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Antibody Response in Rabbits to Adenovirus Type 12*† (28328)

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The report by Trentin and associates(1) on the oncogenic properties of Adenovirus Type 12 has been confirmed by Huebner *et al.*(2) who have further demonstrated the production of tumors in hamsters with Adenovirus Type 18. These studies suggest a need for an *in vitro* test to differentiate strains of a particular Adenovirus type. The following pilot study on antibody production to Adenovirus in rabbits was initiated with the ultimate goal of demonstrating immunological differences among Adenovirus Type 12 strains. Previous reports(3-7) involved hyperimmunization with multiple doses of large amounts of infectious virus. As here shown, satisfactory antibody titers have been obtained following a single injection of relatively small amounts of viral antigen.

Methods. New Zealand rabbits, 3.5 to 4.5 kg, were bled immediately prior to inoculation, and at 1, 7, 15, 21, 28, 30, 35 and 42 days thereafter. Sera were clarified by light centrifugation and stored at –20°C until tested.

Adenovirus Type 12 antigen was prepared by making 3 serial passages in KB tissue culture of the American Type Culture Collection prototype strain (Huie). Infected cells and fluid from the third passage harvest were freeze-thawed 3 times and lightly centrifuged to remove cellular debris. The supernatant virus fluid represented the antigen used in these studies. This pool‡ has since been demonstrated to be oncogenic by Huebner *et al.* (2). Infectivity titrations in HeLa cells have shown the virus pool to titer $10^{3.5}$/0.1 ml after 7 days. Considerably higher titers up to $10^{6.3}$/0.1 ml were obtained when the virus was titrated for 14 days in primary human embryonic kidney (HEK) tissue culture. All virus titers listed in the following discussion and tables are based on titrations in HEK.

Three groups of 2 rabbits each were inoculated intravenously with 1½ ml of Adenovirus Type 12 with 30,000,000 tissue culture infective doses — 50% ($TCID_{50}$); 300,000 $TCID_{50}$; and 3,000 $TCID_{50}$. Rabbits #3012, #3014 and #3016 were given a booster inoculation of 300,000 $TCID_{50}$ intravenously on day 28.

Serum neutralization tests were carried out in 24-hour-old HeLa tube cultures. Cells were maintained for the duration of the test on Eagle's Basal Medium(8) in Earle's Balanced Salt Solution (BSS) containing 5% Agamma Calf Serum.§ All media were supple-

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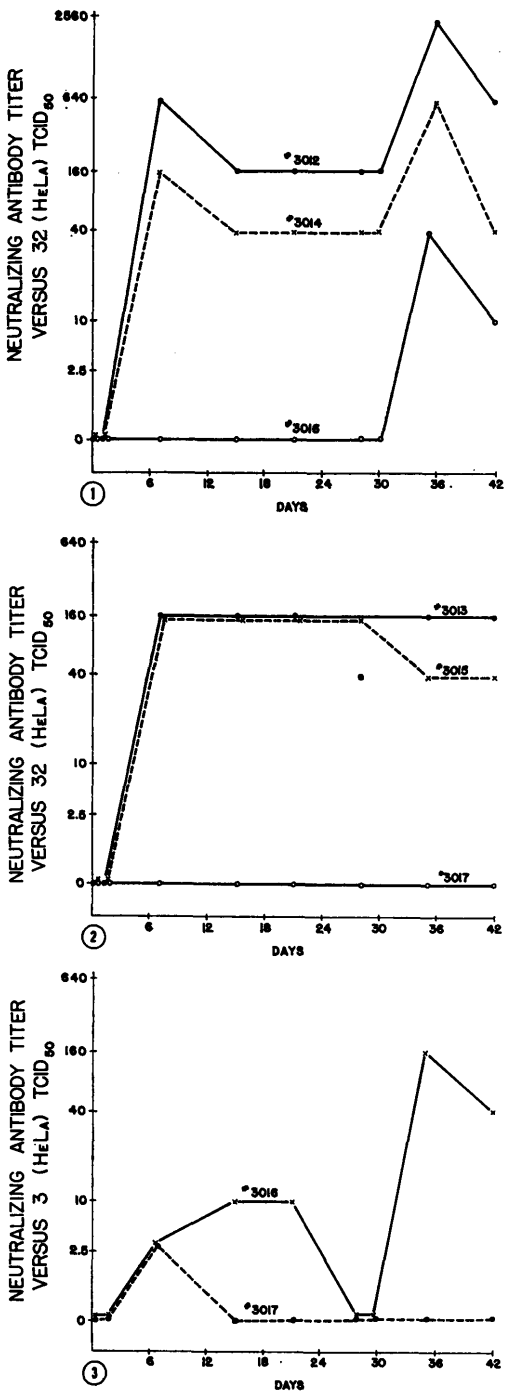

FIG. 1. Neutralizing antibody response of rabbits to Adenovirus Type 12. Rabbits received a single dose intravenously on day 0 as follows: Rabbit #3012, 30,000,000 $TCID_{50}$; Rabbit #3014, 300,000 $TCID_{50}$; and Rabbit #3016, 3,000 $TCID_{50}$. All rabbits received a booster inoculation of 300,000 $TCID_{50}$ on day 28.

mented with 100 μ of penicillin and polymyxin and 100 μg streptomycin per ml.

All sera were inactivated at 56°C for 30 minutes prior to testing. Serial 4-fold dilutions of sera were prepared in Hanks' BSS and each dilution was mixed with equal volumes of virus, diluted to contain approximately 100 (HeLa) $TCID_{50}$ per 0.1 ml at the end of 7 days. Challenge virus was assayed in the actual test. Virus-serum mixtures were allowed to incubate 30 minutes at room temperature before inoculating triplicate HeLa cell cultures with 0.2 ml each. Cell cultures were examined at 3 and 7 days. Appropriate controls were included in all tests. Neutralization titers are defined as the reciprocal of the highest serum dilution causing complete inhibition of virus cytopathology.

Results. Fig. 1 and 2 illustrate the antibody response of rabbits immunized with 30,000,000; 300,000; and 3,000 $TCID_{50}$ of Adenovirus Type 12 virus.

It is apparent that with a single injection of both the higher doses, essentially maximum titers of 640 were attained within 7 days, decreasing slightly to a stable value at day 15. There was no detectable antibody response to the lowest dose of antigen (3,000 $TCID_{50}$). When animals in each group were reinjected with 300,000 $TCID_{50}$ 28 days after the initial injection, a typical secondary response was noted in all animals including that (#3016) initially immunized with 3,000 $TCID_{50}$. In all rabbits receiving a booster injection a significant drop in titer was observed in the 42-day bleeding.

The failure of rabbits to respond to a primary injection of only 3,000 $TCID_{50}$ was apparent (Fig. 3) rather than real. When the sera from these animals were retested against 3 (HeLa) $TCID_{50}$ instead of 32,

FIG. 2. Neutralizing antibody response of rabbits to Adenovirus Type 12. Rabbits received a single dose intravenously on day 0 as follows: Rabbit #3013, 30,000,000 $TCID_{50}$; Rabbit #3015, 300,000 $TCID_{50}$; and Rabbit #3017, 3,000 $TCID_{50}$.

FIG. 3. Neutralizing antibody titer in rabbits immunized with a single dose of 3,000 $TCID_{50}$. Rabbit #3016 received a booster inoculation of 300,000 $TCID_{50}$ on day 28.

both showed significant, if slight, antibody response falling off to undetectable levels 3 to 4 weeks after the initial injection with typical rapid secondary response in the animal reinjected on day 28.

Although the highest levels of antibody were achieved in the 2 animals receiving the highest dosage (1.5 ml of culture fluid—30,000,000 $TCID_{50}$), one of 2 animals receiving 1/100 as much virus (only 0.015 ml of culture fluid) responded equally well (#3015). It is apparent, however, that this is approximately the smallest dose capable of eliciting a maximal response.

Discussion. Studies on the immunologic relationship between strains of Adenovirus Type 12, and on their oncogenicity, will obviously be facilitated through development of an *in vitro* method of identification.

Such tests have been described for Poliovirus by McBride(9); and Rozee and Casey (10). McBride(9) has stated "for best resolution of differences among Poliovirus strains, it is best to use comparatively early antisera"; and it is quite possible that among Adenovirus strains also, maximum resolution will be attained with specific antiserum prepared against small quantities of virus as here described. With the present method also, antiserum of usable titer can be obtained as early as 7 days after injection, with relatively small amounts of virus, and blood can be drawn up to 28 days later without appreciable losses in titer. If higher titered antiserum is desired, a booster inoculation of low dosage (300,000 $TCID_{50}$) raises antibody titers to higher levels for a short time.

Additional studies are in progress to determine the antibody response of rabbits to single doses of other Adenovirus types, as well as other Type 12 strains, and to explore the possibility of strain differences within types.

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Prevention of Ovarian Cyst Formation by Ethamoxytriphetol (MER-25).* (28329)

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Ethamoxytriphetol (MER-25), an estrogen antagonist(1-3) will counteract the enhanced ovarian sensitivity of the hypothyroid rat to chorionic gonadotrophin(4). Furthermore, the compound may be useful in treatment of human polycystic ovaries(5). However, no evidence has been presented to determine whether or not MER-25 will prevent any of the biochemical changes which occur in the ovary in association with ovarian cyst formation. Cystic ovaries of hypothyroid-chorionic gonadotrophin treated rats exhibit a characteristic subnormal concentration of both free and esterified cholesterol whereas the ovaries from euthyroid rats following chorionic gonadotrophin reveal a decrease in esterified cholesterol only(6,7). The present study involves the influence of MER-25 on rats subjected to an experimental regime for ovarian cyst induction. Adrenal cholesterol and hypo-

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