

neutral fats and phospholipid fractions in the serum.

The appearance of these deleterious effects in rabbits would seem to warrant a search for

similar effects in other animal species including man.

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Alterations in Rate of Influenza Virus Proliferation Produced by Growth Hormone and Testosterone.* (17990)

SEYMOUR S. KALTER, DONALD C. STUART, JR.,[†] AND JAY TEPPERMAN

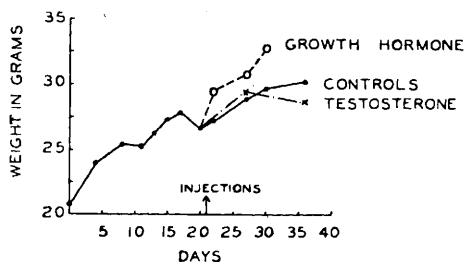
From the Departments of Bacteriology and Parasitology, the Virus Laboratory, Bureau of Laboratories, and the Department of Pharmacology, University of the State of New York Medical Center, Syracuse, N. Y.

Recent studies have revealed marked differences in the susceptibility of mice of different ages to influenza virus infection. With advancing age there is a progressive increase in the amount of virus necessary to produce a lethal infection(1). These observations led to the development of the hypothesis that the rate of virus proliferation is, in part, dependent upon the rate of protein anabolism in the tissues of the host. It was, therefore, decided to study the effect on virus proliferation of deliberate alterations in the protein anabolic activity of the host. Since it is well established that the administration of pituitary growth hormone and testosterone results in increased rates of protein synthesis in the host(2,3), these substances were selected for use. The following report, then, is concerned with the rates of virus proliferation in growth hormone-treated and testosterone-treated mice as compared with appropriate controls.

Materials and methods. Female Swiss mice were employed throughout these experiments. They were obtained from the same source at the age of approximately 8 weeks and maintained in the laboratory on Purina dog chow and water *ad libitum*. The mice were

weighed 3 times weekly, and, after a period of about 20 days, their weight curves "plateaued."

Both hormones were administered by subcutaneous injection. The growth hormone[‡] was given for a period of 10 days, each mouse receiving 0.2 mg in a single injection per day. Testosterone[‡] was given in 3 weekly injections, each mouse receiving 5.0 mg per week of a commercially prepared aqueous suspension. Control mice received appropriate injections of the vehicle in which the hormone was dissolved. The day after the last hormone injection, approximately 35-40 mice of both treated and control groups were inoculated intranasally with a 10^{-5} dilution of mouse-adapted PR 8 influenza virus. This inoculum was equivalent to approximately



THE EFFECT OF GROWTH HORMONE AND TESTOSTERONE ON THE WEIGHT OF MATURE FEMALE MICE.

FIG. 1.

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1. Kalter, S. S., *J. Immunol.*, 1949, v63, 17.

2. Li, C. H., *Growth*, 1948, v12 suppl., 47.

3. Kochakian, C. D., In R. S. Harris and K. V. Thimann, *Vitamins and Hormones*, 1946, v4, 255.

[‡] We wish to thank Irby Bunding of Armour and Co. for a generous supply of Growth Hormone (Lot 22kR1) and Fred Houghton of Ciba Pharmaceutical Products, for the Testosterone used in this study.

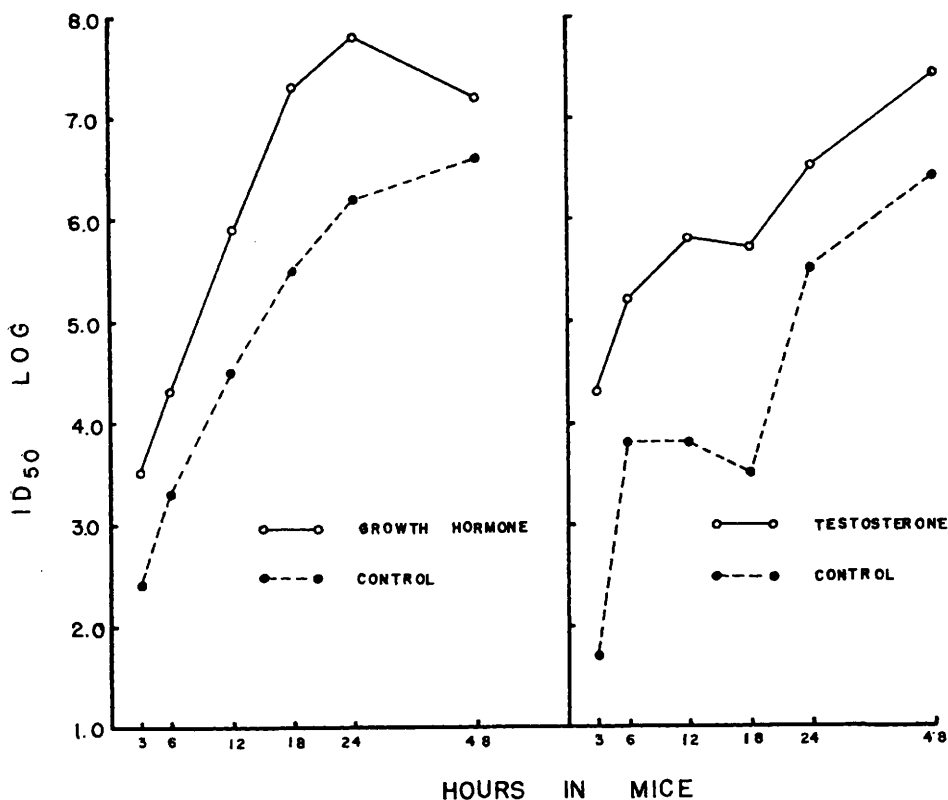


FIG. 2.

The effect of growth hormone and testosterone on the rate of proliferation and amount of influenza virus in mice. The ID₅₀ was determined by titration in chick embryos.

500 x the LD₅₀ dose when titrated in 4-5-week-old mice. Following virus inoculation, 5 treated and 5 control mice were killed, the lungs removed and blotted on filter paper, pooled in their respective groups, weighed, quickly frozen and stored at -70°C. The mice were sacrificed 3, 6, 12, 18, 24 and 48 hours after infection. When all the samples had been collected, 10% suspensions of the lungs were homogenized with buffered saline in a Waring blender and centrifuged. For the egg titrations, dilutions were made in buffered saline, containing sufficient penicillin and streptomycin to insure sterility, and each dilution inoculated into 11-day-old fertile embryos. Five embryos were employed for each dilution. The allantoic fluids were harvested in the usual manner and the LD₅₀ determined by the method of Reed and Muench. Hemagglutination tests were also

made of the mouse lung preparations in the usual manner(4).

Results. The effect of growth hormone and testosterone on the weights of the mice may be seen in Fig. 1. Approximately a 4.0 g increase in weight was obtained by using the growth hormone. No significant weight difference was observed following the use of the testosterone.

Treatment with both hormones resulted in an increased rate of virus proliferation and an increase in amount of virus present. This is demonstrated in Fig. 2. Little difference in virus proliferation was apparent between the growth hormone and testosterone treated animals. These experiments were repeated and the same relationship was observed between the treated and control animals.

4. Hirst, G. K., *Science*, 1941, v94, 22.

Hemagglutination determinations of the lung suspensions also demonstrated a difference between the treated and control animals. However, this method of assay which is known to be less sensitive than the *in ovo* titration, revealed less striking differences between experimental and control groups.

Discussion and conclusions. If virus proliferation is partially dependent upon the host's protein synthetic mechanisms, an increase in the rate of protein anabolism in

the host may be reflected in an increased rate of virus growth. Under the conditions of these experiments, the data obtained are not inconsistent with this hypothesis.

Summary. When the rate of protein anabolism in the host was increased by testosterone or growth hormone, increases in rate of proliferation and amount of influenza virus were clearly demonstrated.

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A Rapid Method for Detection of Influenza Virus During Epidemics.* (17991)

SEYMOUR S. KALTER

From the Department of Bacteriology and Parasitology, University of the State of New York Medical Center, Syracuse, N. Y., and the Virus Laboratory, Bureau of Laboratories, Department of Health, Syracuse, N. Y.

The established procedures for the detection of influenza in a community are: (a) isolation of the virus, (b) serological examination of acute and convalescent phase serum specimens, and (c) the inoculation of susceptible animals with throat washings. Regardless of the method, these procedures require several days as a minimum before identification of the agent may be made. During the current epidemic, a screening method has been employed which enables the laboratory to ascertain the causative agent in one day.

Materials and methods. Twenty-eight throat washings were obtained during the epidemic from patients in the student infirmary. These patients were admitted with the clinical diagnosis of either influenza or respiratory infection. Usually the throat washing was obtained during the first 3 days of illness. The patients first coughed several times and then gargled with approximately 15 ml of nutrient broth. These throat washings were then quickly frozen and stored at -70°C until tested. Control throat washings were obtained from 10 persons who gave no history

of illness during the previous 3 week period. The throat washings from patients and controls were treated in an identical manner.

The throat washings were thawed under running water and 10 ml removed. The remaining 5 ml was used for isolation of virus by amniotic inoculation of 13-day-old chick embryos. Sufficient 1% red blood cells (either chicken or human type O) in buffered saline were added to each throat washing in order to compensate for the hemolysis which sometimes occurred. This was usually in the order of 2-4 ml. After standing at room temperature for $1\frac{1}{4}$ hours, the material was centrifuged and the supernatant fluids discarded. To the red cell sediment was added 0.8 ml saline, the mixture shaken and incubated in a 37°C water bath for 2-4 hours with occasional shaking. Following this period of elution, the material was again centrifuged and the supernatant fluids removed to small tubes. Then 0.6 ml of each supernatant was removed and 0.2 ml placed in each of 3 tubes. To the first tube, 0.2 ml 1% fresh erythrocytes were added, to each of the other 0.2 ml samples, 0.2 ml red cells (1%) in a 1:50 dilution of specific antiserum was added. The antisera employed were PR 8, Lee and FM-1. The FM-1 antiserum was

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