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Factors Influencing the Inactivation of the Rabbit Papilloma Virus by X-Rays.

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Lacassagne found several years ago that the rabbit-papilloma virus is notably resistant to X-rays,[1] and Syverton has more recently reported that a dose of 14,000,000 r was required to abolish the infectivity of a cell-free suspension of the papilloma virus[2] though 1,000,000 r or less sufficed to inactivate certain other viruses and bacteria. In experiments undertaken lately for other purposes we have noted that the amount of irradiation required to inactivate the papilloma-virus is influenced by the virus concentration, as also by extraneous material present along with it in the irradiated suspensions. Berkefeld filtrates, which contained the virus in large amount, were rendered completely non-infectious upon exposure to 2 to 4 million r of irradiation, while only 400,000 to 800,000 r was required when the virus had been partially purified by repeated differential centrifugations.

Virus was obtained by grinding infectious cottontail rabbit papilloma-tissue and extracting it in 10 or 20 volumes of isotonic saline and centrifuging at about 4,400 rpm for 20 minutes in an angle centrifuge, with filtration of the supernatant fluid through Berkefeld V candles. Partially purified suspensions of the virus were prepared by centrifuging the filtrate at 30,000 rpm for 1 hour in an air-driven centrifuge, after which the supernatant fluid—which contained no detectable virus but much extraneous protein, as indicated by nitrogen-determinations—was removed. The small pellet of sediment was then resuspended in isotonic saline to the original volume and spun at about 4,400 rpm for 20 minutes. The high-speed centrifugation was then repeated, the pellet of sediment resuspended in saline, and another low-speed centrifugation done to remove gross particles. The final virus suspensions were very faintly opalescent and contained less than 1% as much nitrogen as the whole filtrate.

Twenty to 30 cc of the virus suspension was irradiated in discoid

1 Lacassagne, A., *Compt. rend. Soc. biol.*, 1936, **123**, 736.

2 Syverton, J. T., Third International Cancer Congress, Atlantic City, New Jersey, 1939, 176.

pyrex flasks, 1.8 cm deep with thin flat top and bottom. They were placed half way between 2 X-ray tubes at a distance from each target of 10 cm. To control the temperature the flask was placed in a Petri dish containing water and the air over it was circulated with a fan. The temperature of the water bath never rose above 30°C. The X-rays were not filtered. The tubes were run at 30 milliamperes and at a peak voltage of 180 to 190 kilovolts. The intensity was 6,200 r per minute and the half value layer of radiation was 0.19 mm of copper. The larger doses of irradiation were of necessity intermittent and the virus specimens were kept in the icebox between exposures to the X-rays. Small samples of the virus fluid were removed at intervals during the irradiation and tested for infectiousness by rubbing them into the freshly scarified skin of domestic rabbits. Control portions of the virus solutions submitted to identical conditions, but not irradiated, remained highly infectious.

Ten comprehensive experiments have been made, in which 4 virus filtrates were used and 7 partially purified virus suspensions procured from the papillomas of as many different cottontails. The findings have been consistent. Only a very small proportion of the virus in Berkefeld filtrates remained infectious after 1,000,000 r of irradiation, the irradiated material giving rise to less than 1/20th as many papillomas as the controls; and 2 to 4 million r rendered the filtrates completely innocuous. Suspensions of virus partially purified by 2 differential centrifugations, as described above, were almost completely inactivated by 100,000 r and completely so by 500,000 to 800,000 r. Whole filtrates or purified suspensions containing much virus have invariably required more irradiation to abolish the infectivity than comparable preparations containing less virus. This apparent influence of virus concentration is probably due to the use of total inactivation as the quantitative measure of radiation effect.

Normal rabbit serum was added to a purified virus suspension prior to irradiation to learn whether it would have a protecting effect. It was found that the virus suspended in 10% normal rabbit serum retained about half of its infectivity after exposure to 100,000 r and about 10% after 800,000 r, whereas the same virus suspended in saline was rendered almost completely innocuous by 100,000 r and was completely non-infectious after 400,000 r. In 2 further tests the non-infectious supernatant fluids removed from virus filtrates after centrifugation at 30,000 rpm for 1 hour were found to protect purified virus suspended therein instead of in saline, these suspensions requiring about as much irradiation to abolish their infectivity as the original virus filtrates.

It is common knowledge that extraneous proteins protect bacteria and viruses against heat and ultraviolet light, and we have found that the papilloma-virus in crude suspension resists more heat[3] and ultraviolet light[4] than after Berkefeld filtration or partial purification by differential centrifugation. It seems probable that the protection of the papilloma-virus from X-rays by normal serum or by extrinsic material present in papilloma-extracts is also due to extraneous protein. Further work must decide what the findings imply as regards the mode of action of X-rays on biologically active materials.

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Minimum Daily Requirement of Rabbits for α-Tocopherol.

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During the past year we have been studying the vitamin E requirement of rabbits for the cure and prevention of nutritional muscle dystrophy. Recently, MacKenzie and McCollum[1] reported that the rabbit requires 0.7 to 1.0 mg of α-tocopherol per kg of body weight, but this does not quite agree with our findings. In our experience, rabbits made dystrophic on the Goetsch-Pappenheimer diet 13 were cured by the daily oral administration of synthetic dl-α-tocopherol acetate in quantities ranging from 0.18 to 1.0 mg per kg of body weight, with most of the cures resulting from doses of 0.2 to 0.5 mg (as free alcohol).

We found that a definite correlation exists between the higher requirements and the *total* α-tocopherol intake. When animals with an apparently high vitamin E requirement were subjected to a new test on a smaller *total* tocopherol intake, their requirement dropped to a lower level (values ranging from 0.2 to 0.4 mg per kg). These findings suggest that the higher values, as well as the wider range of variation in the requirement values, are not so much an outcome of individual variability in the need of the tissues for vitamin E as of variations in the efficiency of absorption, in ability for storing and in the rate of destruction of the vitamin.

3 Friedewald, W. F., and Kidd, J. G., *J. Exp. Med.*, 1940, **72**, 531.

4 Unpublished experiments.

1 MacKenzie, C. G., and McCollum, E. V., *J. Nutrition*, 1940, **19**, 345.