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Adjuvant Arthritis Induced in Germ-Free Rats.* (27959)

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A polyarthritis induced in rats by a single injection of Freund's water-in-oil adjuvant has been under investigation since 1956(1) but the precise mechanisms which underlie its development are still obscure. It is most likely that delayed hypersensitivity is implicated but the antigenic stimulus, if any is involved, has not been found. In the background in all of the experiments lingers the possibility that a latent infectious agent has been aroused by the injected adjuvant (which is itself sterile), thereby causing an infectious arthritis. Because of this possibility considerable effort has been expended by us (2) and others(3) in an attempt to clarify this matter. These studies have not shown bacteria, fungi or pleuropneumonia organisms (PPLO or Mycoplasma) when a wide variety of cultural technics were used on joint fluid, periarticular tissues or visceral organs. The studies did not rule out the presence of viruses, rickettsiae or certain bacteria or other microbes with fastidious growth requirements, but the presence of the latter seemed unlikely.

To pursue this matter further adjuvant was given to a group of "germ-free" animals so that the effect of the normal bacterial flora, or lack thereof, could be assessed in relation to the induced arthritis. Germ-free animals differ physiologically from "conventional" animals maintained in a non-sterile environment in several respects including the presence of lower concentrations of circulating beta and gamma globulins(4) and some retardation in their immune responses(5).

Five male and four female rats of the Lobund strain, which have been maintained in the germ-free state for 12 generations, and housed at the NIH, were used in the study. At time of inoculation their weights ranged from 131 to 166 g. Other details of germ-free existence and measures necessary to maintain the animals in a healthy state have been outlined elsewhere(6). The adjuvant preparation has also been described previously(7). In brief it contained 1 mg of the Wax D fraction† of human *M. tuberculosis* (strain Brevannes) per ml of final mixture; 0.5 ml was given subcutaneously into the proximal half of the tail in a single injection.

Except for slight nodular swelling which developed along the site of adjuvant deposition on about the 8th day and necrosis of the distal 1 or 2 cm of the tail which occurred in 6 of the animals on about the 6th day the animals appeared well until the 16th day when 2 showed minimal arthritic reaction in one or more paws or digits. On the 18th day another 2 animals had arthritis and on the 20th day 9/9 were affected. In all ani-

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mals the disease was progressive with fusiform swelling of the digital and other joints of the paws in a manner that has been described before(7). It was graded as severe, and obviously painful, in 6/9 animals and moderate in the other 3. All of the 5 males developed balanitis and urethritis, a feature which occurs with moderate frequency in other strains of rats(8) and with high frequency in the Lobund strain(8). No other gross lesions were noted.

All animals were sacrificed on the 30th day at which time the arthritis and balanitis were still intense. At autopsy, aside from the articular disease, the cecum was massively distended with a watery dark brown fluid as is commonly found in germ-free animals. No gross visceral lesions were observed. Bacterial cultures were taken from the feces and food several times during the 30-day period, and also from the urine and urethral discharge on the 30th day. ‡Samples of food and feces were placed in sterile screw capped jars, while the urine and urethral discharges were picked up on sterile cotton swabs, placed in plugged test tubes and removed from the isolator. All samples were streaked in duplicate on trypticase soy 5% blood agar (discarded human bank blood) for aerobic growth and emulsified in 25 cc of thioglycollate broth for anaerobic growth. One set of each sample was then incubated at room temperature and another set at 37°C for 14 days. Direct smears, gram stained, were also made on each sample. All bacterial tests were negative.

A control series was hardly necessary for this experiment since this strain has never been known spontaneously to develop arthritis, especially in the germ-free state. Nevertheless 3 small groups served as controls. These included: (a) 2 littermates of the germ-free group that were "conventionalized" just prior to the experiment. Each developed an arthritis and balanitis on the 21st and 28th days after inoculation. These 2 animals were maintained separately in a relatively clean environment completely apart from other animals and undoubtedly had not acquired an extensive bacterial flora; (b) a group of 9 completely conventionalized male Lobund rats which had been studied 18 months earlier in laboratories in Los Angeles. In this series 9/9 developed arthritis by the 14th postinoculation day and in 3 animals it had an onset on the 8th day. Eight of the 9 affected animals also had balanitis (8); (c) 5 rats from the same Lobund colony at the NIH which had been housed in regular animal quarters for several generations. In this group 5/5 developed arthritis, the first animal becoming affected on the 13th day and the last on the 24th day. One of the 3 males showed a balanitis.

The time of onset was delayed slightly in both the germ-free animals (18.7 days) and their "conventionalized" colony mates (19.0 days) over that in other strains (average 13.2 days(7)) or even in the Lobund rats that we studied earlier. The significance of this observation is unclear, but it does not seem to depend solely upon the presence of a germ-free state.

These results indicate that a bacterial flora, either normal or abnormal, is not necessary for development of adjuvant arthritis. On the other hand the study does not eliminate the possibility that a virus, rickettsia or even a fastidious PPLO is involved; however precisely the same uncertainties apply in human rheumatoid arthritis and related diseases. Further studies are now under way.

Summary. The polyarthritis which occurs after injection of water-in-oil adjuvant into laboratory rats was found to develop as readily in the germ-free animal. In addition to the acute arthritis, both groups of animals also developed a florid urethritis and balanitis. In this study repeated cultures of food, feces, urine and urethral discharge were always bacteriologically sterile in the germ-free group. Hence it is apparent that a bacterial flora is not necessary for development of adjuvant arthritis.

‡ These tests were carried out by Dr. Lloyd G. Herman, Division of Research Services, Nat. Inst. Health, and will be reported elsewhere.

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Comparison of Effects of Deuterium Oxide and X-Ray Irradiation on Multiplication of Poliovirus.* (27960)

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In studies on the effects of deuterium oxide on various biological systems we have observed that the multiplication of poliomyelitis virus is enhanced in tissue culture systems in which the medium contains 20-40% of D_2O(1,2). This effect was observed with several strains of poliovirus–Mahoney, MEF-1, and CHAT (an attenuated type 1 virus)–and with various host cells, among them HeLa and stable and primary lines of monkey kidney cells. Using KB cells and poliovirus I, Lwoff and Lwoff have made similar observations(3,4). In speculations concerning the mechanism of action of D_2O(5) we noted the possibility that the D_2O effect may be similar to the actions of X-rays since both deuteration(2,6) and X-ray irradiation(7) result in formation of giant cells and both treatments result in increased virus yield when these cells are infected(1,8). Since CHAT, a virus which normally does not replicate at incubation temperatures as high as 40°C, will reproduce at 40°C in deuterated medium(1,5,9), it was of special interest to ascertain whether this virus would also replicate at 40°C in X-irradiated cells. In preliminary experiments (5) we studied this possibility using only one level of irradiation (300 r). This communication covers a broader investigation into this phenomenon.

Materials and methods. Monolayer cultures were prepared from primary trypsinized monkey kidneys by technics previously outlined(9). Two days after seeding the Petri dishes, the developing monolayers were treated with medium containing D_2O, with X-ray irradiation, or with both. To yield a growth medium containing 25% D_2O, half of the standard growth medium was removed and replaced with a 50% D_2O medium that has been previously described(9). The X-ray irradiation of monolayers was done in covered Petri dishes with the medium present. After the irradiation the pH of the medium, which had risen markedly, was adjusted to pH 7.4 by addition of a few drops of 0.1 N HCl. Control monolayers were treated in the same way except that D_2O or X-ray irradiation was omitted. Five days after addition of medium containing D_2O or the X-ray irradiation treatment, the monolayers were infected and overlaid with agar-overlay medium in a manner previously described(9).

X-ray irradiation was done by using a Keleket X-ray tube operating at 200 KV and 20 m.a. at a distance of 30 cm. No filters were used. The rate of irradiation was monitored and dosage was varied by altering the time of exposure to X-ray.

The technics for determining cell number and volume were as follows: the cell mono-

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