(complement) exists in turtle serum (*2, 4, 9*). In my work, certain of the agglutinating reagents (normal and immune) have been heated when necessary to prevent lysis of the turtle cells being tested. Fresh serums from two Florida snapping turtles (*Chelydra serpentina osceola*) have lysed sensitized sheep cells (*10*).

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References and Notes

1. W. H. Hildemann, in *Blood Groups in Infrahuman Species*, C. Cohen, Ed., *Ann. N.Y. Acad. Sci.* **97**, 139 (1962). See also G. I. A. Svet-Moldavskii, *Biul. Eksptl. Biol. Med.* **38**(9), 54 (1954); E. E. Evans, *Proc. Soc. Exptl. Biol. Med.* **112**, 531 (1963); T. A. Rees, D. J. Perkins, S. D. Elek, *Comp. Biochem. Physiol.* **8**, 123 (1963).
2. H. Noguchi, *Zentr. Bakteriol. Parasitenk.* **33**, 353 (1903).
3. C. M. Downs, *J. Immunol.* **15**, 77 (1928).
4. G. C. Bond, *ibid.* **39**, 125 (1940).
5. Capillary tubes used for orbital bleeding were those supplied with Unopettes® (Becton, Dickinson and Co., Rutherford, N.J.).
6. V. Riley, *Proc. Soc. Exptl. Biol. Med.* **104**, 751 (1960); P. J. Berger, *Turtox News* **40**, 55 (1962).
7. Scientific names of all turtles are from H. Wermuth and R. Mertens, *Schildkröten, Krokodile, Brückenechsen* (Fischer, Jena, 1961). Common names of United States amphibians and reptiles are from R. Conant, *A Field Guide to Reptiles and Amphibians* (Houghton Mifflin, New York, 1958) and *Copeica*, No. 3, 172 (1956).
8. R. J. DeFalco, *Bull. Serol. Museum* No. 19, 1 (1957).
9. H. Noguchi, *Zentr. Bakteriol. Parasitenk.* **33**, 362 (1903); T. Amako, *Z. Chemother.* **1**, 224 (1912); K. D. Jentzsch, *Zentr. Vetmed.* **9**, 600 (1962).
10. Dr. Mabel Boyden performed all extractions from plant tissues and she set up and recorded most agglutinations using *Testudo hermanni, Clemmys caspica, Geoemyda p punctularia*, and *Emys orbicularis*. Dr. Ralph J. DeFalco gave valuable suggestions and advice. Mr. Thomas Mao titrated *Chelydra* complement, and Mr. René E. Honegger supplied *Testudo hermanni* and some other foreign turtles. Most of this research was performed at Rutgers University, New Brunswick, N.J., and much of it is condensed from a portion of a Ph.D. dissertation (1962).

8 April 1963

Poliomyelitis in Monkeys: Decreased Susceptibility after Avoidance Stress

Abstract. *Eleven monkeys were subjected to avoidance stress for 24 hours followed immediately by intravenous inoculation with type I poliovirus. Twelve control monkeys not so stressed were similarly inoculated. Seven of 11 stressed animals survived the infection while only one of the controls lived and their average incubation period was significantly longer than the average for controls. The number of circulating lymphocytes decreased significantly in experimental animals during and immediately after exposure to stress.*

Changes in resistance to herpes simplex virus and to anaphylactic shock in mice exposed to the stress of confinement or avoidance in a shuttlebox have been reported (*1–3*).

We now summarize effects of stress on resistance to poliovirus infection in cynomolgus monkeys.

In these experiments the Sidman avoidance procedure was used (*4*). Animals learned to press a telegraph key at a steady rate to avoid a shock to the tail which would be delivered once every 10 seconds if the lever was not pressed. Monkeys learn this procedure well and when fully trained receive few shocks during long periods of exposure. Despite this fact, the avoidance procedure is stressful for monkeys. During prolonged periodic exposure increased amounts of 17-hydroxycorticosteroids are found in blood and urine (*5*), and fatal gastrointestinal disorders develop (*6*).

The experiment was repeated five times with a total of 23 adult male monkeys. Throughout each experiment the animals lived in chairs (*7*) in which they were held loosely at the neck and waist. All 11 experimental animals and 7 of the 12 controls were trained to make the avoidance response; five controls were not trained. At the end of training all animals were permitted to rest for at least 9 days during which three blood samples were taken for base line total and differential white blood cell counts. Experimental animals were then exposed to the avoidance situation continuously for 24 hours, with a 10-minute interruption for feeding midway. Control animals remained in adjacent chairs but were not subjected to stress. In two experiments blood samples were taken at 3-hour intervals during the stress period to determine changes in white blood counts. At the conclusion of the 24-hour period of stress blood samples were taken, and experimental and control monkeys were inoculated intravenously with 100 tissue culture ID_{50} (50 percent infectious dose) of Brunhilde strain of type I poliovirus. Thereafter, blood samples were taken daily and the animals were observed for the development of fever and paralytic disease.

Exposure to the stressful avoidance situation results in a marked decrease in susceptibility to poliovirus infection. Whereas 7 of 11 stressed animals survived the infection and recovered, only 1 of the 12 controls lived. This difference in survival rates is significant at the .01 level (Fisher exact test). Three of the stressed animals showed little or no residual paralysis. The other four survivors, while appearing healthy, showed some residual leg, arm, or facial paralysis. Except for a higher incidence of quadriplegia and bulbar involvement in controls, paralytic patterns did not differ in the two groups. Prolonged incubation is also evidence of the protective effect of exposure to avoidance stress (Table 1). On the average, stressed animals developed symptoms 2 days later than controls (6.8 days as against 4.8). This difference tested by *t*-test is significant at the .001 level.

The number of circulating lymphocytes decreased in experimental animals during exposure to stress. Values for stressed animals were significantly lowered at the end of the 24-hour avoidance period, as well as 4 hours after its termination. In those instances where blood samples were taken at 3-hour intervals during exposure to stress, mean lymphocyte values were significantly lower for experimental animals than

Table 1. Effects of avoidance stress on susceptibility to poliomyelitis. Signs of experimental infection were tremor and paralysis with difficulty in breathing and swallowing. Four monkeys, two stressed and two controls, died overnight without developing detectable paralysis; all four showed tremor a day before death. The remaining animals that died all exhibited paralysis before death.

Mortality ratios		Mean incubation (days)	Number of monkeys developing poliomyelitis at times after inoculation					
Total	Died		Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
			Stressed monkeys					
11	4	6.8	0	2	3	2	1	2
			Control monkeys					
12	11	4.8	5	4	1	1	0	0

for controls after 3 hours of avoidance stress. Significantly lowered values were observed for control monkeys on the 4th and 5th days after inoculation. Presumably these values reflect a response to the developing stress of infection which occurred earlier in controls.

These results contrast sharply with previous findings on reduced resistance to virus infection in mice subjected to shuttle box stress (*1, 2*). They also contrast with observations of increased susceptibility to poliovirus in hamsters and mice treated with cortisone before inoculation (*8, 9*). One factor which may be important in accounting for the directional difference in susceptibility is the schedule of exposure to stress. In experiments with mice an intermittent "chronic" stress schedule was used in which the animal was exposed for 6 hours daily with 18 hours of rest between exposures for a period of weeks, whereas, in the monkey, exposure was to a single "acute" 24-hour period of avoidance stress. A period of at least 14 days of intermittent exposure to stress was the minimum for producing decreased resistance to virus infection in the mouse in contrast to the 24-hour period which proved effective for increasing resistance in the monkey. In earlier work on the mouse (*10*) it was demonstrated that physiological changes, presumably related to pituitary adrenal function, occurred very early in exposure to intermittent stress, as did increased resistance to anaphylactic shock. Resistance decreased along with thymus and spleen involution only after 14 or more days of exposure to stress.

Seven of the 12 control monkeys received the original avoidance training because of the possibility that it and the stress associated with it might influence subsequent response to stress during the experiment. This did not prove to be true as all of the trained controls succumbed to polio while 7 of the 11 trained stressed animals did not. Similarly, the duration of the rest period (ranging from 9 to 480 days) between original training and the experiment did not affect results.

The effects of shock per se on resistance might be questioned since controls received no shocks. If shock was a crucial factor, some correlation between the number sustained by stressed animals and resistance to poliovirus might be expected. The total number of shocks sustained in the 24-hour period ranged from a minimum of 155 to 6042 in one animal. (The latter resulted from apparatus failure.) The four that died ranked fourth, sixth, eighth, and tenth among the 11 stressed monkeys in terms of the number of shocks. The number clustered about the mean for the group. Similarly there was no correlation between the number of shocks and the length of incubation period (see *11*).

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References and Notes

1. A. F. Rasmussen, J. T. Marsh, N. Q. Brill, *Proc. Soc. Exptl. Biol. Med.* **96**, 183 (1957).
2. T. Johnsson, J. Lavender, J. Marsh, *Federation Proc.* **18**, 575 (1959).
3. A. F. Rasmussen, E. S. Spencer, J. T. Marsh, *Proc. Soc. Exptl. Biol. Med.* **100**, 878 (1959).
4. M. Sidman, *Science* **118**, 157 (1953).
5. R. W. Porter, J. V. Brady, D. Conrad, J. W. Mason, R. Galambos, D. M. Rioch, *Psychosomat. Med.* **20**, 379 (1958).
6. J. W. Mason, J. V. Brady, E. Polish, J. A. Bauer, J. Robinson, R. M. Rose, E. D. Taylor, *Science* **133**, 1596 (1961).
7. J. W. Mason, *J. Appl. Physiol.* **12**, 130 (1958).
8. G. Schwartzman, *Proc. Soc. Exptl. Biol. Med.* **75**, 835 (1950).
9. G. M. Findlay and E. M. Howard, *J. Pharm. Pharmacol.* **4**, 37 (1952).
10. J. T. Marsh and A. F. Rasmussen, *Proc. Soc. Exptl. Biol. Med.* **104**, 180 (1960).
11. Supported in part by grant 2M-6415 from U.S. Public Health Service and research grant FD-GR-60-2 from the Army Chemical Corps. One of us (S.S.C.) had a grant from the International Cooperation Administration under the Visiting Research Scientists Program administered by the National Academy of Sciences.

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Tumors Induced in Primates by Chicken Sarcoma Virus

Abstract. *A suspension of a variant of Rous sarcoma was injected into four adult and eight newborn rhesus monkeys. Seven newborns developed tumors, three of which were diagnosed as fibrosarcomas, in 2 to 6 weeks; none of the adults have tumors after 11 weeks. Virus was demonstrated in two of the tumors by injecting a tumor-suspension preparation into the chick wing-web where tumors subsequently appeared. To the best of our knowledge this is the first time that sarcomas have developed in primates after a virus has been injected.*

A variant of Rous chicken sarcoma was injected into small laboratory animals. Newborn rats developed angiomas and conditioned rats developed fibrosarcomas (*1*), newborn and adolescent hamsters developed pleomorphic giant cell sarcomas, newborn guinea pigs developed fibromas, and adolescent mice developed fibrosarcomas (*2*). The strain of chicken sarcoma was obtained from L. A. Zilber of the Gamaleya Institute in Moscow (*3*). Another strain has been used successfully to induce tumors in hamsters, guinea pigs, and mice (see *4*).

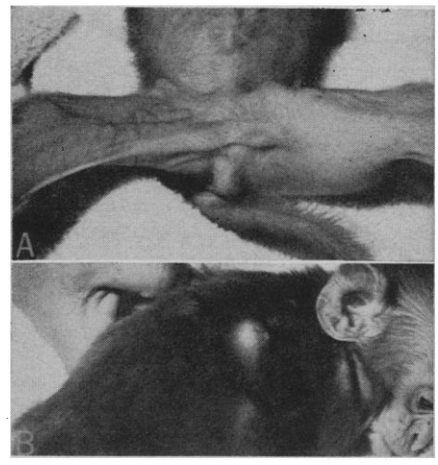

Fig. 1. Tumor in left thigh of monkey No. 403 (top) extending from pelvis to knee and causing a threefold increase in circumference as compared with right thigh. Recurring subcutaneous tumor in the back near the inferior angle of the scapula in monkey No. 397 (bottom). The scar over the lesion resulted from excision of a tumor of similar size at this site 3 weeks previously.

Fourteen monkeys (*Macaca mulatta*) were used in our study. Tissues of two newborn monkeys involved in fatal neonatal accidents were used as noninjected controls. The remaining 12 monkeys were injected with suspensions of chicken sarcoma. Four of these were 5- to 7-year-old adult monkeys; eight were newborn monkeys (*5*).

One of the newborn monkeys, No. 406, a premature, on the 6th day after injection died from what seemed to be a systemic illness with weakness, anorexia, weight loss, leukocytosis, and low-grade fever—all of which began on the day after virus injection. This generalized illness was also noted in most of the virus-injected monkeys, including the adults, within the first 10 days. Virus was demonstrated in the liver and at the site of injection (thigh) of monkey No. 406 by the chick wing-web technique (injection of a suspension of organs or tumor material into the wing web of chickens, 3 to 5 days old, which results in tumors).

All of the other newborn monkeys