SPECIAL ARTICLES

THE PRODUCTION OF MUTATIONS BY ULTRA-VIOLET LIGHT

THE discovery by Muller that x-rays produce mutations led to the suggestion that similar forms of radiation, occurring in nature, might be the cause of natural mutations. Undoubtedly, part of the natural mutation rate is due to the radiation in question, but, as shown by the calculations of Muller and Mott-Smith, the entire rate could not be accounted for in this way. The problem was therefore raised as to what other agencies might cause natural mutations. One such possible agency is ultra-violet light, since it is known that ultra-violet light causes chemical activation. Moreover, it seems probable from the studies of Gurowitch and others that growing and dividing cells give off "mitogenetic" rays, which are in the ultra-violet region of the spectrum. It therefore seemed desirable to test the effect of ultra-violet light on the mutation rate.

Previous experiments which I did on Drosophila indicated that ultra-violet light had a slight positive effect on the mutation rate but in many of these experiments I treated the adults and it was difficult to get the rays to the reproductive cells on account of the high screening effect of the superficial tissues. I therefore decided to use the developing eggs and, in particular, eggs in which the "pole cells" were formed; that is, the germ tract cells at the time that they form a polar cap at the amicropilar end of the egg. Only the polar cap was treated, the rest of the egg having been screened (by a cover glass). A quartz mercury arc lamp was used as a source of the light. It was run at 50 volts. The eggs were at a distance of 150 cm from the lamp, and they were given 3 to 4 minutes treatment. Lethals were looked for in the X-chromosomes of males that developed from the treated eggs, and Muller's Cl B method was used for the detection of the lethals. In case any mutations were produced by the ultra-violet light at the polar cap stage, then these should appear in "bundles"; that is, in a fairly large proportion of the sperm cells. Moreover, they should be "reduplications"; that is, the same mutation multiplied. For, assume that there are 10 pole cells in the polar cap at the time of treatment and that one of the cells is struck in the right way and a mutation produced in its X-chromosome. Then roughly one tenth of the sperm cells of the adult should contain the mutation, and the male in question should transmit the mutation to about one tenth of his daughters.

Some tests already made show just such an effect. The data are as follows. From 108 males treated in the polar cap stage I got 8 cases of "reduplicated" lethals. From 110 males in the controls I got 1 reduplicated lethal.

A certain number of lethals turned up in the controls, but these were apparently lethals that occurred after the polar cap stage (that is, after treatment), and represented the natural mutation rate. For they were either not reduplicated at all, or only to a small extent, just as would be expected if they occurred at a later stage in development. The treated lot also contained these "natural" mutations. As a criterion for a lethal that was produced in a cell at the polar cap stage, I use the number of cells present in the polar cap (10 to 20) at the time of treatment. In other words, I regard as induced lethals any that are reduplicated in about 5 to 10 per cent. (or more) of the reproductive cells, and which are shown by linkage tests to be the same lethal.

On *a priori* grounds it would be very unlikely that a lethal should occur, apart from treatment, at just the polar cap stage, especially in view of the small number of cells at this stage, and the short length of time that it lasts (less than 1 hour). It is therefore very surprising that there should have been a reduplicated lethal in the controls. It is possible that the male which yielded this lethal belonged with the treated lot or that some stray light (reflected from the walls of the room) got around the sides of the plate glass that I had in front of my controls, and that it happened to strike one of the control eggs.

I wish to thank the American Academy of Science for a grant of $500 to defray the expenses of an assistant for the above experiment. I wish also to thank Miss Frances Ward for her capable assistance.

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THE IMMUNOLOGICAL RELATIONSHIP OF EASTERN AND WESTERN STRAINS OF EQUINE ENCEPHALOMYELITIS VIRUS

THE epizootic of equine encephalomyelitis which invaded sections of Delaware, Maryland and Virginia during July, August and September, 1933, has presented characteristics judged to be closely comparable with those seen in the disease as it has occurred in the West, the only apparent material difference being in a more acute course and perhaps greater mortality. Epizootiologically, the diseases have much in common and the syndromes do not differ, except as above noted.

The anatomical changes observed at autopsy have been confined largely to the central nervous system and have not been uniformly different from those seen in cases of the western disease.

The histological alterations appear to differ only in degree, the eastern disease exhibiting a more intense small cell infiltration, and a more marked extravasation of erythrocytes and fluid into the perivascular and pericellular spaces.

The writers have isolated eight strains of a filterable

virus from cases in Delaware and Maryland, and one, a Maryland strain designated Md[1], has been compared in preliminary tests with a South Dakota strain of virus designated S. D., which was isolated by the writers[1] in 1932. Neutralization tests, utilizing a hyperimmune horse serum and a hyperimmune rabbit serum, were conducted with the two vira. The horse serum was prepared by Dr. C. M. Haring *et al.*, of California, through the use of California strains of virus, while the rabbit serum was obtained by the writers, who utilized the South Dakota virus. Both sera had previously been repeatedly shown capable of neutralizing California virus as furnished by Dr. Haring, the S. D. virus and a second strain of South Dakota virus[2] which we recovered from a case occurring during the present 1933 outbreak.

The technique of preparing virus suspensions, mixing and holding serum-virus inocula, was identical to that employed by Howitt[3] in neutralization tests of poliomyelitis and equine encephalomyelitis vira.

A series of three tests was conducted, using S. D. and Md[1] vira on the same days, with controls in the form of normal serum-virus mixtures and saline-virus mixtures of the same virus dilution as that in the immune serum-virus mixtures. The guinea-pigs were inoculated intracerebrally after trephination. In each of the tests applied, the serum completely neutralized the S. D. virus as judged by failure of any inoculated animals to show any signs of illness during an observation period of ten days. Normal serum-virus and saline-virus inoculated guinea-pigs died or developed a moribund condition warranting destruction on the fourth to sixth day. No guinea-pig inoculated with mixtures of Md[1] virus and the above immune sera survived for more than four days (some moribund animals were destroyed on the third or fourth days). Likewise animals inoculated with normal serum and saline control mixtures containing the same dilution of virus succumbed in a manner typical of previous passage inoculations of the same virus.

Two further tests using two volumes of immune serum to each volume of Md[1] virus of the same dilution as previously employed failed to demonstrate neutralization of the virus. As an additional check two tests using three volumes of serum to each volume of Md[1] virus likewise gave no indication of virus neutralization or even partial inactivation. Indeed, in some instances the serum appeared to cause an increased virus activity as evidenced by a shortened incubation period.

With the Md[1] strain of virus well-marked symptoms were often evident on the second day following intracerebral inoculations and death ensued on the third or fourth day after a syndrome indistinguishable from that of the S. D. virus disease.

Of a group of four guinea-pigs which had been shown by at least one intracerebral inoculation to be immune to S. D. virus, two were inoculated intracerebrally with Md[1] virus and two were exposed in the same manner to S. D. virus. The two animals inoculated with the S. D. virus survived without any signs of illness, while those inoculated with Md[1] virus succumbed. Controls inoculated with each virus developed typical encephalomyelitis and succumbed or were destroyed upon reaching a moribund state.

A guinea-pig virus brain (Md[1] strain) was ground in a mortar with sand and saline and centrifuged at 1,000 r.p.m. for 20 minutes. The supernatant fluid was further diluted and guinea-pigs were inoculated intracerebrally with 0.2 cc of dilutions varying from 1:100 to 1:20,000. Those animals which were inoculated with a dilution of 1:7,000 and lower succumbed while those which received dilutions greater than 1:7,000 survived without evidence of illness.

Titrations of S. D. virus similarly prepared have disclosed a M. L. D. of 0.2 cc of a 1:2000–1:5000 dilution, depending upon the particular sample tested.

While anti-serum of the Md[1] type was not available at the time these tests were made, our preliminary observations indicate that the Md[1] virus recovered from the current outbreak of encephalomyelitis in the central Atlantic coast states is not identical to the western virus as exemplified by the S. D. strain. The Md[1] virus disease in the guinea-pigs is of a more acute type than the S. D. virus infection and the vira show certain immunological differences.

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[1] This strain of virus, referred to in SCIENCE, Vol. 78, 2012, pp. 63–64, 1933, was recovered from a specimen submitted by Dr. C. H. Hays, inspector in charge, B. A. I. field station, Pierre, S. Dak., who conducted extensive field studies of the 1932 outbreak in South Dakota.

[2] Recovered from specimen submitted by Drs. C. H. Hays and C. C. Heacock, collected during the 1933 outbreak in South Dakota.

[3] B. Howitt, *Jour. Infect. Dis.*, Vol. 51, No. 3, p. 493, 1932.

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