TABLE II. Excretion Time of Chicks as Affected by Different Carbohydrates.*

Carbohydrate	2 wk minutes	4 wk Group 1 minutes	4 wk Group 2 minutes
Lactose	77 ± 7	86 ± 14	78 ± 6
Sucrose	115 ± 22	133 ± 6	149 ± 17
Dextrin	140 ± 8	177 ± 5	181 ± 11

* Includes stand. error. $\sqrt{\frac{d^2}{n(n-1)}}$.

group to also eliminate entirely the dehydration caused by lactose.

It was thought that a clearer understanding of the intestinal effects would be had by determining the time it takes for food to pass through the digestive tract of the chicken. At 2 and 4 weeks, 5 chicks per group were put in individual cages and given feed and water *ad libitum*. Each bird was given a number zero capsule filled with carmine 40, which is not absorbed by the intestine. The length of time taken for red to first appear in the feces was taken as the excretion time. The results are shown in Table II. The excretion time of the dextrin birds was 20% longer than the sucrose birds. These data lend support to the theory that carbohydrate differences may be explained on the basis of intestinal synthesis or efficiency of carbohydrate breakdown in the gut. It would seem that dextrin allows more time for synthesis to take place, since chicks fed this ration retain the food longer. This extra time may allow additional synthesis of some unknown chick growth factor or factors.

Summary. When the rate of growth of chicks is used to measure the nutritional efficiency of carbohydrates they fall in the following order: dextrin, cerelose, sucrose, and lactose. Cellulose, sulfasuxidine, reticulogen, fish solubles, or vit. B_{12} do not change the significant differences observed when these carbohydrates are fed. However, the lactose-fed birds do give the greatest response to reticulogen, fish solubles and the higher levels of vit. B_{12}. The excretion times of chickens fed these different carbohydrates are in the following decreasing order: dextrin, sucrose, and lactose. The possibility of these nutritional differences being explained by the synthesis of known and/or unknown factors is discussed.

We are indebted to Merck and Co., Inc., Rahway, N. J., for crystalline vit. B_{12}, and crystalline vitamins; to the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., for synthetic folic acid; to Abbott Laboratories, North Chicago, Ill., for haliver oil; to Wilson and Co., Inc., Chicago, Ill., for gelatin; and to E. I. duPont de Nemours and Co., Inc., New Brunswick, N. J., for crystalline vit. D_3.

Received July 28, 1950. P.S.E.B.M., 1950, v75.

Experimental Production of the L. E. Phenomenon in the Skin of Man.* (18165)

JOHN W. REBUCK AND LAWRENCE BERMAN. (Introduced by F. W. Hartman.)

From the Department of Laboratories, Henry Ford Hospital and the Department of Pathology, Wayne University College of Medicine, Detroit.

The L.E. cell, first described by Hargraves, Richmond and Morton(1), was originally observed in incubated marrow aspirates of patients suffering with acute disseminated lupus erythematosus. Sundberg and Lick(2) found occasional L.E. cells in the peripheral blood in acute disseminated lupus erythematosus although again leukocytic concentration in the test tube was employed. Haserick and Bortz

* Presented before the Joint Meeting of the North Central Region College of American Pathologists and the Michigan Pathological Society, Detroit, Mich., May 13, 1950.

1. Hargraves, M., Richmond, H., and Morton, R., *Proc. Staff Meet. Mayo Clinic,* 1948, v23, 25.

2. Sundberg, R. D., and Lick, N., *J. Invest. Derm.,* 1949, v12, 83.

(3) and Hargraves(4) next produced L.E. cells *in vitro* by utilizing control marrows incubated with only the plasma of patients acutely ill with disseminated lupus erythematosus. This report describes the production of the L.E. cell phenomenon (Fig. 1B, 3, 4) in the skin of normal human volunteers following inoculation of experimental windows with the plasma of a patient with acute disseminated lupus erythematosus.

Materials and methods. Five experimental windows were prepared in 2 human male volunteers. The technic of human window preparations has been described in detail elsewhere(5-8). Briefly, the epithelium was slowly scraped away with a sterile scapel over a small area in the forearm until the papillary layer of the corium was exposed. In the present experiments a small drop of diphtheria toxoid or egg-white was next placed on the lesion to increase the toxicity of the local area. The lesion was then covered with a sterile glass cover-slip which in turn was surmounted by a sterile square of cardboard and both were covered with surgical adhesive tape. Within an hour or 2 the cells of the inflammatory exudate migrated to the under-surface of the cover-slip. At timed intervals the cover-slip was removed from the lesion, air-dried and stained with May Grünwald-Giemsa stain. The lesion was again immediately covered by a second cover-slip. In this way samples of the cellular exudate were obtained from the same lesion every few hours throughout the simple inflammatory cycle. Two to 3 drops of the L.E. factor plasma were added to the lesions at from 5.5 to 9.5 hours after the initiation of the lesions. Five control experiments were performed in which 4 lesions were inoculated with diphtheria toxoid without the addition of the L.E. factor. In the final control experiment the human volunteer was inoculated with diphtheria toxoid, followed at suitable intervals by inoculation of normal human plasma.

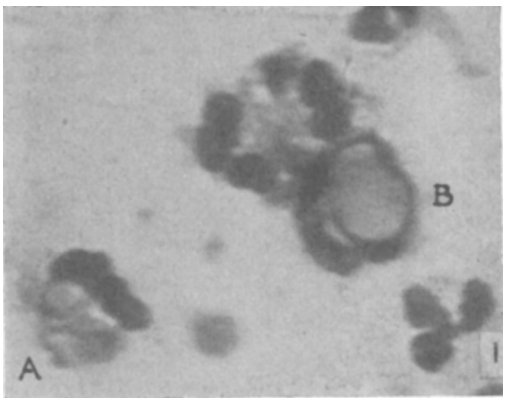

FIG. 1.

Lesion 1. Experimental. (A) Partial lysis of nuclear lobes forming 2 "ring lobes." (B) L.E. cell. 11 hr of inflammation in man. ×1400.

Experimental results. Lesions 1 and 2 were studied in the first human male volunteer; he was Schick negative. In lesion 1 the original inoculant was diphtheria toxoid. Cover-slips were removed at 3.5, 5.5, 9, 11, 14 and 23 hours. The L.E. plasma was introduced at the 9th hour of inflammation, and the L.E. phenomenon was observed in the next preparation, *i.e.* the 11th hour of inflammation or 2 hours after the addition of the L.E. factor to the lesion. In lesion 2 the original inoculant was egg-white. Cover-slips were removed at 3.5, 5.5, 7.5, 11.5, 14 and 23 hours. The L.E. plasma was introduced at 5.5 hours of inflammation and the L.E. phenomenon was observed in the next preparation, *i.e.* 7.5 hours of inflammation or 2 hours after the addition of the L.E. factor to the lesion.

Lesions 3, 4 and 5 and control lesions 9 and 10 were studied in a second human male volunteer who was Schick positive. The original inoculants in these five experiments were the same, diphtheria toxoid. In lesion 3 cover-slips were removed at 3, 6, 8, 8.75, 9.5 and 11 hours. The L.E. plasma was introduced at 8 hours of inflammation and the next preparation, *i.e.* at 8.75 hours of inflammation or 45 minutes later failed to show a

3. Haserick, J. R., and Bortz, D. W., *J. Invest. Derm.*, 1949, v13, 47.

4. Hargraves, M. M., *Proc. Staff Meet. Mayo Clinic,* 1949, v24, 234.

5. Rebuck, J. W., Thesis University of Minnesota, 1947.

6. Rebuck, J. W., and Woods, H. L., *Blood,* 1948, v3, 175.

7. Rebuck, J. W., and Monaghan, E. A., *Fed. Proc.,* 1948, v7, 277.

8. Rebuck, J. W., *Anat. Rec.,* 1949, v103, 497.

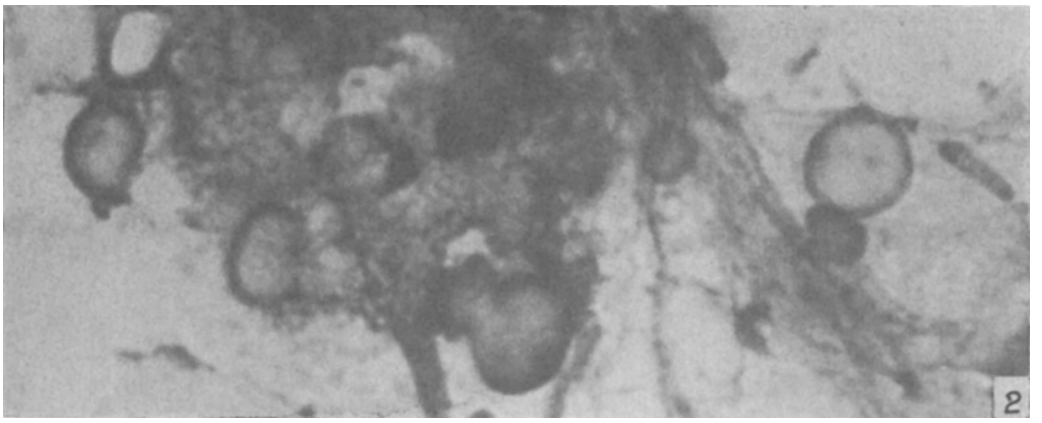

FIG. 2.
Lesion 4. Experimental. Free partially lysed nuclear lobes amid granular and filamentous nuclear debris. 13 hr of inflammation in man. ×2000.

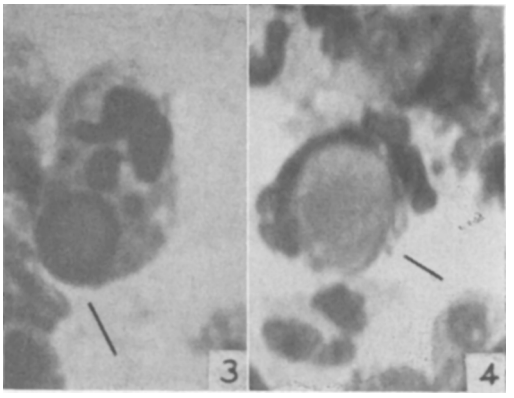

FIG. 3.
Lesion 2. Experimental. L.E. cell with 2 additional small ingested nuclear bodies. 7.5 hr of inflammation in man. ×2000.

FIG. 4.
Lesion 1. Experimental. L.E. cell. 11 hr of inflammation in man. ×1400.

clear-cut L.E. phenomenon. Lesions 4 and 5 were then modified as follows: In lesion 4 cover-slips were removed at 4, 7, 9.5, 11.75 and 13 hours. The L.E. plasma was introduced at 9.5 hours of inflammation, and the next preparation at 11.75 hours again failed to show a clear-cut L.E. phenomenon; thereupon two more drops of the L.E. plasma were added to the same lesion and the next preparation, *i.e.* at 13 hours of inflammation or 1.25 hours after the second application of the L.E. factor presented the L.E. phenomenon. In lesion 5, cover-slips were removed at 4, 6.75, 9.5, 11.25, 12 and 13 hours. The L.E. plasma was introduced at 9.5 hours of inflammation and the next preparation at 11.25 hours again failed to show a clear-cut L.E. phenomenon; thereupon additional L.E. plasma was applied to the same lesion and the next preparation, *i.e.* at 12 hours of inflammation or 45 minutes after the second application of the L.E. factor presented the L.E. phenomenon.

Lesions 6 to 10 comprised the control lesions; in lesions 6 to 9 cover-slips were removed from the second to the twenty-fourth hours of inflammation at intervals of a few hours. In lesions 6 to 9 diphtheria toxoid alone served as the inoculant. In control

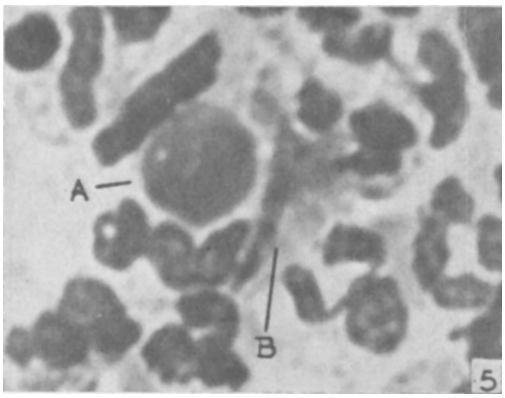

FIG. 5.
Lesion 1. Experimental. (A) Free partially lysed nucleus. (B) Nuclear debris. Note "rosette" of surrounding neutrophilic leukocytes. 11 hr of inflammation in man. ×1700.

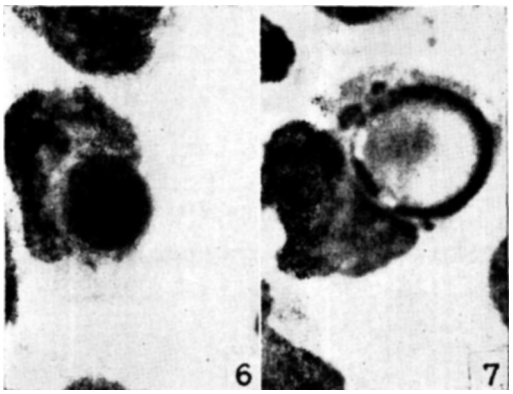

FIG. 6.
Lesion 9. Control. Lymphocyte with ingested nuclear mass. 12 hr of inflammation in man. ×2000.

FIG. 7.
Lesion 9. Control. Histiocyte (macrophage) with ingested nuclear mass. 16.5 hr of inflammation in man. ×20000.

lesion 10 in addition at 7 and 9 hours of inflammation normal heparinized plasma (in place of the L.E. plasma of the experimental lesions) was added to the lesion from which cover-slips were removed at 2.5, 5, 7, 9, 11.75 and 13.25 hours.

In the experimental lesions 1, 2, 4 and 5 then the L.E. phenomenon appeared in the inflammatory exudate in from 2 to 3.5 hours after introduction of the L.E. factor plasma. Because of the dynamics of the window technic the progressing cytolytic processes were as prominent as were the L.E. cells themselves. In the original description(1) the L.E. cell was usually a mature neutrophilic polymorphonuclear leukocyte which had either ingested free exogenous nuclear masses of homogenous, purple chromatin material or had suffered partial autolysis of its own nuclear lobes. The window technic afforded a fuller exposition of the cytolytic phenomenon than previous *in vitro* technics have offered.

The first structural changes observed in the exudative neutrophilic leukocytes exposed to the necrotizing factor of the L.E. plasma were apparent in the neutrophilic granules, the cell body, and polymorphous nucleus. The changes in the granules were non-specific swelling and increased basophilia together with clumping and loss through cytoplasmic budding. Changes in the cell body were likewise largely non-specific, its disintegration was accompanied by vacuolation and cytoplasmic budding or shedding. The nucleus was relatively more resistant to the necrotizing factor and nuclear lysis proceeded in stages. Unlike the pyknosis of ordinary neutrophilic degeneration in the controls which was accomplished by shrinkage and clumping of nuclear lobes, leading to formation of rounded hyperchromatic nuclear remnants, karyolysis of neutrophils in the experimental windows was initiated by early swelling of one or more of the individual nuclear lobes. Swelling of the individual lobes was soon followed by marked changes in the chromatin-parachromatin pattern. The ordinary coarse, chromatin blocks formed a smooth homogenous mixture with the paler parachromatin; gradual effacement of the lobar chromatin pattern than resulted. Commonly such swollen, homogenous waxy nuclear masses were set free into the exudative fluids at this stage of karyolysis and ingested by still viable neutrophilic leukocytes, thus producing one form of the L.E. cell. Not too infrequently lobar lysis proceeded within the originally affected nucleus. Further lysis was signaled by lobar imbibition of fluid which not only brought about further swelling but also resulted in progressively paler staining of the affected structures. The central portion of the lobe became vacuolar, the chromatic mixture was pushed to the periphery with the formation of ring-like lobes, (Fig. 1A) a finding previously described as "ring nuclei" by Ash and Spitz(9) in the polymorphonuclear leukocytes of stools in the Shigella infections.

The second group of structural changes observed were associated with dissolution of the affected neutrophils freeing the affected lobes undergoing varying degrees of lysis. Thus free, partially lyzed nuclei and/or nuclear lobes were scattered among the exudative cells (Fig. 2). The possibility that small portions of the toxic cytoplasm were occasionally still attached to the lysing nuclear material

9. Ash, S. E., and Spitz, S., Pathology of Tropical Diseases, W. B. Saunders Co., Philadelphia, 1945.

could not be ruled out. The relatively intact neutrophilic leukocytes were apparently attracted to the nuclear and lobar bodies.

The third stage in the development of the ultimate inclusion phenomenon was the ingestion of the partially lysed swollen lobes and nuclei presenting partial or complete homogenization of their chromatin and other nuclear materials (Fig. 1B, 3, 4).

The last stage in the development of the inclusion phenomenon was the further digestion by the relatively intact neutrophils of the ingested lobar and nuclear material. The phagocytosed chromatic portions became progressively paler until affinity for the basic dyes was finally lost. There remained a large empty vacuole with a faint lavender pellicle marking the site of the former nuclear or lobar membrane.

Variants were noted in the interplay of the 4 stages. Nuclear lysis and degradation might proceed to the very late stages while the nuclei or their lobes were still free in the exudate. Phagocytosis at this later stage resulted in phagocytosis of "ring lobes" or "ring nuclear remnants." As Hargraves *et al.*(1) originally suggested, there was some evidence that all four stages of karyolysis could go on to completion within the original neutrophilic leukocyte, the individual lobes differing as to their susceptibility to the L.E. necrotizing factor. Thus the lobes were observed in different stages of lysis within a single cell (Fig. 1A). Homogenization of the nuclear material at times preceded the swelling of the lobes. Occasionally the nuclear material was found in the form of numerous small, partially lysed nuclear fragments and bodies much smaller than individual lobes pointing to fragmentation of the typical L.E. bodies prior to (Fig. 2) or subsequent to ingestion (Fig. 3). Host cells may fare quite well (Fig. 3); at other times they too seemed to be deleteriously and rapidly affected either by the necrotizing factor of their general environment or by the fact of ingestion of an affected nuclear lobe (Figs. 1B, 4).

"Rosettes" or polymorphonuclear leukocytes clumped around nuclei (Fig. 5A) or amorphous masses (Fig. 5B) of nuclear debris as described by Haserick and Bortz(3) were also present *in vivo*. However, the masses appeared to be purple and not acidophilic as originally described. The central mass may resemble (Fig. 2) the stringy desoxyribose nucleoprotein studied by Sherry, Tillett and Christensen(10) in purulent pleural exudates.

We were not able to find L.E. cells in the numerous windows obtained from the control lesions. In lesion 9 in which diphtheria toxoid alone served as the inoculant at the 12th hour of inflammation, the lymphocytes were at times phagocytic for nuclear debris (Fig. 6). At 16.5 hours of inflammation in the same lesion an occasional small histiocyte (macrophage) had ingested a round nuclear remnant (Fig. 7). In this instance there had occurred some intracellular homogenization of the phagocytosed nuclear material. Although uncommon in the control material, such a finding as illustrated in Fig. 7 depicting as it does the ability of the histiocyte to bring about intracellular homogenization of phagocytosed nuclear material may afford a clue as to the source of the enzymes responsible for the nucleic acid depolymerization, a depolymerization claimed for systemic lupus erythematosus by Klemperer and his associates(11). Such ingested nuclear materials were never observed within the cell-bodies of neutrophilic leukocytes in the control lesions and conversely histiocytes rarely became the host L.E. cells in the experimental lesions.

The cover-slip preparations obtained subsequent to the production of the L.E. phenomenon contained few or no L.E. cells. In the 5 months since these experiments were completed no evidence of acute disseminated lupus erythematosus has been noted in the two human volunteers. We advise against repetition of these experiments in man until such a time as it is possible to utilize active necrotizing L.E. factor plasma known to be free of any contamination with the virus of homologous serum hepatitis. We are indebted to Dr. R. R. Margulis for his generous help

10. Sherry, S., Tillett, W. S., and Christensen, L. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 179.

11. Klemperer, P., Gueft, B., and Lee, S., *J. Mt. Sinai Hosp.*, 1949, v16, 61.

proffered in these experiments.

Summary and conclusions. The L.E. cell phenomenon has been produced experimentally in the skin of two normal human volunteers following inoculation of windows with the plasma of a patient with acute disseminated lupus erythematosus. *In vivo,* under the conditions of our experiments, the neutrophilic L.E. cell usually developed as the result of ingestion of other neutrophilic nuclear lobes or nuclei which had undergone a previous partial peculiar lysis. Less commonly the L.E. cell may represent the originally affected neutrophilic leukocyte in which lobar lysis is out of step in the individual nuclear lobes. The experiments described should provide a means of testing the proposed identity of the L.E. cells and the "hematoxylin staining bodies" of the diseased tissues in acute disseminated lupus erythematosus, an identity recently suggested by several workers(11,12).

12. Berman, L., Axelrod, A. R., Goodman, H. L., and McClaughry, R. I., *Am. J. Clin. Path.,* 1950, v20, 403.

Received August 7, 1950. P.S.E.B.M., 1950, v75.

The Antipyretic Effect of Cortisone.* (18166)

LILLIAN RECANT,‖ WALTER H. OTT AND EDWARD E. FISCHEL.
(Introduced by R. F. Loeb.)

From the Department of Medicine, Columbia University College of Physicians and Surgeons, the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital, N. Y., and the Merck Institute for Therapeutic Research, Rahway, N. J.

In various febrile illnesses, clinical improvement following the use of adrenocorticotrophic hormone (ACTH) or cortisone is usually associated with a decline in temperature to normal or near-normal levels(1). The decline occurs together with a striking diminution in the clinical and laboratory evidences of inflammatory activity. There have been several reports illustrating an antipyretic effect of ACTH without appreciable alteration in the course of the underlying disease. Thus, in pulmonary tuberculosis(2), pneumococcal pneumonia(3) and subacute bacterial endocarditis(4), the administration of ACTH was followed by apparent clinical well-being and rapid defervescence of fever, but cultures of the blood or sputum continued to be positive. Indeed, in one instance extension of the tuberculous process has been noted to occur during ACTH administration. In view of these observations, it became of interest to determine whether an antipyretic effect of cortisone could be experimentally demonstrated.

Exp. I. Thirty chinchilla rabbits of about 2 kg body weight were divided into two groups of equal number. Each animal of one group received an initial injection of 10 mg of cortisone acetate† intramuscularly, fol-

* This work is supported in part by the Helen Hay Whitney Foundation and the Masonic Foundation for Medical Research and Human Welfare.

‖ Fellow of the Commonwealth Fund.

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2. Freeman, S., Fershing, J., Wang, C. C., and Smith, L. C., *Proc. of the First Clinical ACTH Conference,* p. 509, (Feb.) 1950, The Blakiston Co., Philadelphia, Toronto.

3. Finland, M., Kass, E. H., and Ingbar, S. H., Ibid.(2), p. 529.

4. Bunim, J. J., McEwen, C., Baldwin, J. W., and Kuttner, A. G., *Proc. Amer. Heart Assoc. Scient. Session,* June 22, 1950, San Francisco.

† Obtained from Merck and Company on recommendation of the Committee on Cortisone of the National Academy of Sciences, through funds allocated by the U. S. Public Health Service.