CIRCADIAN ACTIVITY OF A NICKEL-COATED GLASS SLIDE USED FOR CARRYING OUT IMMUNOLOGIC REACTIONS AT A LIQUID-SOLID INTERFACE

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In an article to appear shortly in *Physiological Chemistry and Physics* (see also references 1 and 2) it is shown that the state of the nickel surface coating a glass slide upon which a layer of antigen has been adsorbed plays a major role in the thickness of the antibody layer specifically immobilized on the slide dipped into a dilute solution of antiserum. When the slide before the adsorption of the antigen has been submitted for 2 min to a magnetic field of a few thousand gauss whose lines of force are perpendicular to the metallized surface facing the south magnetic pole ($\perp$S) the thickness of the subsequently adsorbed layer of antibodies is nearly twice that observed when the lines of force of the field are parallel to the surface ($\parallel$). This is generally true provided the serum is diluted at least 1/100 in veronal buffer. Slides submitted to the field $\perp$S or $\parallel$ are called active or inactive slides, respectively. The activation of a slide is reversible. An active slide becomes inactive when placed in the field $\parallel$, and inversely an inactive slide becomes active when submitted to the field $\perp$S. It was consistently observed that it was more difficult and sometimes impossible to activate a slide in the afternoon. This question has now been investigated and is the subject of this note.

Glass slides nickel plated by evaporation in vacuo in the presence of a magnetic field $\perp$S to make them active were kept either at room temperature or at $-40°C$ in a deep freeze. The slides were not submitted to a magnetic field after the formation of the nickel layer. They were all tested under the same conditions with the system bovine serum albumin—antibovine albumin rabbit antiserum diluted 1/200 in veronal buffer pH 7.5. The slides were tested every 2 h for 27 h in the following way. They were immersed for 2 min in an aqueous solution containing 10^{-3} g/ml of bovine albumin. After rinsing, drying, and measuring in the ellipsometer, they were placed in the antiserum solution with stirring for 7 min. After rinsing and drying the thickness adsorbed from the antiserum was determined with the ellipsometer. Then, the used part of the slides (1 cm) was cut off and the slides were placed back either in the deep freeze or kept at room temperature. The results were independent of the temperature at which the slides were kept. The slides were always found fully active when tested in the evening or in the early morning at 6 a.m. The thickness adsorbed from the antiserum was between 70 and 80 Å. There was a gradual inactivation of the slides in the course of the morning, and the slides became fully inactive by 11 a.m., the thickness of the antibodies layer dropping to 42 to 40 Å. 40 Å is the thickness of the antibodies layer adsorbed early in the morning on a slide submitted to the magnetic field with its lines of force parallel to the surface ($\parallel$). This 40 Å thickness was observed for all experiments carried out during the afternoon until shortly after 6 p.m., after which time the slides regained slowly their activity which became maximum at 8 p.m. and remained constant throughout the night until 7:30 a.m. the next morning. Then the whole cycle repeated itself quite regularly every 24 h. These experiments were carried out in mid-August. It was ascertained that light falling on the slide played no role in the phenomenon.

When the slides were kept in a heavy brass cylinder with a 4 cm wall thickness or protected by 1.0 cm of lead they did not regain their activity during the night. They were however partly reactivated if kept in an aluminum or steel tube with 0.1 cm and 0.15 cm wall thickness, respectively. Slides kept in plastic or glass tubes behaved as those not protected. Keeping active slides in the heavy brass container or in a lead cylinder of 1 cm wall thickness did not prevent their inactivation during day time which occurred at about the rate observed when no container was used. However, when the metallic shield consisted of a steel tube of 0.15 cm thickness plus 3.5 cm of lead, the slides kept their activity during daytime. For instance, one slide tested at 6:30 in the morning adsorbed a 71 Å thick layer of antibodies. It was then placed under 3.5 cm of lead, and when tested again at 12:30, the adsorbed antibody layer was 66 Å thick. It would have been 40 Å had the slide not been protected or protected by 1.5 cm of lead only.

Slides exposed for 27 or 5 h to a weak irradiation by γ-rays from a source of 0.09 μCi of radium were fully active at 1 p.m., time at which they would have been inactive if not irradiated. They could be inactivated at that time by a weak magnetic field ($\parallel$) of a few hundred gauss and reactivated by a somewhat larger field ($\perp$S). To be successful, the reactivation should not be attempted too long after the inactivation since a slide which has been inactive for some time cannot be reactivated in the afternoon. It appears that irradiation by γ-rays which prevents the inactivation does not activate an inactive slide since a slide submitted to irradiation during the whole afternoon, starting late in the morning when it has reached the inactive state, remains inactive until the evening as it would if not irradiated.

Similar results concerning the circadian variation of the activity of a slide were obtained when the experiments were carried out 25 miles north of New York City. It would, therefore, seem that artifacts which might have played a role in the laboratories at Rockefeller University may be discounted as an explanation.

The experimental facts just presented indicate that the activation of the slides at night and their inactivation during daytime results from the exposure of the slides to radiation of a penetrating power of the order of magnitude of the radium γ-rays. It would appear justified to call this radiation a soft component of cosmic rays, the inactivating radiation emanating probably from the sun. The energy for inactivation is more penetrating than the energy for activation since it requires 3.5 cm of lead to stop the inactivation and 1 cm to prevent the activation. It is most puzzling to observe that the overall activity of a slide varies periodically within a 24 h period. The inactivation takes place during daytime only, since when a slide activated in the early evening is placed under a shield of 1 cm of lead, and tested early (6 a.m.) the next morning, the slide is found fully active. Had inactivation proceeded at night, it would not have been prevented by a shield of 1 cm of lead, and the slide should have become inactive since activation is stopped by 1 cm of lead. Therefore it can be concluded that the inactivation occurs during the day only and not at night. In all probability, the activation is a continuous process but so far it has been impossible to demonstrate activation during daytime when inactivation predominates. It seems probable that the active and inactive states of a slide correspond to different configurations of the superficial magnetic domains and their boundaries brought about by the magnetic field oriented $\perp S$ (active) or $\parallel$(inactive), respectively. It is most intriguing that cosmic rays may be able to favor one configuration or the other depending on the penetrating power of the rays.

When the glass slides are coated by evaporation with nickel in the absence of a magnetic field, they are inactive and the thickness adsorbed from the antiserum (~45 Å) is independent of the time of the day at which the immunologic reaction is performed. They can be activated by the magnetic field but if kept unprotected they become inactive in the course of one afternoon and do not regain any activity at night. The nightly reactivation is observed only with slides coated with nickel in the presence of the magnetic field oriented $\perp S$.

These observations open up a new field that deserves to be thoroughly investigated both from a theoretical and from a practical angle. There is no doubt that a new and very sensitive method is now available for diagnostic purposes. For routine measurements, slides coated with nickel in the presence of a magnetic field properly oriented should be protected with at least 3.5 cm of lead when they are in the active state. They become inactive and remain inactive if protected with 1 cm of lead only.

Note Added in Proof. Experiments made in mid-October showed that the daily maximum degree of inactivation of the slides was smaller than in August, corresponding to the lower position of the sun above the horizon. The period of inactivation was also shorter, and extended exactly from sunrise to sunset.

Received for publication 19 September 1974.

REFERENCES

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