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Daily Periodicity in the Activity of a Slide Used to Perform Immunologic Reactions at a Liquid-Solid Interface

[circadian activity/eclipse of (the) sun/magnetic field/condensed phases]

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ABSTRACT **Nickel-plated slides were prepared by evaporating a nickel layer (≃ 4000 Å thick) on glass slides in the presence of a magnetic field whose lines of force were perpendicular to the surface of the slides. Such slides are called active. After being coated with a layer of bovine albumin, they could adsorb a layer of antibodies 70–80 Å thick. However, if the active slides before they were coated with bovine serum albumin, were submitted to a magnetic field with lines of force parallel to the surface, the layer of antibodies adsorbed was only 40 Å thick. They had become inactive. It has been found that slides remain active at night but that shortly after sunrise they become slowly inactivated and reach a minimum in their activity at exactly the midday period. They regain full activity at sunset. It is shown that the inactivation results from a solar radiation that can be stopped by 3.5 cm of lead. On December 13th, 1974 there was an eclipse of the sun with 65% occultation at noon (Daylight Saving Time). The activity of the slide at noon was 65% of the maximum activity (83 Å) observed before sunrise. The thickness of the adsorbed layer of antibodies was 75 Å instead of 63 Å observed in the absence of the eclipse. The activation of the slides originates in a radiation of non-solar origin that is adsorbed by 1 cm of lead.**

More than 30 years ago, it was shown that immunologic reactions could be carried out at a liquid–solid interface. A monomolecular layer of antigen becomes adsorbed on a slide when the slide is dipped into a solution of antigen, which can be either a protein or a polysaccharide. The antigen, if it is a protein, can also be spread on a Langmuir trough and the spread monolayer transferred to a slide (1). Antigen-coated slides specifically adsorb multilayers of antibodies when immersed in a solution of antiserum. The thickness of the layer of antibodies is of the order of one hundred Angströms but can be as large as 600 Å. The thickness is determined optically with an ellipsometer. For optical reasons, the glass slides are first coated by evaporating a layer of nickel that is ≃4000 Å thick onto their surfaces.

The sensitivity achieved for detecting immunologic reactions can be tested in two ways. In the first method, a slide that is densely coated with antigen is immersed in successively diluted solutions of antiserum and one notes at which concentration one ceases to observe a specific adsorption. In the second method, the antigen solutions are successively diluted and the concentration of the antiserum is kept constant ($^1/_{10}$ diluted in Veronal buffer). For all solutions of antigen whose concentrations are smaller than 10^{-7} g/ml, a weak electric current (0.3 mA) is used during the adsorption with the slide acting as one electrode and a thin platinum wire as the other electrode.

An active slide is one that can be used to detect immunologic reactions in very dilute solutions (as little as 10^{-12} g/ml) of either antiserum or antigen. The slide is active when the nickel is evaporated in the presence of a magnetic field of a few thousand Gauss (≃ 0.3 T) whose lines of force are perpendicular to the slide, the metalized surface facing the south magnetic pole ($\perp$S). When slides are densely coated with antigen, the solutions of antiserum should be diluted at least 1/100 in order to observe a difference in the thickness of the layer adsorbed from the antiserum by an active as compared to an inactive slide. When a 1:200 dilution of bovine serum albumin is used, the thickness adsorbed on an active slide ranges from 70 to 80 Å and that adsorbed on an inactive one is only 40 Å. An active slide can be inactivated by placing the slide in a magnetic field with its lines of force parallel to the surface of the slide ($\parallel$). An inactive slide can be reactivated by the field if the lines of force of the field are perpendicular to the surface of the slide ($\perp$S). The activation–inactivation process is completely reversible (2).

In a recently published note (3), it was shown that the thickness of the layer of antibodies adsorbed on an active slide depended upon the time of day at which the experiment was performed, i.e., the activity of a slide does not stay constant. The activity, maximum at night, starts decreasing at sunrise, becomes minimum at midday, and begins increasing a few hours before sunset. At sunset, the slide has recovered its maximum activity, and will remain fully active until the next morning. This characteristic 24 hr periodicity of the activity of a slide can be observed week after week.

When the experiments were performed in mid-August, the layer of antibodies adsorbed was only 40 Å thick by noon (12:00). This inactivated state lasted until 16:00, but by 20:00 (Daylight Saving Time) the slides had recovered their full activity. In November, with shorter days, the period of inactivation was also shorter and extended also between sunrise and sunset, the maximum of the inactivation occurring exactly at midday. The thickness of the adsorbed layer of antibodies at midday was ≃55 Å in the middle of October and ≃65 Å in mid-December.

It was most remarkable to observe that a slide, placed in a lead cylinder with walls of 5 or 3.5 cm thickness, was not inactivated during the day. When a slide was placed in a lead cylinder with a wall of 1 cm thickness, it was not only inactivated during the day but it also did not recover its activity at night. An active slide, completely surrounded by a sheet of μ metal of 0.15 cm thickness, remained active during the day. μ metal is a common name for an alloy mainly

of Ni and Fe with magnetic shielding properties. Thus, the protection offered by a thin μ metal sheet is equivalent to that of 3.5 cm of lead. However, there is an essential difference between a slide protected by a thick layer of lead and a slide protected by μ metal. An active slide which has been protected by lead can be completely inactivated in a few seconds by a magnetic field of a few thousand Gauss whose lines of force are parallel to the surface of the slide ($\parallel$). In contrast, if a slide kept active is removed from a μ metal shield, the slide cannot be rapidly inactivated by the field ($\parallel$). If, however, the slide is protected by the μ metal *and* an outside lead cylinder with a wall that is 5 cm thick, the slide can be inactivated by the field ($\parallel$) as in the absence of μ metal. This shows that it is not the direct contact between slide and μ metal that prevents eventual inactivation by the field ($\parallel$).

From all of these observations, it seems fully justified to assume that there is a radiation emanating from the sun capable of inactivating the slide. Furthermore, the higher the sun above the horizon the greater the inactivation. Inactivation proceeds at the same rate whether the slides are kept in the dark or exposed to daylight. The experimental facts show unequivocally that the inactivating radiation is practically stopped by 3.5 cm of lead or a thin sheet of μ metal.

Before discussing the nature of this radiation it is appropriate to present now some other experimental facts that also indicate that the sun is the source of the inactivating radiation. On December 13th, there was a partial eclipse of the sun beginning in New York at 10:25 reaching a maximum of 65% occultation at 12:00 (Daylight Saving Time). In New York on this day, the sky was completely overcast and the sun could not be seen. As could have been predicted from the results presented above, the shielding of the sun by the moon had a strong influence on the activity of a slide. The results have been summarized in Fig. 1. Curve A represents the activity of a slide as a function of the time of day on December 12th and 16th. The activity is measured by the thickness of the layer of antibodies adsorbed in Å units. Of course, the curve changes its shape as the days become shorter but the differences are too small to be noticeable within a 1-week interval. Curve B was obtained the day of the eclipse. From sunrise until 10:30, curves A and B are identical. Shortly after the beginning of the eclipse the activity of the slide, instead of continuing to decline, started to increase to a maximum value at 12:00 when the eclipse was also at its maximum of 65% occultation. Thereafter, the activity dropped sharply to reach a minimum at 13:00, then increased again and from 14:00 on curves A and B were superimposed. When there was no eclipse, the loss of activity from sunrise to midday corresponded to a drop in the thickness of the antibody layer of 20 Å (83 Å–63 Å). Since 35% of the solar disc was not occulted at 12:00 on the day of the eclipse, one can estimate that the drop in the thickness of the layer of antibodies adsorbed at 12:00 on that day should be 20 Å $\times$ 0.35 = 7 Å, as compared to the experimental value of 6 Å (83 Å–77 Å). Thus, there is a good linear relationship between the occulted area of the sun and the thickness of the layer of adsorbed antibodies.

When a slide that was fully active in the evening is placed in a cylinder of lead with a wall thickness of 1 cm, the slide is still fully active the next morning. One cm of lead allows inactivation to occur but prevents the activation of an inactive

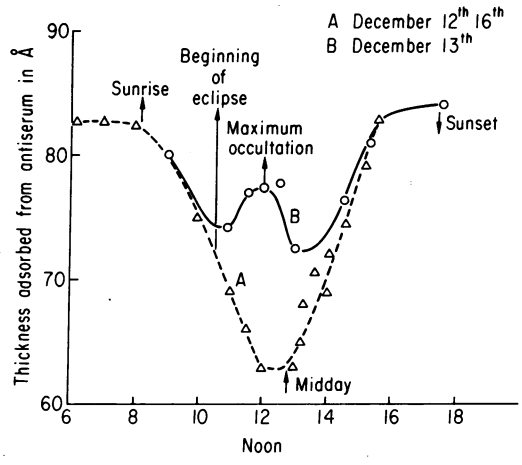

FIG. 1. Influence of the eclipse of the sun on the activity of a slide.

slide. Consequently, no inactivation takes place when the sun is below the horizon since otherwise the slide should have been found inactive in the morning.

The following experiment is good evidence that the activation proceeds continuously day and night. A slide fully inactivated by the field ($\parallel$) on November 13th at 8:14, 37 min after sunrise, and which could then adsorb a layer of antibodies 42 Å thick only, regained some activity during the morning in spite of the fact that inactivation was already taking place. At 10:50, 56 Å of antibodies could be adsorbed. In other words, the thickness adsorbed increased from 42 Å to 56 Å, a rate of 5.6 Å/hr. It is interesting to compare this rate observed at a time when activation and inactivation occur simultaneously to that obtained when no inactivation takes place, that is before sunrise. On October 25th, the layer of antibodies was 38 Å thick just after inactivation at 6:00, but 1 hr later it was 52 Å thick. This rate of 14 Å/hr is nearly three times faster than that of November 13th because the experiment was conducted when inactivation was not present.

What is the nature of the mechanism that allows either the activation of a slide by radiation from outer space or its daily inactivation by radiation from the sun? As often mentioned in the past (4), specific immunologic reactions carried out with very dilute solutions at a solid interface are possible on account of the long-range order of the adsorbed antigen molecules with, as a consequence, the interplay of cooperative phenomena. This long-range order of antigen molecules is obtained when there is an adequate long-range order of the nickel domains that serve as an anchorage for the antigen. The adequate order is achieved when the slide is submitted to a magnetic field with the lines of force perpendicular to the surface of the slide. When the slide is placed in the field with the lines of force parallel to the surface, the required order is not obtained and the slides are inactive. The order necessary for the activity of a slide is easily disrupted by heating for 1 min at 90°. It seems reasonable to assume that the radiation activates or inactivates a slide through a localized magnetic field. One could further suppose that the radiation consists of fast moving particles with the concomitant presence of a localized magnetic field. The results reported above on the very efficient protection of a thin sheet of μ metal against either activation or inactivation strongly support the view that radiation acts through a magnetic field, the adsorption of

the radiation by a thin sheet of μ metal 0.15 cm thick being negligible. This radiation could be called a soft component of cosmic rays.

Two major objections may be raised against this interpretation. Studies of cosmic rays indicate that the sun's contribution to total cosmic radiation is very small. However, in the experiments reported here the inactivation originates only from the sun. The influence of the sun's cosmic rays is so strong that in midsummer a slide remains completely inactivated the entire afternoon, preventing the radiation from outer space from activating the slide. It must be recognized, however, that activation and inactivation are brought about by particles of different origins whose efficiency in altering the state of the nickel surface may not be of the same magnitude. Consequently, the thickness of the adsorbed layer of antibodies would depend on the intensity of the particles $\times$ their efficiency.

The second major objection is the fact that the sun's radiation inactivates the slide whereas the net effect of the radiation from outer space is to activate the slide. The only immediately apparent difference in these two types of radiation is their degree of penetration through lead. The sun's active particles are stopped by 3.5 cm of lead whereas those causing activation are stopped by only 1 cm of lead. It may be that inactivating and activating particles are of opposite charge, and have different energy and masses.

Another difficulty is that the order of magnitude of the field linked to the particles appears much too weak. However, it should be remembered that a slide can be inactivated by a field of 200 Gauss in 1 min or with a field of a few thousand Gauss in 1 or 2 sec. By contrast, in mid-August it took 5 hr to fully inactivate a slide without a field, from sunrise to noon.

Nearly all the information on cosmic radiation has been obtained from its ionizing properties and it has so far been impossible to separate the sun's contribution from that of the total cosmic radiation. No substantial difference has ever been observed in the intensity of ionization between day and night (5). On the other hand, apparently through localized magnetic fields, the sun's radiation contributes exclusively to the inactivation of a slide. This is the first time that the sun's radiation can be completely separated from radiation from other origins. Two explanations are possible. Either the sun's cosmic rays can create localized magnetic fields of different orientation from those linked with cosmic rays from outer space, or the particles effective in activating or inactivating the slides are new particles undetected so far. It may well be that the state of the nickel surface is of such sensitivity to cosmic radiation that the type of experiment described in this paper might become of great usefulness in the investigation of cosmic rays.

Finally, the daily periodicity observed in the thickness of the adsorbed layers of antibodies as a function of the time of day may not be limited to immunologic reactions when the antigen molecules are immobilized on a nickel anchorage with long-range order. Long-range order in a condensed phase is a characteristic of biological structure that affords favorable conditions for the occurrence of cooperative phenomena and long-range interaction. It is not impossible that a circadian activity might be discovered for certain biological interactions taking place *in vivo* in a condensed phase.

The slides were prepared under stringent conditions by Evaporated Metal Film Corp., 701 Spencer Road, Ithaca, N.Y. 14850.

1. Rothen, A. & Landsteiner, K. (1942) "Serological reactions of protein films and denatured proteins," *J. Exp. Med.* **76**, 437–450.
2. Rothen, A. & Kincaid, M. (1974) "The influence of a magnetic field on immunologic and enzymatic reactions carried out at a solid–liquid interface," *Physiol. Chem. Phys.* **6**, 417–427.
3. Rothen, A. (1974) "Circadian activity of a nickel coated glass slide used for carrying out immunologic reactions at a liquid–solid interface," *Biophys. J.* **14**, 987–989.
4. Rothen, A. (1973) "Immunologic and enzymatic reactions carried out at a solid-liquid interface," *Physiol. Chem. Phys.* **5**, 243–258.
5. Condon, E. V. & Odishaw, H. (1958) *Handbook of Physics* **9**, 239