tempted to determine how long the learning would persist in this preparation.

In naïve preparations, the presentation of the CS does not normally evoke activity in gill motor neurons L_7 and LDG_1 (*10*). However, it may be that with classical conditioning the CS will evoke activity in these neurons. If so, a neural correlate of associative learning can be examined.

We have shown for what we believe to be the first time that a well-studied in vitro model system has the capacity for associative learning. All the advantages that the *Aplysia* siphon, mantle, gill, and abdominal ganglion preparation has had for the study of nonassociative adaptive behaviors, such as habituation and sensitization, can now be used to study the neural mechanisms underlying associative learning.

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2. Associative learning is defined in an operational manner and by control procedures [G. J. Mpitsos, S. D. Collins, A. D. McClellan, *Science* **199**, 497 (1978)]. Essentially, an initially ineffective stimulus comes to evoke a response initially evoked only by an innately effective stimulus, after the preparation has experienced the two stimuli in close temporal association.
3. Habituation and sensitization have been defined as types of nonassociative learning because temporal pairing of stimuli are not required to produce a response decrement (habituation) or a facilitated response (sensitization). Many believe, however, that habituation and sensitization are primitive forms of learning that may constitute the base of more complex learning (*1*). For an analysis of the neuronal mechanisms underlying aspects of habituation and sensitization see V. F. Castellucci, T. J. Carew, E. R. Kandel, *Science* **202**, 1306 (1978); M. Klein and E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3512 (1978).
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Immunization of Baboons With *Schistosoma mansoni* Cercariae Attenuated by Gamma Irradiation

Abstract. *Studies on the efficacy of a vaccine against schistosomiasis in young baboons (*Papio anubis*) disclosed that immunization with* Schistosoma mansoni *cercariae attenuated by gamma irradiation induced significant protection against subsequent infection with normal, viable* S. mansoni *cercariae. Such immunization resulted in reduced worm burdens (70 percent) and egg excretion rates (82 percent). These results support immunization as a potential method for schistosomiasis control.*

The most promising vaccines against schistosomiasis have been prepared with gamma-irradiated schistosome cercariae (*1–5*). Recently, however, Taylor *et al.* (*6*) reported that immunization with *Schistosoma mansoni* cercariae attenuated with low levels of irradiation (2.1, 2.4, or 6.0 kilorads) failed to induce significant resistance in baboons subsequently challenged with normal, viable *S. mansoni* cercariae. Schistosomiasis in the baboon is a chronic disease, as it is in man (*7*), and if a vaccine cannot be demonstrated to be effective in the baboon model, it is unlikely to be successful in man.

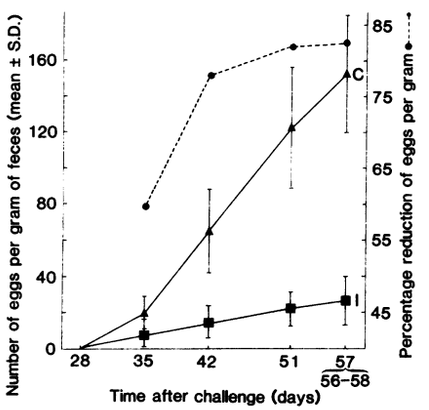

Fig. 1. Comparison of the mean fecal egg load in nine immunized (*I*) and ten nonimmunized (*C*) *Papio anubis* baboons. Each triangle and square dot and associated vertical line represent the mean and standard deviation. The dotted line represents the percentage reduction of eggs per gram of feces calculated according to the formula $[(C - I)/C] \times 100$. Fecal samples were obtained 7, 16, 28, 35, 42, 51, and 56 to 58 days after the baboons received the challenge dose.

The present study was conducted to determine whether attenuation of cercariae with high doses of ionizing radiation might yield an effective vaccine; such a vaccine had proved effective in NIH/Nmri CV mice (*5*). Nineteen young (6 to 8 kg) baboons (*Papio anubis*) from Kenya were used for the experiments. They were first subjected to three stool examinations (*8*) and to screening tests for circulating schistosomal antigen and antibody (*9*). Nine baboons (five males and four females) were immunized and ten baboons (five males and five females) were used as nonimmunized controls. For most studies of schistosome vaccines prepared with irradiation attenuated parasites, the mouse has been used; we therefore conducted a parallel monitoring experiment with 48 mice (6-week-old females of the NIH/Nmri CV strain).

Cercariae of *S. mansoni* (Puerto Rican strain) shed from *Biomphalaria glabrata* snails were attenuated (*10*) and used for

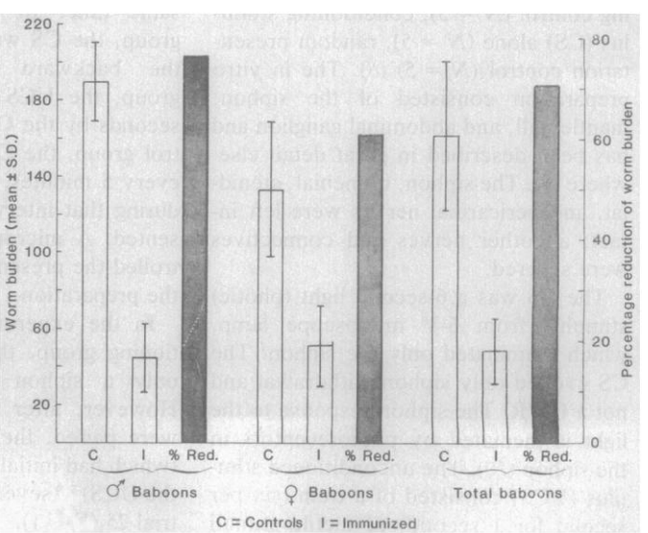

Fig. 2. Comparison of the worm burdens of nine immunized (*I*) baboons and ten nonimmunized (*C*) baboons challenged with 235 ± 22 normal *S. mansoni* cercariae. The open bars represent the mean (± standard deviation) worm burdens, and the shaded bars, the percentage reduction of the burdens calculated according to the same formula as that in the legend for Fig. 1.

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immunization. Nonirradiated cercariae were used for challenge infections. The baboons were immunized and challenged by percutaneous administration of the cercariae. Each baboon was anesthetized with ketamine hydrochloride and a suspension of cercariae was placed in a plastic disk strapped to the shaved abdomen. The mice were immunized by immersing the tail in a plastic tube containing the cercariae. After exposure to the cercariae for 30 minutes, the numbers of cercariae that had not penetrated the skin of the baboons or mice were counted to estimate the cercarial dose each animal received.

The baboons were immunized twice at 21-day intervals. In the first dose each animal received 4500 ± 2800 (mean ± standard error) cercariae, and in the second dose, 4900 ± 1600. All baboons (immunized and control) were challenged with 235 ± 22 normal viable *S. mansoni* cercariae 21 days after the second immunization.

Stool specimens were collected from the baboons for fecal egg load determinations (*7*) at 7, 16, 28, 35, 42, 51, and 56 to 58 days after they received the challenge dose of cercariae.

To ascertain the effectiveness of each immunization and the combined effect of the two immunizations, 12 mice were immunized at both immunization periods with the same batch of irradiation attenuated cercariae used to immunize the baboons; another group of 12 mice received only the first immunization, a third only the second immunization, and the 12 remaining mice constituted the nonimmunized controls. The mean doses were 535 ± 32 irradiation attenuated cercariae for the first immunization and 532 ± 60 for the second. All of the mice were challenged with a mean of 112 ± 12 *S. mansoni* cercariae from the same batch of cercariae as was used for the challenge doses for the baboons.

The baboons were killed and perfused 56 to 58 days after they received the challenge dose. They were first anesthetized with intramuscularly administered ketamine hydrochloride containing acepromazine (10 : 1 by volume), and then given an intracardiac injection of sodium pentobarbital. Heparin, as an anticoagulant, was added to the sodium pentobarbital to facilitate perfusion (*11*), which was carried out by a modified version of the method devised by Yolles *et al.* (*12*).

The mice were killed 42 days after they received the challenge dose. Each mouse was given an intraperitoneal injection of sodium pentobarbital containing heparin. The hepatic-portal vein was then torn and the mouse was perfused with citrated saline by way of the dorsal aorta (*12*).

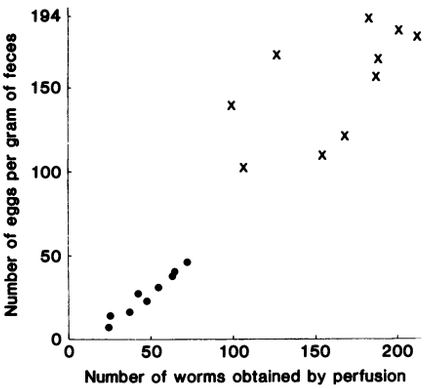

Fig. 3. Individual fecal egg loads plotted against worm burdens in immunized (●) and nonimmunized (×) baboons 56 to 58 days after challenge.

Eggs were first detected in the stool samples of two of the ten control baboons 28 days after challenge, but none of the immunized primates yielded fecal eggs at this time. At 35 days after challenge, all of the control baboons were passing fecal eggs, whereas two of nine immunized animals remained negative for fecal eggs. Figure 1 shows that if one compares the immunized with control groups, the reduction in fecal eggs per gram was 82.5 percent at 56 to 58 days after challenge. Figure 2 depicts the mean adult worm burdens and the percentage reduction of the worm burdens based on a comparison of the immunized and control groups. Fewer cercariae in the challenge doses developed to maturity in the nonimmunized female baboons than in the nonimmunized males ($.0125 > P > .01$); females appeared to respond less well to immunization, although this difference was not significant ($.3 > P > .25$).

The total percentage reduction in worm burden for the immunized baboons was 70.7 percent. Figure 3 shows that in immunized baboons the number of eggs per gram of feces was reduced more than the worm burden. Murrell *et al.* (*13*) also found a greater reduction in fecal egg counts than in worm recoveries in cynomolgus monkeys immunized with irradiated schistosomula. However, the possibilities that disproportionate egg count reductions may be a temporary effect of delayed worm maturation or migration, or a consequence of increased egg retention in the tissues have not been ruled out.

Deterioration of health, including malaise and decreased appetite, were noted in the control baboons by 50 days after challenge. All of the controls were frequently passing mucoid stools containing blood; four of them showed dysenteric symptoms. No obvious clinically deleterious effects were observed in the immunized baboons. Previously, Bushara *et al.* (*14*) demonstrated significantly less clinical manifestations of disease, reduced worm burdens, and fecal egg counts in cattle that had been immunized against *S. bovis*.

Postmortem examinations revealed that the immunized baboons had considerably fewer gross lesions in the liver and intestinal tract compared to the controls. However, the granulomatous reactions seen on histological examination of tissue sections differed only in degree and not in kind. These reactions included granuloma formation with interstitial and periportal plasma cell and macrophage infiltration, slight eosinophilia, and congested areas.

The differences between our results

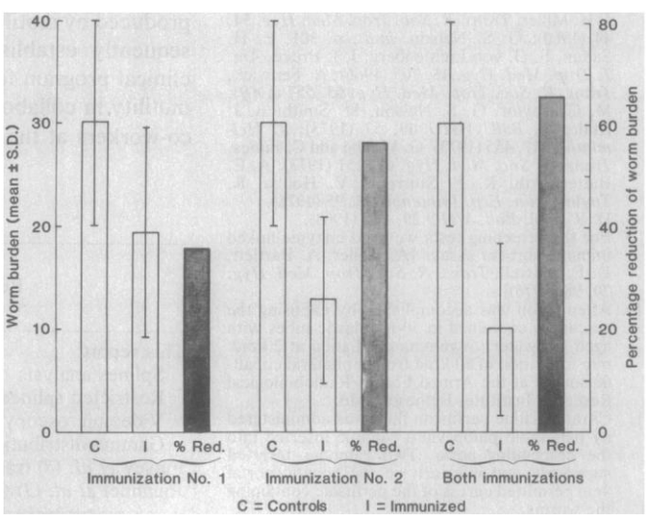

Fig. 4. Comparison of the worm burdens in immunized (*I*) and nonimmunized (*C*) female NIH/Nmri mice. The open bars represent the mean (± standard deviation) worm burdens, and the shaded bars, the percentage reduction of the worm burdens calculated by the same formula as that in the legend to Fig. 1.

and those of Taylor *et al.* (*6*) could be due to greater antigenic stimulation of the hosts in our studies as a result of our using highly irradiated cercariae. Other biological or technical factors might also account for the differences; for example, baboon age, sex, and species, schistosome strain, cercarial handling, time of shedding, water *p*H, or temperature.

Figure 4 shows that in the mice, two immunizations produced greater protection than either immunization alone. This supports the findings of Villella *et al.* (*1*), Erickson and Caldwell (*3*), and Hsu *et al.* (*15*) with other experimental hosts. Further work is necessary to establish the optimum irradiation attenuating dose, number of immunizing cercariae or schistosomules, number of immunizations, and interval between immunizations.

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10. Attenuation was accomplished by exposing the cercariae, contained in 50-ml plastic tubes with aged tap water, to gamma irradiation at 2 krad/min for a total of 60 krad from a bilateral cobalt-60 source at the Armed Forces Radiobiological Research Institute, Bethesda, Md.
11. Citrated saline perfusion fluid was administered by peristaltic pump via a cannula inserted into the descending aorta. Two cannulas inserted superiorly and inferiorly in the hepatic portal vein permitted egress of the perfusate containing the worms.
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Motility Assay of Human Sperm by Photon Correlation Spectroscopy

Abstract. *Microscopic methods of performing motility assays of spermatozoa are slow, subjective, and involve a small number of spermatozoa. Laser light-scattering methods can analyze the motility of many spermatozoa within minutes. The swimming speed distribution of human spermatozoa was investigated by photon correlation spectroscopy. The sperm was diluted in seminal plasma to avoid modifying the viscosity. The swimming speed distribution was reconstructed from the correlation data by Stock's method of splines. When compared with a videomicroscopic assay, the reconstructed swimming speed distribution accurately reflects translational motion between 0 and 80 micrometers per second, while for speeds greater than 80 micrometers per second the distribution is distorted by the effects of rotational motion.*

Sperm motility has been recognized as a significant factor in reproductive biology since MacLeod and Gold (*1*) established that the forward motion of sperm is the most important single factor in fertilization. Although sperm motility assays are of great importance both in clinical investigations of human fertility and in the practice of animal husbandry, traditional motility assay techniques are far from ideal. Visual microscopic assays can only quantify the movement of a small number of individual spermatozoa and include uncontrollable subjective errors. Microcinematography, while far more precise, is a slow and costly procedure.

Bergé *et al.* (*2*) observed that when laser light is scattered by sperm, the spectrum of the scattered light is dramatically modified by the Doppler shifts produced by motility. Bergé's group subsequently established an experimental clinical program for the study of human motility in collaboration with David and co-workers at the Bicetre Medical Center in Paris (*3*), using this technique of "light beating spectroscopy."

A second approach to motility assays based on laser scattering was initiated by Nossal and Chen (*4*) in a study of *Escherichia coli*. By using photon correlation spectroscopy, which is a fully digital technique, they opened the way for development of a rapid computer-based motility assay which could, in principle, be adapted to routine high-speed precise motility assays.

Nossal and Chen's procedure suffered from two difficulties: (i) wobbling and rotational motion of the swimming organisms distort the data and lead to overestimation of swimming speeds, particularly at large scattering angles, and (ii) the analytic procedure of Fourier inversion used to extract the swimming speed distribution $P(V)$ from the reduced correlation data $g^{(1)}(\tau)$ is highly sensitive both to noise and to the limited delay time span of the data. Subsequently, Stock and Carlson (*5*) showed that difficulties due to wobbling motion could be

Table 1. Results of human sperm motility assays.

Reference	Mean speed, V (μm/sec)	Motile fraction, α
This report:		
Splines analysis	75	0.36
Restricted splines (0 to 150 μm/sec)	57	
Videomicroscopy	46	
Gamma distribution fit (Eq. 1)	65	
Finsey *et al.* (*8*) (range, 0 to 250 μm/sec)	130	
Jouannet *et al.* (*3*) (range, 20 to 180 μm/sec)	96	0.47

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