolate guinea-pig white blood cells by the dextran method (23), freeze and thaw them and then make a smear of the disrupted cells. After this process, specific nuclear fluorescence was seen. There was no evidence of inhibiting substances in guinea-pig blood that could account for the failure of human L.E. serum to act on guinea-pig blood smears.

This resistance of guinea-pig white cells to the action of human lupus serum correlated well with the difficulty encountered in producing L.E. cells in the induction tests. L.E. cells could be formed by incubating lupus serum with guinea-pig white cells, but it was not accomplished as easily as previous authors had reported (11). In fact, L.E. cell phenomena could be produced easily and consistently only when lupus serum was incubated with dextran isolated guinea-pig leukocytic nuclei which had been disrupted by freezing and subsequently exposed to viable human leukocytes. When these first two components were presented to viable guinea-pig leukocytes very few phagocytized L.E. bodies were seen. However, many swollen and homogeneous appearing nuclei and hematoxylin-like bodies were seen to be lying free in the smear. This indicated that, although the first stage of the L.E. cell phenomena could be easily produced, the viable guinea-pig leukocytes were unable to complete the second or phagocytic stage. This correlates well with the predominant type of L.E. cell-like phenomenon seen in the preparations from the experimental animals (Figs. 4, 6, 7). These morphologic changes are quite analogous to the findings of

![Graph showing the response of an individual guinea-pig to riboflavin](image-url)

**Figure 2:** Effect of riboflavin-deficient diet on the weight of guinea pigs and the response to subsequent administration of riboflavin.

Fig. 2. This graph showing the response of an individual guinea-pig is representative of the responses of the other animals. After an initial weight loss during adaption to the diet, the animals maintained a steady weight for 4-6 weeks. At this point, they began to lose weight over a 10-14 day period. If riboflavin was not supplied at this time, the animals would die. When riboflavin was given orally while the animals were still on the diet, a prompt and sustained weight gain occurred.

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### TABLE 2

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Survival Time (months)</th>
<th>Died (D) or Sacrificed (S)</th>
<th>Time of Appearance of LE Cell Phenomena</th>
<th>Time of Appearance of Serum Antinuclear Activity</th>
<th>Renal Lesions at Autopsy</th>
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<tbody>
<tr>
<td><strong>Experiment A</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>1</td>
<td>9</td>
<td>D</td>
<td>Rosette formation—7 months</td>
<td>4 + at 9 mo.</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>D</td>
<td>none</td>
<td>4 + at 7 mo.</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>D</td>
<td>none</td>
<td>4 + at 6 mo.</td>
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</tr>
<tr>
<td>4</td>
<td>1.75</td>
<td>D</td>
<td>Nuclear swelling—1 month</td>
<td>1 + at 0.5 mo.</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>D</td>
<td>Hematoxylin bodies—4 months</td>
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</tr>
<tr>
<td>6</td>
<td>3</td>
<td>D</td>
<td>none</td>
<td>1 + at 3 mo.</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>D</td>
<td>none</td>
<td>none</td>
<td>+</td>
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<tr>
<td><strong>Experiment B</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>3</td>
<td>S</td>
<td>none</td>
<td>1 + at 3 mo.</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>S</td>
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<td>4 + at 3 mo.</td>
<td>0</td>
</tr>
<tr>
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<td>D</td>
<td>Hematoxylin bodies—1.5 mo.</td>
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<td>+</td>
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<td>3</td>
<td>S</td>
<td>Nuclear swelling—3 mo.</td>
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<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>S</td>
<td>none</td>
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<td>2</td>
<td>D</td>
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<td>D</td>
<td>none</td>
<td>none</td>
<td>+</td>
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<tr>
<td><strong>Experiment C</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>3</td>
<td>S</td>
<td>none</td>
<td>none</td>
<td>+</td>
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<td><strong>Experiment D</strong>&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>Nuclear swelling—2 mo.</td>
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<td>+</td>
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<tr>
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<td>3</td>
<td>S</td>
<td>LE cell—2 mo.</td>
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<td>+</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>S</td>
<td>none</td>
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<tr>
<td>4</td>
<td>3</td>
<td>S</td>
<td>none</td>
<td>none</td>
<td>+</td>
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<sup>1</sup> Experiment A—normal diet, hydralazine treated. 19 animals total. 4 animals died of pneumonia in the first 4–6 weeks and are not included in this total.

<sup>2</sup> Experiment B—riboflavin deficient diet, hydralazine treated. 17 animals total.

<sup>3</sup> Experiment C—normal diet, hydralazine treated, UV irradiation. 17 animals total.

<sup>4</sup> Experiment D—riboflavin deficient diet, hydralazine treated, UV irradiation, female guinea pigs. 18 animals total.

Robineaux (24) and Rifkind and Godman (25) who were able to follow the development of the LE cell phenomenon. They noted that the immediate action of lupus serum on white cells was swelling and homogenization of lymphocytic or polymorphonuclear leukocytic nuclei. The swollen body was extruded into the media while the cytoplasm was left behind. It was then phagocytized by a viable leukocyte to become the classic LE cell.

These findings in the induction tests may in part explain why the guinea-pig sera reacted with homologous white cells and not with human white cells. It is possible that, just as the guinea-pig white cell is resistant to the action of human lupus serum, so is the human white cell resistant to the action of guinea-pig anti-nuclear serum. Unfortunately, these guinea-pig sera were not tested against frozen and thawed human white cells.

A four plus anti-nuclear reaction means that 80–100% of white blood cells show nuclear fluorescence. A one plus reaction means that 10% of white blood cells exhibited the same reaction. It may be a weakly positive reaction because...
Fig. 3. Cell produced by the action of human lupus serum on guinea-pig leukocytes. Note that the inclusion body is fragmented and lumpy but still homogeneous. This was the predominant type of L.E. cell produced in the induction tests. × 2160.

Fig. 4. Homogenization and swelling of white cell nuclei produced by the action of human lupus serum on guinea-pig leukocytes. Compare with figures 6 and 7. × 2160.

Fig. 5. Homogeneous basophilic bodies lying free in an L.E. cell test from a hydralazine treated animal. They are morphologically indistinguishable from hematoxylin bodies seen in human L.E. cell tests. (Experiment A, animal No. 5). × 2160.

Fig. 6. Homogenization and nuclear swelling present in L.E. cell test. (Experiment B, animal No. 4). × 2160.

Fig. 7. Many leukocytes undergoing nuclear swelling and homogenization. (Experiment A, animal No. 4). × 2160.

Fig. 8. Two polymorphonuclear leukocytes are in close proximity to a basophilic homogeneous body, as if about to phagocytize it. (Experiment A, animal No. 5). × 2160.
Fig. 9. This L.E. cell test shows a similar homogeneous basophilic body in the process of being phagocytized by a guinea-pig white cell. Note the lack of chromatin structure in these bodies in figures 8 and 9. (Experiment B, animal No. 3). X 2160.

Fig. 10. Rosette formation with L.E.-like cell present. Note that the inclusion material although homogeneous is lumpy and fragmented, and similar to that seen in figure 3. (Experiment A, animal No. 1). X 2160.

Fig. 11. L.E.-like cell with smaller inclusion body. Note its similarity to the inclusion material in figure 3. (Experiment D, animal No. 2). X 2160.

Fig. 12. P.A.S. stained section of renal glomerulus from a control guinea pig. Note fine delicate basement membrane and the moderate cellularity present. X 960.

Fig. 13. P.A.S. stained section of renal glomerulus from another control animal. Arrows indicate degree of irregular basement membrane thickening. X 960.

Fig. 14. The arrows show the degree of glomerular basement membrane thickening seen in 13 hydralazine treated guinea-pigs. The basement membrane stained somewhat more deeply and homogeneously with P.A.S. stain than did the corresponding area of control animals. The basement membrane thickening was also of slightly greater degree. P.A.S. stain. X 960.
when control smears incubated with normal guinea-pig serum are examined, only 1–2% of the white cells show nuclear fluorescence. This same percentage is seen in preparations from normal human beings. None of the sera obtained from these animals before the onset of the experiment showed anti-nuclear activity.

In a few animals, the renal glomeruli showed slight thickening of the basement membranes with the P.A.S. stain (Fig. 14). It is difficult to assign significance to these findings because normal guinea-pig kidneys show some irregular thickening of the basement membrane (Figs. 12, 13). The thickening found in the hydralazine treated animals was only slightly more than that of the controls. These findings were not correlated with any changes in the urine or blood urea nitrogen. The remainder of the kidneys as well as the other organs did not show abnormalities compatible with lupus erythematosus. Additional experiments are required to determine whether or not these changes are analogous to wire loop lesions seen in renal tissue from patients with lupus erythematosus.

**DISCUSSION**

These studies support the most significant claims of previous investigators in this area: hydralazine does produce an illness in experimental animals and this illness closely resembles lupus erythematosus. However, the criteria for the evaluation of L.E. cell phenomena in the experimental animal must be revised. Inclusion bodies may be fragmented and not as completely homogeneous as usually described for the classical L.E. cell. L.E. cell induction tests performed with human lupus serum and guinea-pig leukocytes make this clear. In addition, only the first stage of the L.E. cell phenomenon may be present. A phagocytic defect in the animal's own white cells may prevent the second stage from occurring to any significant degree.

Two other investigations are relevant: Miescher (26) was able to produce a few L.E.-like cells in a guinea-pig that was immunized with liver cell nuclei. Finch et al. (27) produced L.E.-like cells by incubating human leukocytes with a rabbit anti-human leukocyte serum. The inclusion bodies in the L.E.-like cells in these experiments very closely resemble those shown in Fig. 10. These experiments in addition to our own L.E. cell tests and L.E. induction tests constitute further morphologic evidence for the presence of an anti-leukocytic factor in the sera of hydralazine treated guinea-pigs.

In previous investigations, it was necessary to incubate the animal's serum with human white cells to produce L.E.-like cells. In our work, L.E.-like cells were produced by the action of guinea-pig serum on guinea-pig leukocytes. On a few occasions guinea-pig sera were incubated with human leukocytes, but no L.E.-cells were observed.

Anti-nuclear factors have been demonstrated for the first time in hydralazine disease in a laboratory animal. Unfortunately, these factors were labile when frozen and thawed several times. Friou (28) tested sera from two patients with hydralazine disease and found that titers of anti-nuclear activity were low when compared with those of patients having lupus erythematosus. Although the presence of anti-nuclear activity in the animal's serum did not always correlate with the demonstration of L.E. cell phenomena, this finding was not unexpected. A recent review by Larson (29) contains data which show that approximately one-sixth of patients with lupus erythematosus have positive L.E. cell tests without demonstrable anti-nuclear factors. The other combinations of anti-nuclear activity alone or in conjunction with L.E. cell phenomena also are well known in human clinical material. The results in our animals are similar.

Although the riboflavin hypothesis was not supported, it may be worth pursuing further. Only 60% of the adult guinea-pigs were made riboflavin deficient. If they could be kept in a borderline deficient state for longer periods of time, perhaps hydralazine disease could be produced more easily. Synthetic riboflavin antagonists in conjunction with the diet might produce a much higher percentage of riboflavin deficient animals and help to enhance the production of this lupus-like syndrome.

The renal glomerular changes are difficult to interpret. Normal guinea-pig kidney has basement membrane changes that are slightly abnormal by human standards (Fig. 13). It is on this base that further abnormalities are superimposed. The irregular basement membrane thickening seen in hydralazine treated animals is only slightly greater than that of the control animals. Further experiments may clarify this problem. A similar difficulty may play a role in
the controversy over the renal lesions in the hydralazine treated dogs. From what we know of the clinical course of hydralazine patients, and from autopsy findings in one case (30), there is no reason to think that "wire loop" lesions should be produced by hydralazine in an experimental animal. Patients who developed the syndrome showed a decrease in albuminuria and an improvement in urinary sediment. The one autopsy sied case did not show glomerular basement membrane thickening. Another case of "hydralazine poisoning" which was examined at autopsy showed wire loop lesions in the kidneys (31). However, it was a very atypical case and it might not represent hydralazine toxicity.

It has not been possible to duplicate many of the findings of Siguier et al. One major difference exists between their protocol and ours. They put hydralazine in the drinking water so that the animals had constant exposure to the drug whereas we administered it once daily by mouth. This difference could account for our failure to confirm all their findings.

Since only 17% of our animals developed laboratory changes similar to those found in lupus erythematosus, we do not yet have a practicable experimental model. It is possible that the guinea-pig is a poor animal to study since its leukocytes are relatively resistant to the action of human L.E. serum as a substrate for either fluorescent antiglobulin or L.E. cell induction tests.

SUMMARY

1. Hydralazine was administered to a large group of guinea-pigs. L.E.-like cells and serum anti-nuclear factors developed in 17% of 70 animals. The most striking abnormalities occurred in animals that received hydralazine for at least six months.

2. Riboflavin deficiency, ultraviolet irradiation, and the use of female guinea-pigs did not enhance the production of a lupus erythematosus-like syndrome with hydralazine.

3. Minimal thickening of the glomerular basement membranes occurred in some animals. This finding was difficult to interpret since there were no associated abnormalities in the urine or blood urea nitrogen.

4. No changes in the hemogram or serum proteins occurred. There was no distinctive clinical illness associated with the abnormal laboratory findings.

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