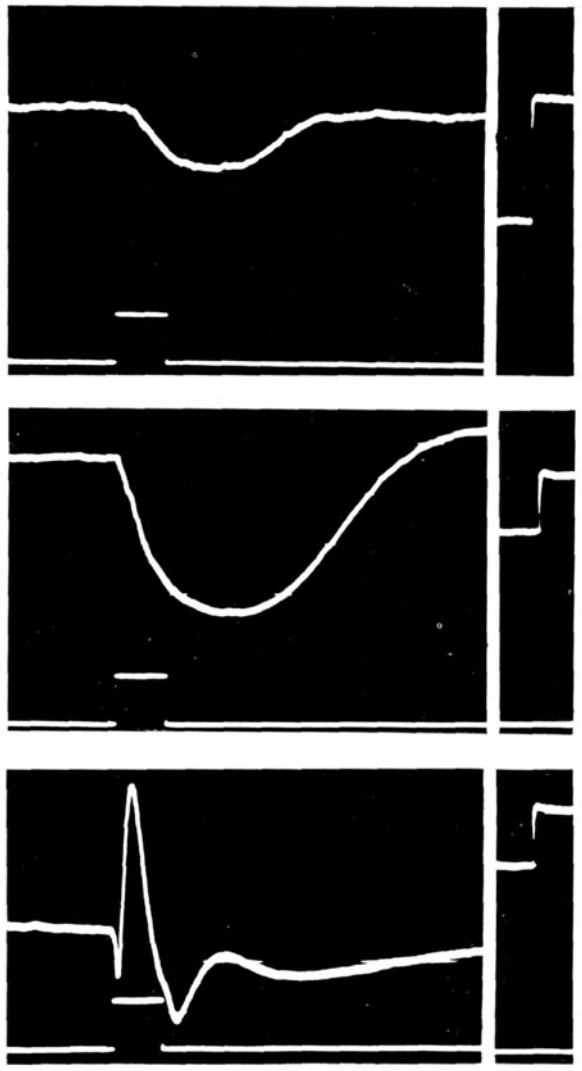

ERG der isolierten umströmten Kaninchennetzhaut. Umströmung mit a, modifizierter Tyrode-Lösung (20°C), b, Plasma-Lösungsgemisch (20°C), c, wie b, aber bei 30°C. Reizintensität 0,2 lx, Reizdauer 1 sec. Eichung (rechts): 130 μV.

stark vergrössert und erreicht eine hohe Stabilität (Figur b). Die für ein normales ERG charakteristische positive b-Welle ist dagegen erst dann voll ausgebildet, wenn Präparat und Plasma-Tyrodelösung auf 30°C gehalten werden (Figur c). Wurde die Umströmung bei 30°C ohne Plasma-Zusatz durchgeführt (ähnlich der Methode von AMES und GURIAN[4]), so konnte nur in einzelnen Fällen mit hohen Reizintensitäten eine kleine und überdies instabile b-Welle registriert werden, ebenso bei 20°C und Plasmazusatz. Aus der erfolgreichen Verwendung artunspezifischen Plasmas ergibt sich die praktische Konsequenz, stabile ERG auch von isolierten Netzhäuten kleiner Warmblüter zu erhalten, deren Plasmavolumen sehr gering ist.

Durch Variation der Milieubedingungen können verschiedene Komponenten des ERG getrennt untersucht werden. So ist von der isolierten, mit Plasma-Tyrodelösung umströmten Kaninchenretina bei Raumtemperatur eine stabile negative Komponente abzuleiten, während bei Thermostasierung auf 30°C die positiven ERG-Komponenten vorherrschen. Andererseits muss mit differentem Verhalten der einzelnen Warmblüternetzhäute gerechnet werden, da das ERG der menschlichen Netzhaut unter gleichen Umströmungsbedingungen schon bei Raumtemperatur eine positive b-Welle aufweist[5].

Summary. The isolated perfused retina of the rabbit produces a normal electroretinogram only if the perfusion fluid contains blood plasma and is kept at 30°C. At a temperature of 20°C, the electroretinogram consists mainly of a negative component (P III) which is greatly diminished in absence of plasma in the perfusion fluid.

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Physiologische Institute der Karl-Marx-Universität Leipzig (Deutschland) und der Universität Wien (Österreich), 24. Mai 1965.

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STUDIORUM PROGRESSUS

In vitro and in vivo Interaction of Nuclear Antibodies with Corresponding Antigens[1]

One of the interesting problems in the study of diseases characterized by the presence of circulating auto-antibodies is concerned with the direct demonstration of antibody and its effects in the diseased or target tissues[2–6]. If successful, this approach might aid in the search for knowledge of the role of these antibodies in autoimmune disease.

In the course of efforts to characterize tissues of patients with systemic *Lupus erythematosus* (SLE) by direct immunofluorescent staining methods[7,8] for tissue-fixed γ-globulin, we observed that freshly prepared smears of buccal mucosal cells of such patients exhibited no specific nuclear reaction; nor did sections of needle biopsy of liver which had been immediately frozen[4]. In contrast, it was found that films of leucocytes from the same cases gave definite nuclear staining. The leucocyte films were prepared from the buffy coat of oxalated venous blood – a procedure requiring a minimum time interval of approximately 15 min from withdrawal of blood to finished (dried) preparations. The obvious disadvantage of the latter procedure was that the leucocyte preparations could be tested only after in vitro exposure of the cells to plasma proteins and oxalates.

To circumvent this undesirable feature the white blood cell preparations were made by the finger-prick technique. Such blood films were accomplished within 5–10 sec. In addition, films of liver cells from a selected case (PAT)[4] were prepared at biopsy from the same tissue used for the preparation of previously mentioned frozen sections.

A small thread of liver was rubbed lightly with the aid of forceps on several glass slides within about 5 sec after biopsy. These smears were then quickly dried under a current of air. Direct staining for the presence of γ-globulin was carried out with a conjugate of fluorescein isothiocyanate and ammonium sulfate-precipitated globulin of a goat immunized with human γ-globulin. Dried smears were dipped in phosphate-buffered (pH 7.2) saline for 5 min and, following acetone-fixation and airdrying, were flooded with the conjugate for 1 h. The slides were rinsed once again and examined under phosphate-buffered glycerol by means of a Zeiss microscope equipped with a 35 mm camera. Positive reactions were scored as 1+ to 4+ according to the intensity of specific staining.

Interpretation of the leucocyte staining was complicated by two factors: (1) The concentration of leucocytes in the blood was inordinately low – a fact not infrequently observed in SLE; hence, these cells were widely scattered in the films. (2) Non-specific cytoplasmic staining tended to obscure the specific nuclear staining of the leucocytes.

Nuclear staining (intensity of 1+ or more) was observed in rare isolated leucocytes in a proportion amounting to less than 0.1% of all cells counted on single slides. Most blood films, however, showed no nuclear staining whatever. In contrast, liver biopsy films revealed some cells with nuclear staining scattered singly and in clusters, in several slide preparations. The exact identity of the stained cells was not established. The majority of such reactive cells exhibited cytoplasmic staining similar to that seen in perisinusoidal cells in liver sections described by BEUTNER et al.[4]. A few of the stained cells in liver films, in addition showed a distinct nuclear reaction. The nuclear staining of these cells ranged in appearance from granular (1+) in some to smooth and moderately strong (3+) in others. The serologic specificity of the immunofluorescent reaction was confirmed by negative results obtained with similar slides treated with γ-globulin-neutralized conjugate.

In order to investigate the possibility that the presence of nuclear-specific globulin detected by direct staining was due to absorption of antibody from serum in vitro, carried with and by the cells to the slides, experiments were performed by the indirect (two-step) staining method to determine the influence of time on the nuclear reaction. The procedure was carried out with undiluted sera containing anti-nuclear antibodies and unfixed frozen sections of non-SLE human liver and normal monkey liver. With the aid of a stop-watch, duplicate liver sections were exposed to serum for intervals of time ranging from less than 2 sec up to 60 min. The serum was applied with a medicine dropper equipped with a rubber bulb and, at pre-set intervals, the sections were rinsed precisely and thoroughly under a forced stream of buffered saline and dipped in buffered saline with agitation for 2 min. The slides were fixed, dried and exposed to the anti-human conjugate for 1–1 1/2 h, followed by washing and by extracting for at least 30 min in 50% citrate-buffered glycerol for reduction of non-specific staining[4]. Also, serum titrations were done by exposed fixing monkey liver sections to serial dilutions of serum for 1 h and completing in a similar manner to that employed in the previous experiment. Readings were done by a blindfold method, usually independently by 3 different observers.

It was observed (Table I) under these partially simulated conditions that a typically varying staining pattern of specific nuclear fluorescence with different degrees of intensity was obtained with human liver in relation to increased serum-exposure intervals. While exposure of liver to non-SLE (negative) serum for 1 h yielded negative results, exposing liver sections to the positive serum for an interval slightly longer than 1 sec resulted in distinct nuclear staining. The pattern and intensity of nuclear staining progressed from a weak, granular (1+) reaction for the shortest interval, through an intermediate stage for increasing exposure times, to a bright (3+) smooth appearance of the nuclei for the longest period of exposure of tissue to serum. The appearance of many of the nuclei representing intermediate intervals of exposure suggested a correspondence to nuclear staining observed in direct liver films of the SLE patient. The distribution and proportion of stained nuclei obtained by the indirect staining method, however, were in every way more uniform and greater respectively, than were these same two parameters in the SLE peripheral blood and liver films studied by the direct method. The general staining pattern of SLE sera with monkey liver was similar to that obtained with human liver. In addition, it was observed with SLE sera and monkey liver (Table II) that the rate of nuclear staining was roughly proportional to the serum titers.

Our experimental results reveal that the nuclear reaction can be obtained in less than 2 sec and are in agree-

Table I. Nuclear reaction of SLE (PAT) serum with human liver

Time	Staining reaction	
	Intensity	Appearance
< 2 sec	+	Granular
5	+	Granular
10	+	Smooth-Granular
20	+	Smooth-Granular
60	+	Smooth-Granular
2 min	+	Smooth
5	++	Smooth
10	++	Smooth
30	+++	Smooth
60	+++	Smooth

[1] This investigation was supported in part by a training grant (2E-130) from the U.S. Public Health Service. Taken in part from thesis (G.W.B.) submitted to the Faculty of the Graduate School of Arts and Sciences of the University of Buffalo (N.Y., USA) in partial fulfillment of the requirements for the degree of Doctor of Philosophy (1962). Present address (G.W.B.): Department of Pathology, State University of New York at Buffalo.

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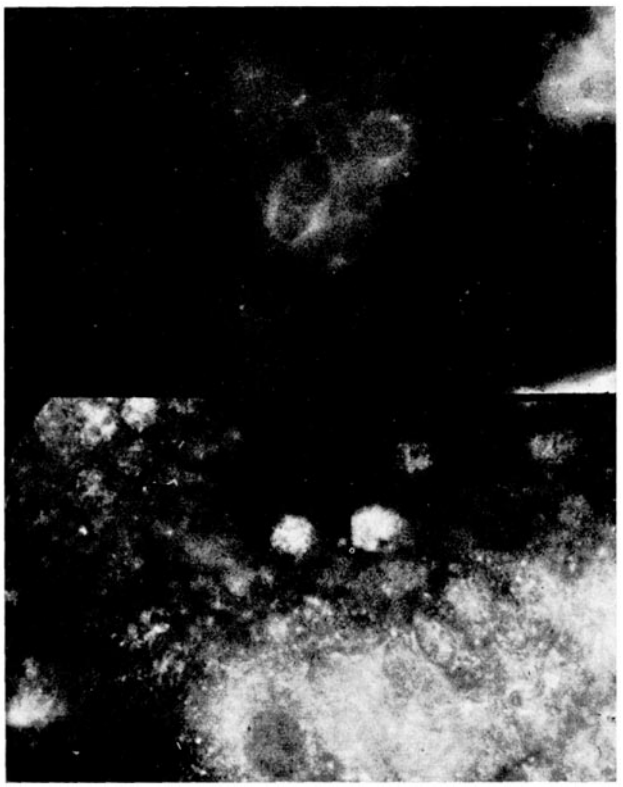
Table II. Nuclear reaction of SLE sera with monkey liver

Patient	Serum-titer	Reaction time[a] < 2 sec	5 sec	10 sec	20 sec	1 min	10 min	1 h
GEO[b]	–	–	–	–	–	–	–	–
AND	1:150[c]	wk +	wk +	+	+	+++	++++	++++
HUN	1:150	±	wk +	+	+	++	+++	++++
ORZ	1:500	+	++	++	+++	+++	++++	++++
STE	1:1000	++	+++	+++	+++	+++	++++	++++
PAT[d]	1:1000	+	+	+	+	++	++	++++

[a] Obtained with undiluted sera. [b] Discoid lupus. [c] Variable titer: 150–300. [d] Tested at an earlier date.

ment with the observation of MAYER and HEIDELBERGER[9] that the liquid precipitin reaction of pneumococcus polysaccharides with homologous antisera at 0°C was almost complete in less than 3 sec. The granular-smooth transition which characterized nuclear staining observed in our time study suggests that structural features of the nucleus on or near its surface prevent complete formation of visible precipitate in briefly exposed preparations. Such inhibiting features might include surface pits and crevices which provide unfavorable spatial orientation of antigenic sites, thus presenting a situation in which limited access by antibody is overcome only through increased exposure of the nucleus to the serum. As a further complication, the bases of DNA which may participate as antigenic determinants along with other chemical groupings in nucleoprotein, are concealed within the structure of the Watson-Crick double helix, rather than being surface elements.



Viable cells are resistant to penetration by or conc en tration of homologous antibody as demonstrated by HIRAMOTO et al.[10]. HOLMAN et al.[11] have noted that antinuclear factors do not penetrate 'intact' cells. Indeed, in our studies of SLE the vast majority of cells in liver films as well as those in films of peripheral blood, exhibited no evidence by direct immunofluorescent staining of being coated by γ-globulin or antibody. On this basis, it would appear that only highly susceptible cells – that is, cells which have lost their 'intactness' through traumatizing experiences such as mechanical injury, disease, or through mechanisms incidental to physiological exhaustion or aging – were penetrated by antibody and these cells constituted a relatively small proportion of the total population. Such an explanation would, of course, include the possibility that nuclear-positive cells observed in liver films were parenchymal or other liver cells damaged in the process of preparing the films and/or that susceptible leucocytes spilled onto the slides from the liver vascular system.

The results of our time study also are in agreement with the generally accepted fact that antigen-antibody reactions for the most part – especially in the primary stage – are quite rapid and governed in their course only by general physico-chemical laws as applied to interaction of macromolecular systems; and hence introduce doubt that direct nuclear staining obtained in this study represents true in vivo activity of antibodies. On the other hand, our observation of random distribution of nuclear-positive cells and cell-clusters in blood and liver preparations would indicate that although only a small number of cells were affected, due care ought to be exercised in attempts to exclude circulating antibody(s) as playing a role in determining the course of SLE. It would seem reasonable to suppose that circulating antinuclear factors, while apparently unable to gain entry into the vast majority of circulating cells, would have less difficulty gaining access to the nuclei of weakened, dead or dying cells which arise through the usual physiologic attritions suffered by circulating cell populations. Complexes of nuclei or nuclear components and antibody may thus be formed continuously and might contribute in some undetermined manner to the production of exacerbations of certain features of SLE such as nephritis, hepatitis, pericarditis, etc. In this connection it may be pertinent that soluble antigen-antibody aggregates have been shown to be pathogenic

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in experimental animals[12]. In addition, it has been noted that reduced levels of serum complement were observed in patients with active SLE[13], supporting the thesis that antigen-antibody complexes which absorb complement are being formed. Experiments designed to demonstrate direct nuclear staining in the absence of serum titers or, in any case, to demonstrate circulating and/or tissue-sequestered complexes of antibody and nuclear fragments would provide an important step toward the solution of the problem.

Zusammenfassung. Es wird mit indirekter Immunofluoreszenz gezeigt, dass Leberzellkerne mit Antikörpern bedeckt werden können, wenn sie systemischem *Lupus erythromatosus*-Serum (SLE) für weniger als 2 sec ausgesetzt werden, und dass die Färbungsintensität vom Titer des Antikörpers abhängt.

Diese Ergebnisse deuten darauf hin, dass das in SLE-Geweben durch direkte Immunofluoreszenz nachgewiesene nukleare γ-Globulin hauptsächlich nukleare post-Biopsiereaktionen wiederspiegelt und weniger in vivo-Reaktionen, und dass in beiden Fällen nur abgestorbene oder nicht intakte Zellen vom Antikörper durchdrungen werden.

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E. H. BEUTNER, and E. WITEBSKY

Departments of Pathology, Medicine, Bacteriology, and Immunology, State University of New York at Buffalo (USA), February 22, 1965.

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CORRIGENDA

In Experientia *21*, fasc. 5, p. 290 (1965) the heading of the paper by T. KEMÉNY, GY. SIMON, and MAGDOLNA BEDÖ reads correctly as follows: *Absorption Tests on Protein-Deficient Rats. Absorption of S^{35}-Methionine and I^{131}-Triolein.* The absorbed material in Figure 1 reads correctly: S^{35}-methionine and in Figure 2 I^{131}-triolein.

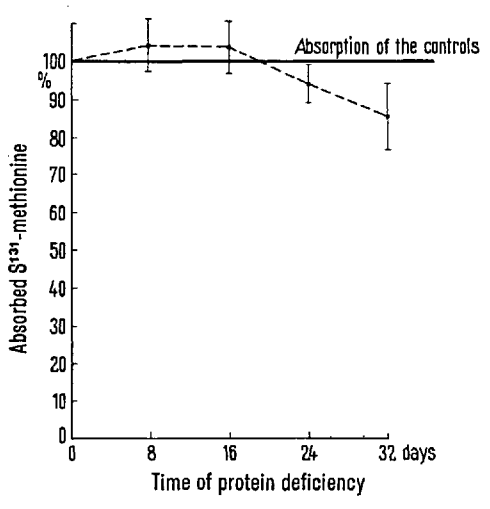

Fig. 1. S^{35}-methionine absorption in protein-deficient rats.

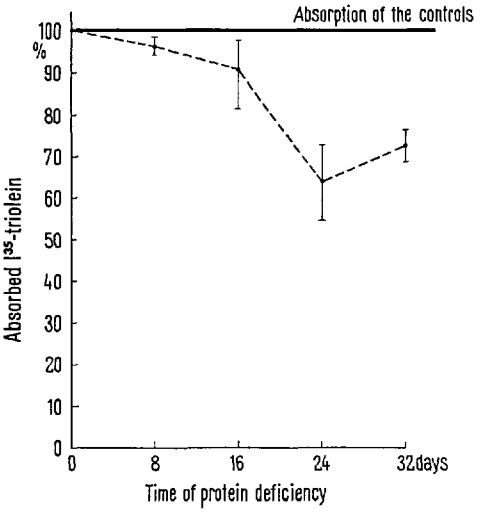

Fig. 2. I^{131}-triolein absorption in protein-deficient rats.

G. BALDRATTI, G. ARCARI, and G. K. SUCHOWSKY: Studies on a Compound Antagonistic to 5-Hydroxytryptamine. Exper. *21*, fasc. 7, p. 396 (1965).

The chemical compound studied was not the 1-methyl-8β-carbobenzyloxy-aminomethyl-10α-ergoline but the

1,6-dimethyl-8β-carbobenzyloxy-aminomethyl-10α-ergoline.