REACTION OF LUPUS ERYTHEMATOSUS SERUM WITH NUCLEI OF CIRCULATING AND NON-CIRCULATING CELLS

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[WITH SPECIAL PLATE]

Friou (1957) was the first to demonstrate that tissue cells brought in contact with serum from patients suffering from disseminated lupus erythematosus (D.L.E.) and exposed subsequently to fluorescein-conjugated anti-human-globulin (A.H.G.) serum showed specific nuclear fluorescence. This application of Coons's fluorescent antibody technique has been further studied by Friou, Finch, and Detre (1957, 1958) and by Holborow *et al.* (1957). In addition, it has been demonstrated by Mellors *et al.* (1957) and Holman and Kunkel (1957) that the inclusion bodies in L.E. cells fluoresce on exposure to fluorescein-conjugated A.H.G. serum.

These results provide strong evidence for the belief that the serum in D.L.E. contains a globulin with an affinity for the nuclei of tissues and leucocytes. It has been suggested that this globulin is identical with the factor responsible for the formation of L.E. cells. Friou (1958) has shown that the capacity of serum from cases of D.L.E. to induce both phenomena is abolished by absorption of the serum with nucleohistone.

In the studies referred to attention has been mainly directed to the reaction of D.L.E. serum with tissues derived from other sources than patients with D.L.E. With the exception of reports on fluorescence of L.E. cells exposed to fluorescein-conjugated A.H.G. serum, no comment on the effect of D.L.E. serum on D.L.E. tissue has been found in the literature. It seemed important to determine whether D.L.E. tissue differed from normal tissue on exposure to D.L.E. serum, and in this report the results of such a study are presented.

Materials

Human Sera.—Serum was obtained from eight patients suffering from D.L.E. In all cases L.E. cells had been found on more than one occasion. All patients but one (Case 3) were receiving prednisolone or cortisone at the time of investigation, the daily dose being shown in the Table. Control sera were obtained from healthy members of the hospital staff. Sera were not heated before use, and when necessary were stored at -15°C . In a preliminary experiment it was found that sera retained their reactivity on storage for periods of at least six weeks.

Leucocytes.—Blood was obtained by ear-prick, films being spread on microscope slides as for routine haematological examination. Films were not fixed, and were used on the day of preparation.

Skin.—Portions of skin were obtained by biopsy from two patients (Cases 4 and 7) and from two healthy donors of corresponding blood groups. One patient (Case 4) and one control were of blood group O, and all sera were tested on sections from these individuals. Within one minute of biopsy the skin was frozen in test-tubes dipped in solid

CO_2 -acetone mixture. Sections were cut at $4-6\ \mu$ in a cryostat at -20°C . The sections were then transferred to slides, thawed, dried by fan, and used on the day of biopsy.

Buccal Mucosa.—Squamous epithelium was obtained by gently scraping the buccal mucous membrane with a metal spatula. The scrapings were transferred to slides and allowed to dry at room temperature. As with blood films and tissue, the cells were examined on the day of preparation.

Fluorescein-conjugated Anti-human-globulin.—Commercial A.H.G. (Coombs's) serum was conjugated with fluorescein isocyanate by the method of Coons and Kaplan (1950). The conjugated serum was absorbed with sheep-liver powder to reduce non-specific precipitation in tissues.

Methods

Sections of tissue or preparations of cells were exposed to human serum in a moist atmosphere at 37°C . Except when otherwise stated, the time of incubation was 30 minutes and the sera were undiluted. The preparations were then washed in two changes of phosphate-saline buffer at pH 7.0 and covered with a few drops of undiluted fluorescein-conjugated A.H.G. serum. After 30 minutes at room temperature the preparations were again washed in buffered saline and mounted in buffered glycerol at pH 7.0. Cover slips cut from OY12 (ultraviolet-absorbing) glass were used.

The specimens were examined in the fluorescence microscope as described by Alexander (1958), with the exception that plain glass microscope slides were used in the present work. When required the preparations were photographed and the field plotted with the aid of the graduated mechanical stage of the microscope. The cover slips were floated off and the tissue fixed in undiluted methyl alcohol. The preparations were then stained with Giemsa's stain and mounted in balsam. Fields were identified and rephotographed using a source of visible light.

Results

In most experiments the presence or absence of nuclear fluorescence was readily recognized, and examples are shown in Figs. 1, 2, 3, and 5 on the Special Plate. Figs. 4 and 6, which correspond to Figs. 3 and 5 respectively, are included as an aid to identification of individual nuclei.

Nuclear fluorescence as seen in Figs. 2 and 5 was observed only in tissues or cells which were pretreated with L.E. serum. Pretreatment with serum from healthy individuals or the direct application of fluorescein-conjugated A.H.G. serum to the preparations invariably gave negative results.

Table Showing Nuclear Fluorescence in Tissues and Cells Pretreated with Sera from D.L.E.

Serum from Case No.	Reaction of Serum with				Daily Dose of Steroid (P = Prednisolone) (C = Cortisone) (mg.)
	Autologous Leucocytes	Group O Leucocytes (Healthy Donor)	Skin from D.L.E. (Group O)	Skin from Healthy Group O Individual	
1	—	+	—	+	62.5 (C)
2	—	+	—	—	5.0 (P)
3	+	+	—	+	—
4	+	+	+	—	15.0 (P)
5	+	+	—	—	5.0 (P)
6	+	+	+	+	50.0 (C)
7	+	+	+	+	5.0 (P)
8	+	+	—	+	30.0 (P)

+ = Nuclear fluorescence present. — = Nuclear fluorescence absent.

The sera from eight patients suffering from D.L.E. were applied to autologous blood films, blood films from healthy donors, sections of skin from a patient with D.L.E., and sections of skin from a healthy individual. All but the autologous cells were obtained from individuals of blood group O. The results of this experiment are shown in the Table. Leucocytes from the healthy subject pretreated with all sera tested showed nuclear fluorescence when subsequently exposed to fluorescein-conjugated A.H.G. serum. Nuclei of autologous leucocytes fluoresced on pretreatment with six of the eight sera. Nuclei in sections of skin from a healthy subject

fluoresced when pretreated with six of eight sera, but nuclei in skin from a patient with D.L.E. (Case 4) showed fluorescence when pretreated with only three of the eight sera.

In view of the apparent diminution of affinity of L.E. serum for nuclei in L.E. tissue, skin obtained from another patient (Case 7) was examined. Pretreatment of this material with autologous serum gave an equivocal result, as shown in Fig. 7 on the Special Plate. Although diffuse fluorescence of epidermal cells was seen, selective fluorescence of nuclei did not occur. This finding cannot be explained by failure of autologous tissue and serum to react, as nuclear fluorescence was observed when tissue from Case 4 was pretreated with autologous serum. It was established by two experiments that the serum from Case 7 had not lost its potency. The serum was used to pretreat leucocytes from a healthy individual, and nuclear fluorescence was observed when the serum was diluted to 1:4,000. Undiluted serum produced nuclear fluorescence when applied to the cells for one minute.

The necessity for using fresh material in this type of investigation has been stressed by Holborow *et al.* (1957). The difficulty of obtaining regular supplies of tissue by biopsy is considerable, and it was decided to examine cells obtained by scraping the buccal mucosa. These could be taken as representative of fixed tissue cells as distinct from the circulating leucocytes. However, the nuclei of squamous cells from healthy individuals or patients with D.L.E. exposed to D.L.E. serum failed to show fluorescence on subsequent treatment with fluorescein-conjugated A.H.G. serum (Special Plate, Figs. 9 and 10). When, however, the time of exposure to D.L.E. serum was extended to 1½ hours nuclear fluorescence was observed in cells which had disrupted. As there was also evidence of diffuse bright fluorescence in structures thought to be of cytoplasmic origin, no conclusion can be drawn from these experiments. Further investigation with specific counter-stains for nuclear material is planned.

Discussion

These results confirm the findings of other workers that the nuclei of cells exposed to serum from patients suffering from D.L.E. adsorb a globulin detectable by application of fluorescein-conjugated A.H.G. serum. This property of L.E. serum is not apparently influenced by administration of steroid hormones.

It has been shown that the phenomenon is readily demonstrable in unfixed blood films. This technique, which avoids the need for preparing films from "buffy coats," would be useful in any large-scale investigation of the phenomenon in other diseases.

The observation that nuclear fluorescence is not present in tissue from cases of D.L.E. exposed directly to fluorescent A.H.G. serum is in agreement with the findings of Mellors *et al.* (1957) in the D.L.E. kidney, and supports the hypothesis that absorption of L.E. globulin on nuclei occurs only *in vitro*. The fact that tissues and leucocytes derived from patients with D.L.E. appear to be less susceptible to uptake of globulin from L.E. serum may be explained by the relatively small number of patients available for study, but the anomalous behaviour of the serum from Case 7 toward nuclei in autologous skin and the nuclei in the skin of another patient is of interest. Recent experiments, which are still incomplete, suggest that the time of incubation of tissue and L.E. serum may have a considerable influence on the intensity of nuclear fluorescence and that individual sera and tissues may differ appreciably in the rate of reaction.

The results of experiments with squamous epithelium suggest that intact tissue cells resist invasion of the specific globulin in L.E. serum. It has been noted in examination of thick sections of skin that nuclear fluorescence may be apparent in the superficial layer, but not in deeper layers. Leucocytes, however, seem to be extremely susceptible to the specific globulin in L.E. serum, and this susceptibility may well be associated with their natural property of phagocytosis.

Summary

With the use of Coons's fluorescent antibody technique it has been confirmed that sera from patients suffering from D.L.E. contain a globulin which combines with nuclei of tissue cells and leucocytes. In a study of sera from eight cases of D.L.E. it was observed that nuclei of leucocytes from healthy individuals showed the greatest affinity for the specific globulin. No evidence of uptake of globulin in tissue from D.L.E. was found *in vivo*, but *in vitro* such tissue had less affinity for the specific globulin than tissue from healthy subjects. Nuclei of intact squamous epithelial cells pretreated with D.L.E. serum did not fluoresce on exposure to fluorescein-conjugated anti-human-globulin serum.

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LIQUID PARAFFIN AS A CAUSE OF OIL ASPIRATION PNEUMONIA

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Liquid paraffin has, for various reasons, been under suspicion at different times since it began to be used to any extent medicinally at the beginning of this century. It has been charged with preventing intestinal absorption of food in general, and of vitamin A and its precursor, carotene, in particular, and on this latter count there is some justification for the charge. Since liquid paraffin is a mixture of liquid hydrocarbons obtained from petroleum, the possibility that it might contain carcinogenic fractions has been investigated (Boyd and Doll, 1954). At one time there was a practice of instilling liquid paraffin into the peritoneal cavity with the avowed object of preventing adhesions. The result has been to produce, in a number of cases, an oil granuloma of the peritoneum (Bennett and Collins, 1952). Probably, however, the most common danger arising from the medicinal use of liquid paraffin is the production of oil aspiration pneumonia.

The pneumonia which results from aspiration of oils and fats has been dealt with extensively in American literature, but has not received the same attention in Britain, although its incidence in this country may not be any less. The diagnosis of the condition in life is not

W. R. M. ALEXANDER AND J. J. R. DUTHIE: REACTION OF L.E. SERUM WITH CELL NUCLEI

FIG. 1.—Blood film from healthy donor pretreated with normal serum. No nuclear fluorescence.

FIG. 3.—Skin from healthy donor pretreated with normal serum. No nuclear fluorescence.

FIG. 4.—Field shown in Fig. 3 stained with Giemsa.

FIG. 2.—Blood film from same donor as Fig. 1 pretreated with L.E. serum. Nuclear fluorescence present.

FIG. 5.—Skin from healthy donor pretreated with L.E. serum. Nuclear fluorescence present.

FIG. 6.—Field shown in Fig. 5 stained with Giemsa.

FIG. 7.—Skin from D.L.E. pretreated with autologous serum (Case 7). Diffuse fluorescence in epidermal cells, but nuclei not selectively involved.

FIG. 9.—Buccal mucosa from healthy individual pretreated with L.E. serum. No nuclear fluorescence. (Brightness of centre of field is due to optical properties of dark-ground condenser.)

FIG. 8.—Field shown in Fig. 7 stained with Giemsa.

FIG. 10.—Field shown in Fig. 9 stained with Giemsa.