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Lending - Article

mouse lung after inhalation of
umen (*L*), showing a red blood
swollen endothelial lining (*E*),
e recognized in the endothelial
ws only localized swellings (*A1*).
l control mouse lung, showing
othelial cytoplasm (*E*) and the
ling (*A*)

icrotome. These were
100 microscope.
reparations, the capillary
ed or completely absent
ial cells are swollen, their
protoplasmic layer around
etween the indentations,
ped and even crushed.
ar wide open because of
Fig. 1*b*).
s are also considerably
lling of their cells. The
idedly enlarged and show
cytoplasm is often filled
nlarged connective tissue
guished from the swollen
since at many places the
ally separating the endo-
e tissue space, is absent.
e alveolar lining shows
nly occasional, localized
he basement membrane
connective tissue space is

oles intact leucocytes are
ed histiocytes, filled with
nbling the swollen con-
naltered red blood cells.
n, no slide showed any
les.

fflard[4] examined lungs of
r minute silica particles
trachea. They noted the
al cells, the narrowing of
s and the disappearance
between the endothelium
pace. But they first saw
lining. Contrary to this,
old induced in the lungs

of white mice a simultaneous reaction of the endothelial layer and of the connective tissue of the septa, while the alveolar lining scarcely reacted at all. We believe that this fusion of the first two layers is a strong indication of their common origin.

Other evidence points in the same direction. In our contention, any infection or other irritation induces simultaneous morphological and dynamic transformations in the connective tissue and in the endothelial cells. Their appearance shows a regression towards a more primitive state, common to both kinds of cells. But this regression makes possible far more useful defence reactions: such as local phagocytosis or migration towards the threatened area followed by phagocytosis.

Regression is well known as a typical reaction of the organic world. But what may perhaps sound a little more unusual is the idea that this property of all living things may provide an indirect method for studying the histogenesis of certain cells. Besides comparative histology and embryology, it may be possible to verify how morphological differences and specialization of living cells are affected by experimental irritative action. Whenever the tissue reacts with a true regression as a mechanism of mobilization and defence, it becomes possible to watch a previous stage of development which may prove to belong to several tissues, which, in normal circumstances, appear to differ widely.

Our experimental study of lung tissue from white adult mice shows that endothelial and connective tissue cells may re-acquire both the appearance and properties of fibroblasts. This is good evidence for their common mesenchymatous origin. The negative evidence of the very weak reaction of the alveolar lining towards the same experimental irritation is in itself not devoid of interest: indeed, we believe rather that it speaks in favour of the epithelial origin of alveolar cells.

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Antibody Production by Single Cells

Fagreus[1] and others[2,3] have shown that certain tissues from pre-sensitized animals can form antibody *in vitro*. This communication describes a technique whereby antibody production by single cells isolated in microdroplets can be detected. The technique is based on specific immobilization of *Salmonella* serotypes by anti-flagellar antibody. It was observed that single cells from a rat, simultaneously stimulated with two antigens, formed detectable amounts of one or the other antibody.

Two monophasic *Salmonellae* were used: *S. adelaide*, flagellar antigen $H_1 f g$, and *S. typhi*, $H_1 d$. They were maintained at maximum motility by frequent passages through a semi-solid nutrient gelatin agar medium[4]. A formalinized broth culture containing about 10^9 organisms per ml. was used as the antigen. Adult Wistar rats were injected with 0·25 ml. of a mixture of equal parts of both antigens into both hind foot-pads. Usually the animals were given three pairs of injections at three weekly intervals. Three days after the tertiary injections, they were killed by exsanguination under anæsthesia. Both popliteal lymph nodes were removed, pooled, and processed[6] to give dispersed cell suspensions in Earle's saline buffered to *p*H 7·0 with *tris*, and supplemented with 20 per cent normal rat serum. The cells were sedimented by gentle centrifugation, and washed three times to remove free soluble antibody. Single cells were then isolated in microdroplets by a simple modification[4,5] of de Fonbrune's oil chamber method[7]. This consisted essentially of depositing tiny droplets (volume 10^{-7}–10^{-6} ml.) on the surface of a coverslip and immersing them in paraffin oil. The coverslip was then inverted over a chamber filled with oil. The easiest method for preparing droplets containing one cell was to dispense a large number of droplets by free-hand manipulation from a suspension containing 1 : 400 by volume of lymph node cells. These droplets contained from nought to six cells; each droplet was later recorded for its cell content. Larger droplets containing up to 100 cells could also be prepared. Alternatively, droplets containing exactly one cell each could be prepared by micromanipulation, but this was more tedious, due to the adhesion of the cells to the micropipette. The oil chamber was then incubated at 37° C. for 4 hr. At the end of this time, the chamber was placed on a microscope and the droplets surveyed at one hundred-fold magnification, dark ground. With a micropipette controlled by de Fonbrune micromanipulator, about ten bacteria were introduced into each droplet. Half the droplets were inoculated with *S. adelaide*, and the other half with *S. typhi*. After twenty minutes at room temperature, the droplets were observed for motility of the organisms. Total loss of motility of all the organisms was recorded as 'inhibition'. If even one organism in the droplet remained motile, this was recorded as 'no inhibition'. For control purposes, the suspending medium, the final supernatant from the washings, and the whole cell suspension prior to incubation were all shown to be free of inhibitory activity. Droplets prepared from the final cell suspension but containing no cells were also scored and found to lack inhibitory activity. Cells from several untreated rats were tested and these failed to elaborate a factor inhibiting the motility of the bacteria. Antisera against each serotype showed negligible cross-reaction with the other.

A proportion of the cells from immunized animals developed a factor immobilizing the test bacteria, and this was presumed to be antibody. All droplets containing single cells which were seen to immobilize the first serotype were then inoculated with about ten organisms from the second. After a further twenty minutes at room temperature, they were again observed for motility. The results of a typical experiment are recorded in Table 1. They indicate that none of the single cells was able to immobilize the organisms of both strains. To date 456 single cells have been tested for antibody production, 228 against each of the two organisms. Out of these, 33 were active against *S. adelaide* and 29 against *S. typhi*, but none of the 62 immobilized both strains.

Table 1. Antibody Production by Isolated Cells

No. of cells in drop	No. of drops inhibitory	No. of drops tested
First tested versus *S. adelaide*		
1	6*	39
2	5	25
3	7	24
4	6	21
5	6	10
6–10	17	33
First tested versus *S. typhi*		
1	3*	18
2	6	26
3	0	14
4	3	14
5	1	8
6–10	22	42

Lymph node cells from rats presensitized to *S. adelaide* plus *S. typhi* were dispensed in micro droplets and incubated for 4 hr. They were then tested by the introduction of motile bacteria.
* These droplets were also tested for activity against the alternative serotype and were negative.

These results imply that when an animal is stimulated with two contrasting antigens, individual cells tend to form one species of antibody. We cannot exclude a residual production of other antibodies at lower rates. The experiments were provoked by current hypotheses on the role of clonal individuation in antibody formation[5,6], with which they are consistent so far as they go. However, further studies will be needed to determine whether the assortment of antibody-forming phenotypes reflects a genotypic restriction or whether it is more akin to such phenotypic effects as interference between related viruses, or diauxie and competition in enzyme formation.

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Similar Effects of Blue and Infra-Red Radiation on Light-sensitive Seeds

Evidence that blue spectral regions affect the germination of light-sensitive seeds has existed for some years, and has recently been confirmed for both light-inhibited[1] and light-promoted seeds[2,3]. Borthwick *et al.*[2] obtained both promotion and inhibition of germination of lettuce seed by blue radiation, depending on the duration of the period the seeds had been allowed to imbibe water prior to irradiation. Evenari *et al.*[3] have also reported both stimulation and inhibition by blue light, depending on the imbibition period. It is well known that infra-re is also inhibitory to the germination of lettuce see and that there is a specific interaction between infra- red and red radiation, the effects of red being revers ible by infra-red and *vice versa*. A similar specific interaction between red and blue light has apparently not previously been reported, although Flint and McAlister[4] showed that a long exposure of lettuce seed to blue spectral regions nullifies the promoting effect of a previous exposure to red. In the following experiments it was shown that the action of blue is effectively similar to that of infra-red.

In these experiments the primary light sources consisted of (1) red fluorescent tubes in conjunction with red 'Perspex' (*R* 400) and (2) blue fluorescent tubes in conjunction with blue 'Perspex' (*B* 705) together with a 1 cm. screen of *M*/3 copper chloride, a combination which transmits the band 400–520 mμ. The experiments were carried out at 25–26° C. Preliminary experiments indicated that with irradiation periods of 30 min. or longer, blue light is inhibitory when the imbibition period exceeds about 12 hr., and hence in the following experiments imbibition periods of about 24 hr. were used.

The seeds were first exposed to 1½ min. of red light at an intensity of 100 μW./cm.², followed by various periods of blue (at the same intensity) ranging from ½ to 4 hr. Some inhibition of germination was obtained with periods of 1–2 hr., but much more effective inhibition was obtained with 4 hr. of blue. In a further experiment, using 1½ min. of red and 4 hr. of blue, the effects of a succession of irradiations with red and blue were investigated. It was found that repeated photo-reversal can be obtained (Table 1) and that the response of the seeds is determined by the nature of the last irradiation, as in the interaction between red and infra-red. The energy required to produce 50 per cent inhibition of germination by blue is about thirty times greater than for infra-red, however. By using a series of Schott interference filters with peak transmissions at 405, 452, 483 and 496 mμ respectively, in conjunction with a 1,000-watt projector lamp, maximum inhibition was found to occur at 452 mμ.

Table 1. Lettuce, var. Grand Rapids. Photoreversal of Promotion and Inhibition of Germination by Red and Blue

Irradiation	Germination
R	77·7
R—*B*	33·0
B—*R*	75·5
R—*B*—*R*	86·1
R—*B*—*R*—*B*	49·0
Dark control	29·0

R, 1½ min. red at 100 μW./cm.² from fluorescent source. *B*, 4 hr. blue at 100 μW./cm.² from fluorescent source. Treatments were commenced 26 hr. after sowing.

Both blue and infra-red appear to be effective also with the light-inhibited seed of *Nemophila insignis*. In previous experiments reported by us[1], it was shown that *Nemophila* seed is inhibited by blue light and we have since found that maximum effectiveness occurs in the region of 450 mμ. There is a further inhibitory band in the infra-red, with strong inhibition in the region of 710 mμ and less at 760 mμ. Our previous failure to obtain inhibition from an infra-red source transmitting wave-lengths longer than 730 mμ appears to have been due to the employment of too low an intensity at less effective spectral regions. The inhibition obtained when using red fluorescent tubes, on the other hand, appears to have been due to the content of near infra-red from this source.