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MUTAGENS AND INFECTIOUS NUCLEIC ACIDS

BY JOHN H. NORTHROP* AND FILIPPO CAVALLERO†

THE UNIVERSITY OF CALIFORNIA AT BERKELEY AND THE ROCKEFELLER INSTITUTE

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Cultures of bacteria usually contain a few cells which differ from the rest in some hereditary character. The difference may be colony type, resistance to antibiotics, virus production, or other properties.

The cells which are resistant to antibiotics may be proved by Lederberg's[1] replica technique to be mutants of the sensitive cells.[2] These resistant cells produce a nucleic acid (transforming principle) which is able to transfer the hereditary character to another cell, and at the same time cause more of itself to be formed.[3, 4] This nucleic acid, therefore, appears as a result of the mutation (or perhaps its appearance is the mutation).

The virus-producing cells also contain a nucleic acid[5] (the virus) which can transfer genetic information to another cell and also cause more of itself to be formed, exactly like the transforming principle. The viral nucleic acid, however, is generally assumed to be formed from a hypothetical "pro-virus,"[13] which had infected the culture at some time in the past.

There are, therefore, two entirely different hypotheses for the origin of transforming-principle nucleic acid and viral nucleic acid, although both have the same chemical and biological properties. This appears improbable and, in any event, violates the principle of economy of hypotheses.

One of the writers suggested,[14, 15] therefore, that the virus-producing cell also is a mutant, like the antibiotic-resistant cell. The fact that the number of such cells is generally increased by mutagenic agents[13] was cited as evidence.

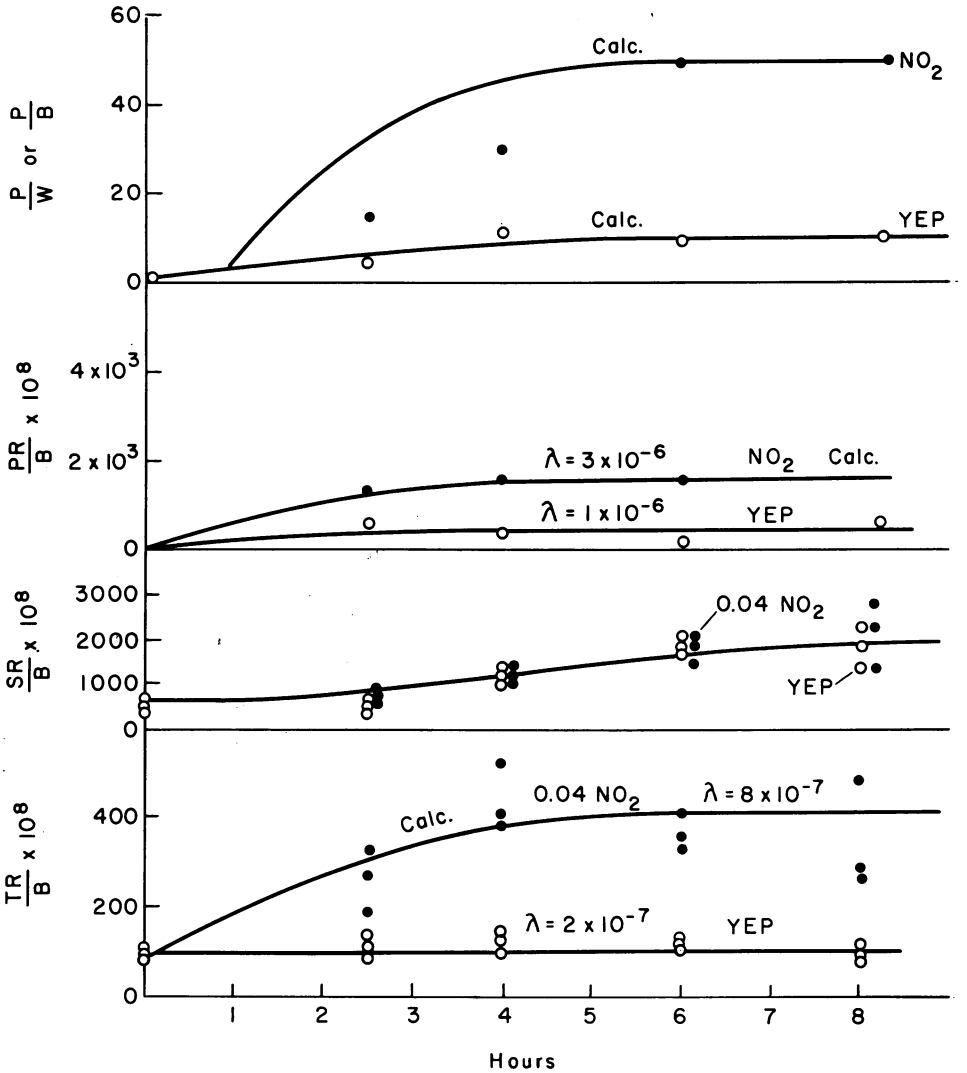

FIG. 1.—Increase in the proportion of various mutants in culture of *megatherium 899* growing in 0.04 *M* $NaNO_2$ in yeast extract peptone medium (YEP). Experimental procedure as in Northrop.[18] Terramycin-resistant (TR) and phage-resistant (PR) curves calculated[21] from

$$\frac{M}{W} = \frac{2\lambda A}{B - A}(e^{(B-A)t} - 1) + \frac{M_0}{W_0} e^{(B-A)t}$$

A = growth rate of wild (determined by direct measurement) = 1.0. B = growth rate of mutants = 0.6. λ = mutation frequency rate constant. M/W = ratio of mutants to wild cells.

Mutant	Culture medium	λ
TR	YEP	2×10^{-7}
	YEP + NO_2	8×10^{-7}
SR	YEP ± NO_2	3×10^{-5}
PR	YEP	1×10^{-6}
	YEP + NO_2	3×10^{-6}

Ratio virus to cells calculated from

$$\frac{P}{W} = \frac{lC}{C - A}(e^{(C-A)(t-1)} - 1) + \frac{P_0}{W_0} e^{(C-A)(t-1)}. \quad (1)$$

P/W = ratio virus to cells. l = virus produced per cell = 200, by direct determination. C = mutation time rate constant (0.04 in YEP), (0.20 in YEP + NO_2).

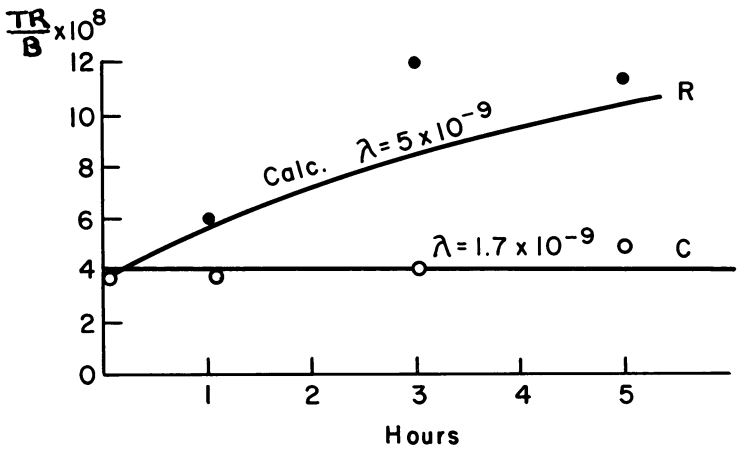

FIG. 2.—Increase in the terramycin-resistant mutants in cultures of *megatherium 899* growing in yeast extract peptone after exposure to 5,000 Roentgens. A = 2.0 (direct determination). A − B = 0.4. $\lambda = 5 \times 10^{-9}$. This experiment was carried out four months later than the nitrite experiments, and the proportion of terramycin-resistant mutants in the culture was then 4×10^{-8} instead of 100×10^{-8}.

Furthermore, the mutagenic agents, ultraviolet light, hydrogen peroxide,[15] heat,[16] and manganese[17] increase the proportion of antibiotic-resistant mutants and virus-producing cells to approximately the same extent. This is the usual effect of mutagenic agents on bacterial mutants.

These experiments have now been repeated using sodium nitrite and X-rays as mutagens. Nitrite was chosen because it is known to cause mutation in viral nucleic acid[18–20] and, therefore, according to the present point of view, should also cause mutations in transforming nucleic acid.

The results of these experiments are shown in Table 1 and in Figures 1 and 2.

TABLE 1

EFFECT OF NITRITE OR X-RAYS ON THE PROPORTION OF VARIOUS MUTANTS

Mutants	Number of experiments	Ratio of mutants in nitrite to mutants in YEP	Number of experiments	Ratio of mutants after exposure to 5,000 R to mutants in control tubes
PP (phage-producing) cells	17	6 ± 0.9	10	4 ± 0.9
PR (phage-resistant) cells	20	3.5 ± 0.6	3	5 ± 1
TR (terramycin-resistant) cells	21	6 ± 1.2	18	2.4 ± 0.2
SR (streptomycin-resistant) cells	20	1.2 ± 0.1	8	1.1 ± 0.1

Experimental procedure: *B. megatherium* 899 grown with shaking at 37° in yeast extract peptone ±0.04 *M* $NaNO_2$. Culture diluted and grown up repeatedly for 6 to 8 hr and mutants determined as described previously.[18]

The table shows that growth in the presence of sodium nitrite or exposure to 5,000 Roentgens X ray results in an increase of 2 to 6 times in the number of phage-producing cells (PP), phage-resistant cells (PR), and terramycin-resistant cells (TR), but neither mutagen has any appreciable effect on the number of streptomycin-resistant cells (SR).

Figure 1 shows the rate of appearance of the various mutants when growing in

peptone containing 0.04 *M* sodium nitrite. The curves were calculated by means of the equation derived by Northrop and Kunitz[21] for the rate of appearance of mutants in a culture.[22]

Figure 2 shows the increase in terramycin-resistant mutants after exposure to 5,000 Roentgens.

Effect of Increasing the X-Ray Dosage or the Concentration of Nitrite.—Increasing the exposure to X-ray causes a rapid increase in the production of virus, and exposure to 20,000 Roentgens or more may cause complete lysis.[24] This effect is predicted by equation (1) (in caption to Fig. 1), since when C (the mutation rate) is small compared to A (the growth rate), the equilibrium value of P/W will increase as C increases, but when C approaches A, a small increase in C will cause a very large increase in P/W and if C is larger than A, the ratio approaches infinity.[15]

The other mutants cannot be determined under these conditions.

Increasing the concentration of nitrite, on the other hand, does not increase the proportion of any of the mutants but merely results in decreasing the growth rate of the culture.

Summary.—Exposure of *megatherium 899* cultures to 5,000 Roentgens or 0.04 *M* sodium nitrite results in an increase of 2 to 6 times in the number of terramycin-resistant cells, phage-resistant cells, and phage-producing cells.

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* The Donner Laboratory of Biophysics and Medical Physics and the Department of Bacteriology, University of California, Berkeley 4.

† Present address: Istituto di Microbiologia dell' Università di Genova.

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The distinction between a transforming principle and a virus has, therefore, become arbitrary. Both may be defined as nucleic acids capable of transferring genetic information from one cell to another. If the genetic character transferred is harmful, as are most mutations, the nucleic acid is called a virus; otherwise, it is called a transforming principle.

The transforming principles which carry antibiotic resistance are strikingly beneficial and, therefore, represent a rare type of mutation.

The discovery of the nucleic acid nature of the viruses may be of great practical importance. In any event, it is necessary, as Herriott[25] has pointed out, to reexamine virus disease from this point of view.

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No assumption as to cell division is necessary in the case of the other mutants, since the mutation rates of these are very low and in this case the equations are the same, whether the mutation is assumed to appear with or without cell division.

Transformation of a sensitive cell after infection with either transforming nucleic acid[23] or virus nucleic acid occurs without cell division.

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*ISOLATION OF PEPTIDES FROM AN ANTIBODY SITE**

BY DAVID PRESSMAN AND OLIVER ROHOLT

DEPARTMENT OF BIOCHEMISTRY RESEARCH, ROSWELL PARK MEMORIAL INSTITUTE, NEW YORK STATE DEPARTMENT OF HEALTH, BUFFALO

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Antibodies against several different haptenic groups appear to contain a tyrosine residue in their specific combining portion. Part of the evidence for a tyrosine residue is based on the loss of antibody activity when antibodies are iodinated.[1, 2] That the loss is due to iodination in the specific combining portion has been shown by the fact that the antibody specific combining portion can be protected from the effects of iodination by combining the antibody with the specific hapten prior to iodination.[2] Making use of this principle of specific protection, we have now been able to isolate polypeptide portions which must have come from the specific combining portion of the molecule. This was done in experiments reported here using as starting material a univalent fragment (Fraction I (Porter)[3]) of the original anti-p-azobenzoate antibody. One portion of the fragment preparation from specifically purified antibody was iodinated with iodine labeled with I^{125}. A second portion was combined with hapten and iodinated with iodine labeled with I^{131}. The iodinations were carried out to the same extent for both portions. Subsequently, the iodinated fragments were combined and digested with pepsin to yield peptides. The peptides were separated by high-voltage electrophoresis, and it was found that the I^{125}-to-I^{131} ratio in some peptides isolated was different from that of the whole digest and this can be the case only for peptides derived from the specific combining portion of the antibody molecule.

Materials and Methods.—Specifically purified antibodies: Pooled rabbit antiserum against bovine γ-globulin coupled with diazotized p-aminobenzoic acid was used. The antiserum gave a