levels of these tropins were largely restored within 11 hours after mating. Pituitary FSH was not significantly influenced by copulation. The results indicate that the mating stimulus is not specific for ovulating hormone release and suggest that other pituitary tropins may affect reproductive function near the time of ovulation.

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Antibody Response in Rabbits to Antigenic Stimulation During Amantadine Treatment.* (32359)

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The anti-influenzal effect of amantadine hydrochloride was initially demonstrated by the absence of a serologic response in volunteers challenged with attenuated live Influenza A_2 virus(1). To determine whether the effect could have resulted from drug inhibition of antibody synthesis rather than the prevention of infection, rabbits were treated with amantadine, dextrose, or 6-mercaptopurine and were inoculated with a viral and non-viral antigen.

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Materials and methods. Animals. Female New Zealand white rabbits weighing 2 kg each were used.

Drug regimen. Amantadine hydrochloride was administered intramuscularly (IM) to 6 rabbits in a dose of 12.5 mg/kg/day in 1.0 ml of normal sterile saline solution and to 6 rabbits intraperitoneally (IP) in a dose of 25.0 mg/kg/day in 1.0 ml of saline solution. As controls, 3 rabbits were given 12.5 mg/kg/day of dextrose in saline solution IM, and 3 received 25 mg/kg/day in 1.0 ml, IP. Six rabbits were given 6-mercaptopurine (6-MP) in a dose of 6 mg/kg/day, intramuscularly. A fresh solution of 6-MP was made each day by dissolving 84 mg of drug in 1.19 ml of 1.0 N sodium hydroxide, which was diluted with 5.81 ml of saline solution.

TABLE I. Antibody Response in Rabbits Given Dextrose, 6-MP or Amantadine to Asian Influenza.

Treatment	Time of serum collection: Day 16 No. with rise/total	Day 16 Titer of reactors	Day 28 No. with rise/total	Day 28 Titer of reactors	Day 42 No. with rise/total	Day 42 Titer of reactors
Exp 1						
Dextrose	4/5	80	5/5	256	5/5	320
Amantadine, IM 12.5 mg/k	5/5	48	5/5	160	5/5	144
IP 25 mg/k	5/5	72	5/5	128	5/5	128
6-MP	3/5	136	5/5	192	4/4	144
Exp 2						
Dextrose	6/6	160	5/5	768	5/5	1280
Amantadine, IM 25 mg/k	7/7	128	6/6	832	6/6	2304
IM 50 mg/k	4/4	272	4/4	1088	4/4	1920

All solutions were sterilized by filtration through a 450 mμ Millipore® filter and given once daily, beginning 24 hours before the initial injection of the antigen and continuing for 16 days.

Antigens. All animals were immunized with 50 mg of bovine serum albumin given intramuscularly 1, 3, and 8 days after drug treatment was started. Simultaneously with injection of BSA, all animals were injected, subcutaneously, with 0.75 ml of Influenza A_2/Japan/305/57 containing 450 CCA units emulsified with 0.25 ml of complete Freund's Adjuvant.

Serology. Blood was collected one day before and 3, 7, 11, 16, 23, 30, 35, and 42 days after the initial inoculation of antigens. Serologic response to BSA was titrated by hemagglutination of sensitized tanned erythrocytes (HA). The titer of antibody for Influenza A_2/Jap/305/57 was measured by hemagglutination inhibition (HI) using chicken erythrocytes and 4 HA units of homologous antigen.

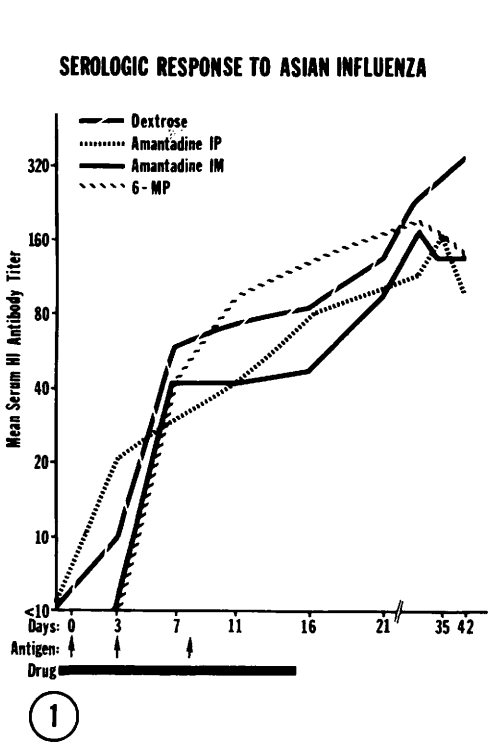
Destruction of nonspecific inhibitors. Inhibitors of viral hemagglutination in normal rabbit serum were removed by treatment of the serum with trypsin and periodate(9), and followed by incubation of 0.6 ml of diluted serum with 0.4 ml of 25% acid-washed kaolin which, after standing for 20 minutes, was removed by centrifugation. The resultant serum dilution was considered as 1:10.

Results. All rabbits reacted with a significant serologic response to Asian Influenza virus by 28 days after initial antigenic stimulus regardless of drug regimen (Table I, Experiment 1). Of 20 rabbits tested, only 3 (one given dextrose and 2 given 6-MP) failed to respond while the drug was being administered. The mean serum HI antibody titer of those animals which reacted to virus antigen on each of the test drugs is plotted in Fig. 1. Titers were calculated only when 2 or more rabbits reacted. It can be observed that responses were observed in all groups by 7 days after initial antigenic stimulation, and never was the difference between the groups 4-fold or greater.

A trend toward suppression of antiviral antibody synthesis during and shortly after administration of 6-mercaptopurine was observed in the number of animals which failed to react. This was more pronounced when bovine serum albumin (BSA) was the antigen. While drug was being administered, no animal had detectable antibody titers specific for BSA, and 2 animals failed to respond by the 27th day after drug was stopped (Table II, Exp. 1). The mean titer of 6-MP-treated rabbits was significantly reduced throughout the 42-day test period (Fig. 2).

Animals injected with BSA and given amantadine IM had a slight depression in antibody synthesis as seen by the number of animals which reacted by day 28, and the mean titer on those which did respond when compared to the dextrose-treated control rabbits. The decreased mean titer of rabbits which reacted was greater than a 4-fold difference in comparison to the control rabbits only on day 16. This suppression was not observed in rabbits given amantadine IP.

To validate the observed effect of amanta-



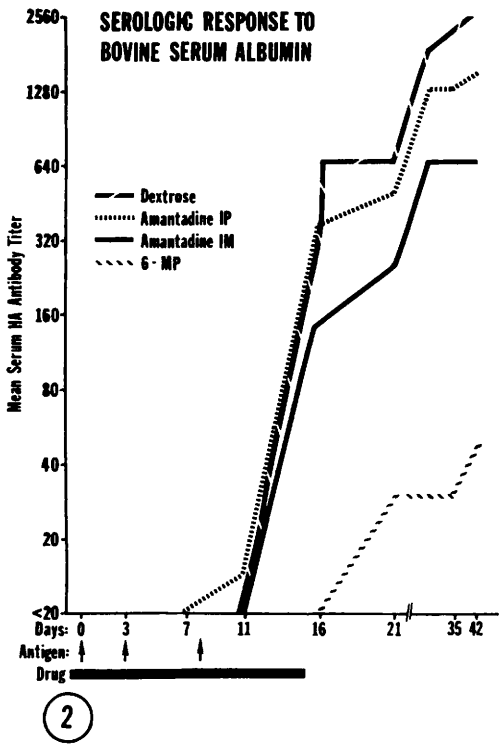


FIG. 1. Serologic response to Asian Influenza.
FIG. 2. Serologic response to bovine serum albumin.

dine, a second series of immunizations was performed using twice and four times the dose of drug given in Experiment 1. Also, nearly twice the dose of viral antigen was given without adjuvant, but the same dose of BSA was used. Control animals were given dextrose and all drugs were administered IM for a period of 12 days, beginning the day prior to initial antigen injection, as in the first experiment. Antigen was administered 1, 2, and 5 days after initiation of the drug regimen rather than on days 1, 3, and 8, as in the first experiment.

The response to influenza virus is shown for both experiments in Table I. Variation in the number of animals reacting, the time of response, or the mean titer in the first experiment was not confirmed and was probably

TABLE II. Antibody Response in Rabbits Given Dextrose, 6-MP or Amantadine to Bovine Serum Albumin.

	Time of serum collection					
	Day 16		Day 28		Day 42	
Treatment	No. with rise/total	Titer of reactors	No. with rise/total	Titer of reactors	No. with rise/total	Titer of reactors
Exp 1						
Dextrose	5/5	640	5/5	2048	5/5	2560
Amantadine, IM 12.5 mg/k	3/5	144*	3/5	640	5/5	640*
IP 25 mg/k	4/5	384	5/5	1280	5/5	1536
6-MP	0/5	<20*	2/5	30*	3/5	52*
Exp 2						
Dextrose	5/5	144	5/5	384	5/5	1024
Amantadine, IM 25 mg/k	7/7	80	6/6	320	6/6	832
IM 50 mg/k	4/4	160	4/4	480	4/4	1088

* 4-fold or greater difference in titer as compared to dextrose.

due to the small number of animals. All HI titers in the second experiment were within a 2-fold difference of the control animal regardless of the dose of the drug. The difference in the antiviral antibody titers is considered to be the result of an increased antigenic mass used in the second immunization.

The response to BSA after drug administration is shown in Table II. In the second experiment, all animals had a response while being given drug, and the mean titers observed were within a 2-fold difference even though a total of 50 and 100 mg of drug was given. The difference in the anti-BSA titers in the 2 experiments may be due to the difference in time of injection of 2 different lots of BSA.

Discussion. On the basis of these studies, the reduction that occurred in the number of serologic responses in volunteers pretreated with amantadine was correctly interpreted as evidence of antiviral activity(1). Analysis of the mean rise in antibody titers in volunteers given drug as compared to placebo also indicated that the reduction was from the antiviral activity of amantadine and not caused by an inhibition of antibody synthesis. A similar conclusion was derived from analysis of data of amantadine-treated virus infected mice(2). In these experiments employing rabbits given a measured antigenic stimulus, the results provide direct evidence that viral HI antibody synthesis specific for Asian Influenza was not suppressed by amantadine. At the time when the drugs were stopped, all animals, regardless of route or dose, had a significant rise in titer to Asian Influenza, and all animals proceeded to synthesize antibody to the same level within the expected statistical variation among groups. Differences between the two sets of animals used are within one standard deviation of the control. The action of 6-mercaptopurine on non-viral antigens has been reviewed elsewhere(3,4,5).

Little action of 6-MP on antiviral antibody synthesis was found in these experiments using rabbits nor in others using monkeys or guinea pigs. The reason does not seem to be antigenic potency of different antigens, routes of injection, variance of antigenic mass, or disposition of antigen. It appears, however, that the type and sensitivity of the serologic test to measure the response of animals treated with immunosuppressive drugs are of great importance.

With respect to viruses, the severity of disease induced in variola-challenged monkeys was enhanced by 6-mercaptopurine(6). A similar effect was observed in rabbits infected with vaccinia, but not with variola(7). The antibody levels, however, were depressed. In 6-MP-treated guinea pigs, antibody levels against Adenovirus type 5 were significantly lower 4 to 19 days after infection, but later they were comparable to the titers observed in control animals(8).

The most striking immunosuppressive effect was seen in the animals treated with 6-MP and tested for anti-BSA antibody. This group had no significant serologic rise in antibody while on drug, and the number of animals which responded later was lower than expected. In this test system, the effects of 6-MP and amantadine were strikingly different. The latter caused no delay in the response and insignificant lowering of the peak titer.

Summary. Rabbits were treated with dextrose, amantadine hydrochloride, or 6-mercaptopurine and simultaneously injected with Influenza A_2/Jap/305/57 and bovine serum albumin. Amantadine administered by different routes and in several doses had no effect on the rate or titer of antibody synthesis of these two antigens. A definite immunosuppressive effect was observed in rabbits treated with 6-MP, but this effect was only observed in anti-BSA synthesis. No effect was observed using influenza virus as antigen.

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Effects of Psychic Stress on Atherosclerosis in the Squirrel Monkey (*Saimiri sciureus*).* (32360)

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Evidence is increasing that psychological factors influence both naturally occurring and experimentally induced atherosclerosis of animals. Ratcliffe and Cronin(1) presented evidence that an increase in the prevalence of arterial lesions found among animals and birds dying at the Philadelphia Zoological Garden was related to intraspecies social pressures. More recently, Ratcliffe and Snyder(2) demonstrated a relationship between the social grouping of White Leghorn chickens and the occurrence of myocardial infarcts in these birds. Pick and coworkers(3) found that the extent of atherosclerosis in cholesterol-fed cockerels could be increased by placing them in unnatural environments. Additional evidence for a relationship between psychological factors and atherosclerosis has recently been reviewed by Sherman(4).

The purpose of this paper is to describe the long-term effects of psychic stress on serum cholesterol levels and coronary artery atherosclerosis in squirrel monkeys (*Saimiri sciureus*).

Method. The squirrel monkeys used in this study were the Brazilian type and were supplied by the Tarpon Boo, Tarpon Springs, Florida, from their supply source at Leticia, Colombia, South America. On the basis of dentition and size the monkeys were estimated to be about one year of age at beginning of the experiment.

Eighteen monkeys were arbitrarily divided into 3 groups, each consisting of 4 males and 2 females. The groups were designated as (I) psychic stress group, (II) box control group and (III) cage control group.

Each of the monkeys in the first 2 groups was placed in a Skinner box for 1 hour per day, 5 days a week. During this time the monkeys in Group I were subject to additional psychological stress by the Sidman avoidance procedure(5,6). In this procedure a brief electric shock is administered without warning stimuli. In our experiment a 2-volt shock 3 seconds in duration was delivered to the tail if the animal failed to press a lever within a period of time which varied from 30 to 50 seconds on a day-to-day basis. Each time the monkey pressed the lever, the cycle would automatically return to its beginning. The monkey could not avoid the

TABLE I. Composition of Diets (g/100 g).

Ingredient	Period 1	Period 2
Purina Monkey Chow	45	40
Boiled eggs	48	24.5
Dried egg yolk	—	15
Sucrose	—	10
Casein, U.S.P.	5	8
Vitamin mixture*	1.75	2
Cholesterol, U.S.P.	.25	.50

* Complete Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp.

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