The Cytochromoxydase Activity in the Uterus of Experimental Animals

The enzymatic activities of the uterus and genitals have been comparatively little studied: particularly poor are the records covering the cytochromoxydase activity, about which only indirect references are available on the basis of the cytochrome *C* concentration or the respiratory activity of this organ.

JUNOWICZ-KOCHOLATY and HOGNESS[1], by using the spectroscopic method for their investigations, succeeded in determining the amount of cytochrome *C* occurring in the various organs and tissues: they have found that only minimal traces of cytochrome *C* occur in the human uterus.

FERRONI[2] in 1941 stated that the respiratory activity of the female rabbit uterus is higher during pregnancy and decreases during puerperium to minimal values during the period of rest in the organ.

According to this author, such variations should be regarded as due exclusively to hormonal factors.

DAVID[3] ascertained that the respiratory activity of the experimental animal uterus is higher during oestruation.

In the present experiments, the cytochromoxydase activity in the rat uterus was determined during the various phases runs of their lives.

The albino rat was used.

Animals of different ages were chosen and kept under identical experimental conditions. The uterus was removed under ether anaesthesia. After immediate weighing on a GALILEO-SARTORIUS balance, 100 mg were weighed separately. In the animals where the uterus weight was lower than 100 mg, the entire organ was used and the result obtained was corrected to 100.

The 100 mg uterus was later mortar homogenized and retaken with 5 cm^3 phosphate buffer 0·067*M* (pH 7·4).

The cytochromoxydase activity was determined according to STOTZ[4]: 0·3 ml of the homogenate were used as an enzymatic suspension.

The system in the first vessel was composed of: 1 ml of phosphate buffer 0·067*M* (pH 7,4), 1 ml of a solution of cytochrome *C* in phosphate buffer (concentration of cytochrome *C* $2{\cdot}4 \times 10^{-4}M$ 0·3 ml uterus homogenate (prepared by 100 mg tissue in 5 cm^3 phosphate buffer), 0·20 ml KOH 30% in central well, and 0·30 ml of a solution of sodium ascorbate (prepared by dissolving 20 mg ascorbic acid in 1 cm^3 Na OH n/10) in the side arm.

The second vessel contained all reagents with exception of the solution of sodium ascorbate.

The third vessel was identical with the first one, but the uterus suspension was boiled 3 min.

The values given by the vessels 2 and 3 were subtracted from the result obtained in the first vessel.

Cytochrome *C* was prepared from horse-heart according to KEILIN and HARTREE[5].

The experiments performed show that the cytochromoxydase activity of the uterus of the animals employed is greater the smaller the weight of the uterus. This applies to young or even impuberal animals, and is probably due to the intense metabolic processes which develop in organs during the various stages of growth.

Experiment	Uterus weight in milligrammes	Result (cytochromoxydase activity in mm^3 O_2)
1	65	86·48
2	72	80·26
3	76	81·44
4	84	76·78
5	85	60·79
6	93	59·74
7	103	58·28
8	100	32·22
9	105	33·21
10	126	52·27
11	136	40·53
12	140	33·28
13	157	33·83
14	155	28·52
15	190	34·54
16	203	40·20
17	207	17·29
18	221	12·89
19	223	21·00
20	270	8·39
21	325	7·25
22	415	5·28
23	420	3·18
24	470	6·16
25	472	0·76

In middle-aged animals, this activity decreases, the minimal values in the larger uteri corresponding to the adult animals.

R. ROSSI

Department of General Pathology and Bacteriology, University of Genoa, Italy, June 12, 1952.

Résumé

L'auteur a déterminé la valeur de l'activité de la cytochromoxydase dans la matrice des rats dans les diverses périodes de leur vie. Il a trouvé que cette activité est plus élevée dans les jeunes animaux, et elle diminue avec la croissance du poid de la matrice et de l'âge des animaux, jusqu'à arriver à des valeurs très petites dans les matrices des animaux adultes.

[1] R. JUNOWICZ-KOCHOLATY and T. R. HOGNESS, J. Biol. Chem. *129*, 569 (1939).

[2] A. FERRONI, Lo Sperimentale *365*, 375 (1941).

[3] L. DAVID, J. Pharmacol. Baltimore *43*, I (1931).

[4] E. STOTZ, A. E. SIDWEL, JR., and T. R. HOGNESS, J. Biol. Chem. *124*, 733 (1938).

[5] D. KEILIN and E. F. HARTREE, Proc. roy. Soc. London [B] *122*, 298 (1937).

Inhibition of Bacteriophage Development in Bacteria Illuminated with Visible Light

Photoreactivation[1] of the induction to lyse and liberate phages of lysogenic bacteria produced by ultraviolet light (U.V.) and by X-rays has been described by JACOB[2], LATARJET[3], and CANTELMO[4]. It appears from the last two communications that photoreactivation of induction may differ from photoreactivation of other effects, since it counteracts the effect of X-ray treatment (LATARJET) and is produced by exposure to white light *before* induction by U.V. (CANTELMO). We wish to call attention to the fact that these results may not be due to

[1] Photoreactivation has become a general term meaning the reversion by visible light of an effect due to ultraviolet light.

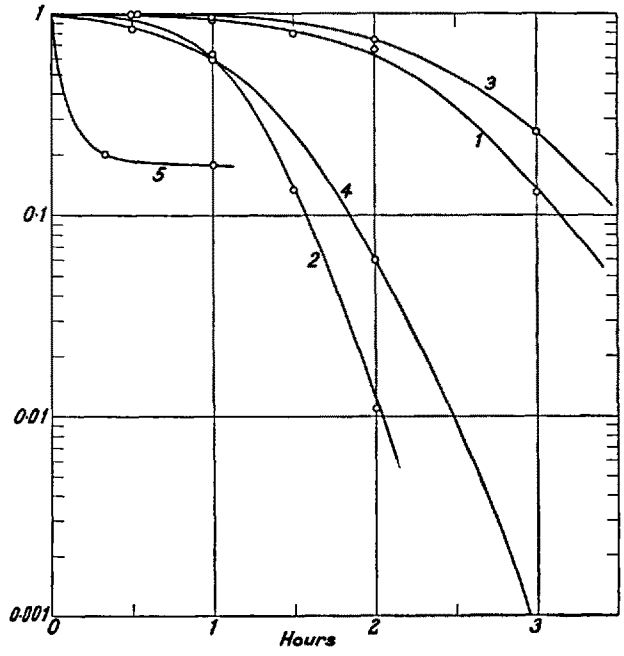
[2] F. JACOB, C. r. Acad. Sci. *231*, 1885 (1950).

[3] R. LATARJET, C. r. Acad. Sci. *232*, 1713 (1951).

[4] P. CANTELMO, C. r. Soc. Biol. *145*, 1882 (1951).

photoreactivation, because white light inhibits the ability of bacteria to support the growth of bacteriophage, independently of a previous treatment with U.V. or X-rays.

(1) *Inhibition of growth of phage T2 in Escherichia coli B irradiated with white light.*—The cells of a bacterial culture grown in tryptone broth to the concentration of 10^8 cells/ml were washed by centrifugation and resuspended in a saline solution. The final concentration was 10^9 cells/ml. Two milliliters of this suspension were exposed at 37°C to the light of a G.E.H5, medium pressure, mercury discharge lamp, under condition previously described (DULBECCO[1]). At various times of illumination samples were withdrawn, diluted 1:10 into the same saline solution, assayed for colony forming bacteria on nutrient agar and then mixed with phage T2 (multiplicity of infection of 0·5); 8 min were allowed for phage adsorption at 37°C, then strong anti-T2 serum was added to inactivate free phage; after 5 min the mixture was diluted and assayed for the number of bacteria yielding phage.



Effect of White Light on the Development of Bacteriophage.

The ordinates represent the logarithm of the "surviving" fractions and the abscissae the dose of white light in hours of illumination. Curve *1*, *Escherichia coli* B as colony former, curve *2* as T2 grower. Curve *3*, *Escherichia coli* K12 as colony former, curve *4* as carried phage grower when illuminated before induction, curve *5* when illuminated after induction.

We determined therefore the change in the number of colony-forming bacteria, and of the number of bacteria supporting the growth of the phage, as functions of the time of exposure to the white light. The results are given in the Figure (curves *1* and *2*).

The graph shows that both classes of bacteria decrease with the time of illumination, giving rise to curves of very high "number of hits". The light suppresses more effectively the ability to support the growth of the phage than the ability to form colonies: only 1% of the cells allow phage multiplication when 70% are still able to form colonies.

[1] R. DULBECCO, J. Bact. *59*, 329 (1950).

Bacteria kept in darkness for a time longer than the time of illumination maintained both properties unaltered.

(2) *Inhibition of the inducibility of Escherichia coli K 12 irradiated with white light.*—A relatively small dose of U.V. induces lysogenic K 12 to lyse and to liberate its carried phage (after a latent period of 60 min at 37°C) (WEIGLE and DELBRÜCK[1]). In the present experiments the bacteria were grown to a density of 10^8 cells per milliliter in tryptone broth, centrifuged, washed twice and resuspended in saline. A part of the suspension was illuminated for different lengths of time with white light using the apparatus described in the preceding section; aliquots were then plated for surviving, colony-forming bacteria and other aliquots were induced with a standard U.V. dose of 80 s at 80 cm from a 15 W G.E. "germicidal" lamp, and plated with sensitive bacteria to determine the proportion of induced cells. This dose of U.V. induced at least 99% of the cells not treated with white light.

The results are shown in the Figure (curves *3* and *4*). One sees that only 1% of the bacteria can be induced when 50% of them can still form colonies. Bacteria kept in darkness for the same length of time showed no detectable change in inducibility.

(3) *Photoreactivation by white light of induction of Escherichia coli K 12 by U.V.*—Bacteria were prepared and induced as in the previous experiment, and treated *afterwards* by white light. As shown in the Figure (curve *5*), after 20 min of illumination the proportion of induced bacteria was reduced from 99% to 20%, without appreciable change at longer exposures. The effect of illumination *after* induction is thus very different from that of illumination before induction.

We conclude that white light inhibits the ability of bacteria to support the growth of infecting lytic bacteriophage and of induced carried phage, as shown by the identical results obtained in experiments 1 and 2. In bacteria infected with lytic phages this effect reduces the number of cells liberating phage and therefore *decreases* the observable amount of photoreactivation; in bacteria carrying prophage the same effect reduces the number of cells liberating phage after U.V. irradiation and therefore *increases* the observable amount of photoreactivation and may even simulate its existence. Photoreactivation of the induction of K12 by U.V. has however been shown to exist by experiment 3. To prove photoreactivation in the case of induction produced by X-rays the same series of experiments should be carried out.

R. DULBECCO and J. J. WEIGLE

Kerckhoff Laboratories of Biology, California Institute of Technology Pasadena, California, August 1, 1952.

Résumé

Les auteurs montrent que des bactéries illuminées avec de la lumière visible perdent leur capacité de multiplier les bactériophages; cela aussi bien lorsque la bactérie (*Escherichia Coli* B) a été infectée de l'extérieur par un phage virulent (T 2) que lorsque la bactérie lysogène (*Escherichia Coli* K 12) a été induite à multiplier le phage (λ) qu'elle portait. Dans ce dernier cas cet effet de la lumière peut simuler une photoréactivation.

[1] J. J. WEIGLE and M. DELBRÜCK, J. Bact. *62*, 301 (1951).